

The effect of processing temperature on microbial safety and antioxidant activity of minimally processed “raw food”

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Summary

The assessment of the effect of dehydration temperatures (40 °C, 50 °C, and 60 °C) on the microbiological quality, antioxidant activity and oxidative stability of lipids of buckwheat-based minimally processed “raw food” (MPRF) was performed. Buckwheat flakes with various ingredients were soaked in sterile distilled water for 20 h, then MPRF samples were formed and dehydrated at constant temperature. Total viable counts, coliform, fungi, yeasts and aerobic spore-forming bacteria counts were evaluated in dehydrated products. While fungi were effectively reduced at all drying temperatures, higher total viable and coliform counts were found in MPRF samples after drying at 40 °C and 50 °C. Generally, antioxidant activity of MPRF samples did not significantly differ, and superoxide dismutase activity remained constant with the increase of drying temperature. The inhibition of lipid peroxidation was significantly higher in MPRF samples dehydrated at 40 °C than in those dehydrated at higher temperatures. Lipid oxidation stability measured by peroxide value, conjugated dienes content and thiobarbituric acid-reactive substances assay was not substantially affected during the dehydration process. Preparing MPRF by dehydration at 40 °C and 50 °C was considered as representing a microbial hazard whereas overall antioxidant activity was found to be minimally influenced by the dehydration temperatures.

Keywords

minimal processing; microbial hazard; antioxidant; lipid stability; dehydration temperature

The proponents of healthy lifestyle, particularly vegetarians or vegans, frequently prepare meals from various plant-based ingredients applying moderate temperature of dehydration/drying in order to protect nutritional composition from thermal damage. Numerous recipes for preparing of raw food are available in internet. The preparation of bars or cakes from buckwheat containing various ingredients was the most abundant. A mixture of ingredients is usually allowed to soak in water overnight, and then cakes are prepared and dried in food dehydrator at a moderate temperature. According to the popular book of Russo [1], a propagator of raw food diet, the

thermal treatment should not exceed 46 °C, which purportedly keeps the enzymes active and helpful for the further digestion of the meal. It was well described that temperature treatment of raw fruits and vegetables resulted in loss of essential nutrients, and thus decreased the overall quality of the final product [2]. On the other hand, the effect of drying temperature on the content of bioactive components, such as vitamins, carotenoids or phenolic compounds, and the corresponding antioxidant activity was not conclusively established at temperatures ranging from 30 °C to 60 °C [3–9]. For instance, the decrease of ascorbic acid content with the increase of drying temperature was ob-

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served in papaya [3], pineapple [4], but an increase was observed upon drying of apricot [5] or jaboticaba fruits [6]. Various effects of drying temperatures on the contents of β -carotene [7], vitamin E [8], vitamin A [5, 9] and vitamin B [7, 9] were also determined. It was found that antioxidant activity and the content of related compounds were influenced by the combination of several factors such as drying temperature, drying time, flow rate of drying air or geometry of the samples. Various trends of antioxidant activity values with the increase of drying temperature were described, for example their decrease in pomegranate seeds [10], increase in quinoa seeds [8] or constant values in maqui berries [7]. Exposing food products rich in lipids to elevated temperature may also result in formation of primary and secondary products of lipid peroxidation. It is well known that the oxidation rate of lipids is temperature-dependent [11, 12] and the extent of lipid oxidation measured as peroxide value, thiobarbituric or *p*-anisidine values reflects the quality of the final products.

With respect to the overall quality of final products, the growth or survival of microorganisms during the processing of plant-based meal is of a great importance. Since several outbreaks of infections associated with foods of plant origin occurred in past years, European Food Safety Agency (EFSA) defined three priority groups with relation to the food/pathogen interaction [13]. The combination of sprouted seeds and fruit-nuts with *Salmonella* and pathogenic *Escherichia* was identified as one of the priority. Soaking of various foods of plant origin in water was found to be a risk factor with regard to the proliferation of bacteria, yeasts and fungi [14–16]. In addition, drying food products at the moderate range of temperatures (i.e. 40–60 °C) may not be effective to provide microbiological safety [17–20].

The aim of this study was to examine the effect of dehydration process at moderate temperatures (40 °C, 50 °C and 60 °C) on the microbial quality, lipid stability and antioxidant activity of buckwheat-based minimally processed "raw food" (MPRF) samples.

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazil (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), potassium persulphate, ferrous chloride, ferric chloride, ammonium thiocyanate, polyvinylpyrrolidone (PVPP),

pyrogallol, Tris(hydroxymethyl)aminomethane, linoleic acid, butylated hydroxytoluene (BHT), disodium salt of ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid, thiobarbituric acid (TBA), and methanol (Chromasolv for HPLC, $\geq 99.9\%$) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Ethanol (96%, v/v), chloroform, acetic acid, hydrochloric acid, and diethyl ether were purchased from Lach-Ner (Brno, Czech Republic). All the reagents were of analytical grade. Nutrient agar No. 2, dichloran medium base with rose bengal, violet red bile agar and peptone glucose with bromocresol purple were obtained from HiMedia Laboratories (Mumbai, India).

Preparation of minimally processed "raw food" samples

Buckwheat flakes, hazelnuts, cashew, dried goji berries (Lifefood Czech Republic, Prague, Czech Republic), flaxseeds and chia seeds (Vega Provita, Frýdek-Místek, Czech Republic) were purchased in the local market in Czech Republic. The ingredients were manufactured in agreement with the Council Regulation No. 834/2007 on organic production and labelling organic products [21]. Buckwheat flakes (120 g), brown flaxseeds and chia seeds (each 50 g), hazelnuts, cashew nuts and dried goji berries (each 15 g) were soaked in sterile distilled water (weight ratio 1:1) at laboratory temperature (22.5 ± 0.5 °C) for 20 h followed by mixing and blending in Sterilmixer 12 rotary blender for 2 min at 15000 min^{-1} (P.B.I., Milan, Italy) in order to obtain homogenous matter. MPRFs were then aseptically prepared (60–70 mm in diameter, 3–4 mm in height) and allowed to dehydrate at 40 °C, 50 °C and 60 °C (± 0.4 °C) in an incubator with forced air circulation (Cooled Incubator ST equipped with heating system; Pol-Eko-Aparatura, Wodzisław Śląski, Poland) for 20 h. The preparation of MPRF was done by sterile kitchen utensils in freshly UV-irradiated laboratory. All working surfaces, including the interior of the incubator, were treated with disinfection solution prior to use.

Microbiological analysis

Total viable count (TVC), yeasts, fungi, total coliform count (TCC) and aerobic spore-forming bacteria count (ASBC) were determined in MPRF samples dehydrated at different temperatures. Following nutrient media were used (HiMedia): Nutrient agar No. 2 for TVC; dichloran medium base with rose bengal for yeast and fungi contents; violet red bile agar for TCC, and peptone glucose with bromocresol purple for ASBC. Ten grams of

sample were homogenized in a plastic bag with 90 ml of physiological saline using peristaltic masticator (IUL Instruments, Barcelona, Spain). An aliquot of appropriate dilution was transferred to the surface of agar plates in duplicates and incubated at 30 °C for 24–48 h (TVC), 25 °C for 6–7 days (total yeast count and total fungi count), 30 °C for 24–48 h (TCC), and 37 °C for 24 h (ASBC). For ASBC, living cells were inactivated at 90 °C (10 min) prior to the inoculation to agar plates. The results were expressed as logarithm of colony-forming unit per gram.

Moisture content and water activity determination

Moisture content of the samples was determined gravimetrically using moisture analyser MLB50-3 (Kern & Sohn, Balingen, Germany). It works on the principle of drying the sample (5 g) using two internal halogen lamps until weight constancy measured by internal laboratory balance (sensitivity ± 0.02). The drying temperature was set to 102 °C. Water activity was measured at (25.0 ± 0.2) °C using Thermoconstanter TH 500 (Novasina, Axair, Switzerland). All measurements were done in triplicate.

DPPH radical-scavenging activity

For the purpose of DPPH radical-scavenging activity determination, 1.0 g of the sample was extracted in 40 ml of ethanol solution (50 % v/v) in an ultrasonic bath for 30 min, followed by filtration to remove solid particles using Whatman filter paper No. 42 (GE Healthcare Life Sciences, Chicago, Illinois, USA). Extracts were prepared in triplicate. The DPPH radical-scavenging activity assay was adopted from the experimental procedure of Mišan et al. [22]. 5.0 ml of a methanol solution containing DPPH radical (\bullet DPPH, 25 $\mu\text{g}\cdot\text{ml}^{-1}$) was mixed with 0.5 ml of sample extract solution, and left to stand for 15 min in the dark before the decrease of absorbance at 517 nm was measured against methanol. DU-530 UV/Vis spectrophotometer (Beckman Coulter, Brea, California, USA) was used for measurements. As a control, 0.5 ml of 50 % (v/v) ethanol solution was used instead of the sample extract in the reaction mixture. The scavenging activity (SA_{DPPH}) was calculated using Eq. 1 and expressed as percentage of inhibition:

$$SA_{\text{DPPH}} = \left(1 - \frac{A_{\text{sm}}}{A_{\text{bl}}}\right) \times 100 \quad (1)$$

where A_{bl} is the absorbance of \bullet DPPH with ethanol; A_{sm} is the absorbance of \bullet DPPH with sample extract. Trolox equivalent antioxidant capacity (TEAC) was expressed in milligrams of Trolox

equivalent (TE) per kilogram of dry mass (DM).

ABTS radical-scavenging activity

The same extracts as for DPPH radical-scavenging activity were analysed. An ABTS radical-scavenging assay based on the method previously described by Mišan et al. [22] was used. ABTS radical cation ($\text{ABTS}^{\bullet+}$) was prepared from ABTS solution (5.0 ml, 50 $\text{mg}\cdot\text{l}^{-1}$) and 100 μl of potassium persulphate (64 $\text{mmol}\cdot\text{l}^{-1}$). The mixture was stored in the dark for 16 h at laboratory temperature before used. $\text{ABTS}^{\bullet+}$ solution was diluted by distilled water to give an absorbance of 0.70 ± 0.20 at a wavelength of 734 nm. After addition of 0.5 ml of sample extract to 5.0 ml of $\text{ABTS}^{\bullet+}$ solution, the mixture was allowed to stand for 30 min in the dark, and the absorbance was measured against the blank. Ethanol solution (0.5 ml, 50 % (v/v)) combined with 5.0 ml of $\text{ABTS}^{\bullet+}$ served as a negative control. The $\text{ABTS}^{\bullet+}$ -scavenging activity (SA_{ABTS}) was calculated according to Eq. 2, and expressed as percentage of inhibition:

$$SA_{\text{ABTS}} = \left(1 - \frac{A_{\text{sm}}}{A_{\text{bl}}}\right) \times 100 \quad (2)$$

where A_{bl} is the absorbance of the control, and A_{sm} is the absorbance in the presence of the sample. TEAC was expressed as milligrams of TE per kilogram DM.

Superoxide dismutase activity

The sample (4.0 g) was thoroughly mixed in a ceramic mortar with 40.0 ml of cold extraction solution containing 0.1 $\text{mol}\cdot\text{l}^{-1}$ phosphate buffer solution (pH 7.8), 0.1 $\text{mol}\cdot\text{l}^{-1}$ EDTA and 1.0 % PVPP. Solids were removed by filtration using Whatman filter paper No. 42 followed by centrifugation at 10000 $\times g$ for 15 min at 4 °C using Hermle Z300K (Gosheim, Germany). Superoxide dismutase (SOD) activity was determined using the method of Marklund and Marklund [23] based on the ability of SOD to inhibit autoxidation of pyrogallol. Sample extract (200 μl) was mixed with 3.0 ml of 50 $\text{mmol}\cdot\text{l}^{-1}$ Tris-HCl buffer (containing 10 $\text{mmol}\cdot\text{l}^{-1}$ EDTA, pH 8.5). The reaction was started by the addition of 200 μl of fresh pyrogallol solution (7.2 $\text{mmol}\cdot\text{l}^{-1}$ in 10.0 $\text{mmol}\cdot\text{l}^{-1}$ HCl) and the decrease in absorbance between 0.17 min and 1.17 min was recorded at 420 nm. The reaction mixture without sample was used as a control. One enzyme unit (1 U) was defined as the activity that inhibited the reaction by 50 %. The results were expressed as enzyme units per gram DM, and represented the mean of three independent experiments.

Lipid peroxidation inhibition assay

The same extract as for SOD activity was analysed. Lipid peroxidation inhibition assay was adopted from the study of PERVIN et al. [24]. One millilitre of the sample extract was mixed with 1.0 ml of 50 mmol·l⁻¹ linoleic acid solution (dissolved in ethanol (95 % v/v)) and 1.0 ml of distilled water. Distilled water and BHT (2.0 mg·ml⁻¹) were used for negative and positive control, respectively. Then the mixture was incubated at 40 °C in a screw-capped glass vial and the degree of oxidation was evaluated using ferric thiocyanate values. An aliquot of the solution incubated with linoleic acid (50 µl) was added to the mixture of 2.5 ml ethanol (75 % v/v), 50 µl of 30% ammonium thiocyanate and 50 µl of 20 mmol·l⁻¹ ferrous chloride (dissolved in 3.5% v/v HCl). Then, the absorbance at 500 nm was measured after 2 min of reaction at laboratory temperature. The thiocyanate value was monitored at regular intervals and the scavenging activity (*S*_{PEROX}) was calculated using Eq. 3 and expressed as percentage of inhibition:

$$S_{\text{PEROX}} = \left(1 - \frac{A_{\text{sm}}}{A_{\text{bl}}}\right) \times 100 \quad (3)$$

where A_{sm} is absorbance of the sample at 500 nm and A_{bl} is absorbance of the control.

Peroxide value assay

Oil was extracted by means of Soxhlet extraction: 10–15 g of the sample was extracted with 120 ml of diethyl ether containing 10 mg·kg⁻¹ BHT to prevent incidental lipid oxidation. After 4.0 h of extraction, the remaining ether was removed under a stream of nitrogen gas and extracts were stored at -20 °C until analysed. Each sample was extracted in duplicate. Spectrophotometric assay based on the oxidation of Fe²⁺ to Fe³⁺ by the peroxides present in the oil extract was used [25]. The oil sample (0.05 g) was dissolved in 1.0 ml of chloroform/acetic acid solution (2:3) with addition of 100 µl Fe²⁺ solution (36 mmol·l⁻¹), mixed vigorously for 15 s, and left in the dark for 10 min. For pigment and oil extraction, deionized water (2.0 ml) and 4.0 ml of diethyl ether (containing 10 mg·kg⁻¹ of BHT) were added. The organic phase was discarded and 1.0 ml of the aqueous phase was mixed with saturated potassium thiocyanate solution. After 10 min, absorbance at 470 nm was measured against a blank. A correction of the spectrum baseline at 640 nm was performed. A reaction blank containing all the reagents without sample was utilized, its absorbance being subtracted from that of the sample. A calibration plot of absorbance (470–640 nm) versus Fe³⁺ concentration was used for quantification of peroxide

value (*PV*). The following equation (Eq. 4) was used:

$$PV = \frac{A_{\text{sm}} - A_{\text{bl}}}{58.84 \cdot 2 \cdot m \cdot W_{\text{sm}}} \quad (4)$$

where *PV* is expressed as milliequivalents of peroxide per kilogram of sample, A_{sm} is the absorbance of the sample at 470 nm; A_{bl} is the absorbance of the blank at 470 nm; 58.84 is the atomic weight of Fe; 2 is the factor for conversion of milliequivalents of Fe to milliequivalents of peroxide; *m* is the slope of the Fe³⁺ calibration plot, W_{sm} is the sample weight in grams. Three replicates for each extract were analysed.

Assay of thiobarbituric acid-reactive substances

The sample (0.5 g) was extracted by 25.0 ml of 80% (v/v) ethanol solution, which was chosen as the best solvent for malondialdehyde extraction from plant material [26]. The extraction procedure was enhanced by sonication for 5 min, then 2.0 ml of supernatant (after centrifugation at 3000 ×g for 25 min) was added to the test tube with equal volume of the reaction mixture (20% trichloroacetic acid, 100 mg·l⁻¹ BHT and 6.5 g·l⁻¹ TBA). The test tube was then heated at 70 °C for 30 min in a block heater, cooled, and absorbances were read at 532 nm and 600 nm. The values of malondialdehyde equivalents (*MDAE*) were calculated according to the equation:

$$MDAE = \left[\frac{(A_{532} - A_{600})}{155000} \right] \times 10^6 \quad (5)$$

where *MDAE* is expressed in nanomoles per millilitre, A_{532} represents the maximum absorbance of TBA-MDA complex, A_{600} the correction for non-specific turbidity, 155000 the molar extinction coefficient for MDA. Three extracts were prepared from each sample, and *MDAE* was measured for each extract in triplicate. Thiobarbituric acid-reactive substances (*TBARS*) were expressed as micromoles of *MDAE* per kilogram DM.

Determination of conjugated dienes

Conjugated dienes were quantified in 0.05 g of extracted oil diluted in 25 ml of isooctane by measuring the absorbance at 233 nm [27]. The content of conjugated dienes was expressed as grams per kilogram DM.

Statistical analysis

The results were represented by mean with standard deviation of repeated measurements. Analysis of variance (ANOVA) was used to determine the effect of drying temperature on the

Tab. 1. Microbiological load of ingredients and minimally processed “raw food” samples dried at different temperatures.

Ingredient	Total viable count	Total yeast count	Total fungi count	Total coliform count	Aerobic spore-forming bacteria count
	[log CFU·g ⁻¹]				
Buckwheat flakes	3.72 ± 0.63 ^a	< 1	3.52 ± 1.03 ^a	3.45 ± 0.55 ^a	3.14 ± 0.81 ^a
Flaxseeds	> 7.48 ^b	3.85 ± 0.65 ^a	2.65 ± 0.72 ^a	5.85 ± 0.63 ^b	< 1
Chia seeds	3.14 ± 0.71 ^a	< 1	3.10 ± 0.72 ^a	< 1	< 1
Hazelnuts	3.92 ± 0.47 ^a	< 1	2.98 ± 0.97 ^a	2.91 ± 0.98 ^a	< 1
Cashew nuts	4.08 ± 0.87 ^a	< 1	4.31 ± 0.97 ^a	< 1	< 1
Dried goji berries	3.58 ± 0.34 ^a	< 1	3.70 ± 0.57 ^a	2.8 ± 1.63 ^a	< 1
Drying temperature					
40 °C	> 7.48 ^b	3.73 ± 0.24 ^a	< 1	7.89 ± 0.65 ^c	4.13 ± 0.36 ^a
50 °C	> 7.48 ^b	< 1	< 1	4.22 ± 0.73 ^{ab}	4.05 ± 0.85 ^a
60 °C	3.95 ± 0.45 ^a	< 1	< 1	< 1	3.93 ± 1.07 ^a

Values represent the mean of two independent experiments ± standard deviation. Values followed by the same letter in superscript within a column are not statistically different ($p > 0.05$) using Tukey's test.

(< 1) – below limit of detection, (> 7.48) – uncountable in 10⁶-fold diluted sample extract.

results of chemical analysis. Multiple comparison process among means was performed using the Tukey's method, and non-parametric Spearman correlation coefficient was calculated in order to determine the relationship between variables. Statistical treatment of the data was done at the probability level of $p = 0.05$ by OriginPro 9.0 (OriginLab, Northampton, Massachusetts, USA).

RESULTS AND DISCUSSION

The effect of drying temperature on microbiological quality

The microbiological quality of each ingredient used for the preparation of MPRF sample was determined in terms of total viable counts, yeasts, fungi, coliforms, and aerobic spore-forming bacteria (Tab. 1).

Fungi were present in all the ingredients in the range from 2.65 log CFU·g⁻¹ to 4.31 log CFU·g⁻¹, yeasts were only found in flaxseeds (3.85 log CFU·g⁻¹). On the contrary, yeasts were the most prevalent microorganisms found in 184 of fresh and minimally processed vegetable samples, ranging from 2.00 log CFU·g⁻¹ to 8.60 log CFU·g⁻¹ [28]. In general, flaxseed was heavily contaminated showing high TVC (> 7.48 log CFU·g⁻¹) and TCC (5.85 log CFU·g⁻¹) in comparison with other ingredients. Although the microbial load of raw matter (i.e. after 20 h soaking in sterile distilled water) was not determined, significantly higher TVC ($p = 1.236 \times 10^{-4}$), and TCC ($p = 0.022$)

were observed in MPRF samples dried at 40 °C in comparison with ingredients. It may imply that proliferation of some microorganisms occurred during the soaking period probably due to the favourable temperature and sufficient time (22.5 °C, 20 h), and high water activity of the mixture ($a_w = 0.96$ –0.98). Soaking of cassava pieces in potable water was identified as a hazard for production of traditional meal in Nigeria with respect to the growth and contamination by pathogenic and spoilage microorganisms [14]. The significant increase in aerobic bacteria, coliforms, yeasts and fungi was also observed during soaking of rice at ambient temperature for 2–4 h [15], and 11-fold rise of *Fusarium oxysporum* was detected after soaking of oil palm seeds for 7 days [16].

Fungi were not found in MPRF samples prepared by dehydrating at all the temperatures in this study. The decline of fungi content below the limit of detection, particularly at 40 °C, can be explained by releasing of antifungal compounds from both buckwheat and flaxseed during thermal treatment [29, 30]. This finding is not in agreement with our previous study where fungi were present in buckwheat cookies (made of buckwheat seed and golden flaxseed) dried at 60 °C [20].

Increasing the drying temperature resulted in a decrease of yeast and total coliform counts in our study. It should be noted that a synergistic effect of both drying temperature and low water activity has to be taken into account. A decrease of water activity to 0.75 ± 0.08 , 0.32 ± 0.12 and < 0.11 in MPRF samples was observed when dehydrated

at 40 °C, 50 °C and 60 °C, respectively. From the microbiological safety point of view, buckwheat-based MPRF samples prepared by dehydration at 40 °C and 50 °C pose a risk for consumption. While 6.6 log reduction of *E. coli* O157:H7 was observed in radish seeds after heating at 64.5 °C for 17.7 h, only 2.78 log reduction was achieved when heated at 55 °C for 14 h [17]. Moreover, higher inactivation rate was determined in case of applying drying-air with a higher relative humidity. Dehydrating at 40 °C may rather act as an incubation temperature, for example, the specific growth rate of *Salmonella Typhimurium* on chicken skin was found to be optimal at 40.3 °C [18]. In our study, *TVC* and *TCC* in MPRF samples dehydrated at 50 °C were $> 7.48 \log \text{CFU}\cdot\text{g}^{-1}$ and $4.22 \text{CFU}\cdot\text{g}^{-1}$, respectively, which may have implications for human health. Reduction in *TCC* was apparent in MPRF samples dried at 60 °C, while ASFB counts did not change significantly with the increase of the drying temperature ($p = 0.151$).

DPPH and ABTS radical-scavenging activities

The *TEAC* value determined by means of DPPH-scavenging activity in raw matter after 20 h of soaking in sterile distilled water was $193.95 \text{mg}\cdot\text{kg}^{-1} \text{DM}$ and, when measured using $\text{ABTS}^{\bullet+}$, it was $1356.65 \text{mg}\cdot\text{kg}^{-1} \text{DM}$. The drying temperature was identified as the main factor affecting the antioxidant activity of MPRF samples determined by using $\text{ABTS}^{\bullet+}$ ($p = 1.820 \times 10^{-3}$), but not by $\bullet\text{DPPH}$ ($p = 0.182$) scavenging activity assays. As can be seen from Tab. 2, the ability to scavenge $\bullet\text{DPPH}$ significantly decreased upon drying in comparison with the raw matter ($p = 1.613 \times 10^{-3}$), followed by gradual but not significant decrease ($p > 0.05$) with the increase in drying temperature. Using ABTS method, MPRF samples lost 33.9% of their antioxidant activity when dried at 40 °C for 20 h. Significantly lower ability of MPRF sample extracts to scavenge

$\text{ABTS}^{\bullet+}$ was observed upon drying at 50 °C ($p = 3.311 \times 10^{-5}$) in comparison with those dried at 40 °C and 60 °C.

Superoxide dismutase activity

In addition to non-enzymatic defence system against reactive oxygen species, such as ascorbate or phenolics, plants have developed an enzymatic system composed of a wide range of enzymes. SOD activity of raw matter after soaking in sterile distilled water for 20 h at laboratory temperature was significantly higher ($p = 1.103 \times 10^{-4}$) than those observed in minimally processed “raw food” upon drying treatment. The drying temperatures did not affect the activity of SOD in buckwheat-based MPRF samples in our experiment ($p = 0.826$). Similar oscillating behaviour of the activity of SOD was observed during sun-drying (with temperature ranging from 20.7 °C to 38.6 °C) of Arabica coffee beans [31] referring to the relative stability of the enzyme at higher temperatures.

Lipid peroxidation inhibition

The antioxidant activity of MPRF samples was also determined by its inhibiting effect towards linoleic acid peroxidation using the thiocyanate method. The antioxidant activity of the extracts in linoleic acid system is depicted in Fig. 1. Raw matter had stronger inhibitory properties towards lipid peroxidation ($p = 3.501 \times 10^{-5}$) in comparison with dried samples, reaching 69% of its inhibition activity after 2 days of incubation in comparison with BHT ($2.0 \text{mg}\cdot\text{ml}^{-1}$). Drying of MPRF samples led to a decrease of their ability to inhibit linoleic acid peroxidation, showing 25% of inhibition after 2 days of incubation. Small but significantly higher linoleic acid peroxidation inhibition was observed in MPRF samples dried at 40 °C than those at 50 °C and 60 °C after 3 days of incubation ($p = 2.782 \times 10^{-3}$ and $p = 5.094 \times 10^{-3}$, respectively). The opposite effect was found by

Tab. 2. Effect of drying temperature on the antioxidant activity of minimally processed “raw food” samples.

	<i>TEAC</i> _{DPPH} [mg·kg ⁻¹]	<i>TEAC</i> _{ABTS} [mg·kg ⁻¹]	SOD activity [U·g ⁻¹]
Raw matter	193.95 ± 13.26 ^a	1356.65 ± 50.42 ^a	64.54 ± 4.73 ^a
Drying temperature			
40 °C	115.68 ± 5.88 ^b	897.02 ± 4.77 ^c	15.47 ± 1.50 ^b
50 °C	109.57 ± 16.45 ^b	769.97 ± 4.31 ^b	15.61 ± 0.40 ^b
60 °C	86.85 ± 3.38 ^b	875.60 ± 15.10 ^{bc}	16.54 ± 3.09 ^b

Results are expressed as the mean ± standard deviation of three independent experiments. Values followed by the same letter in superscript within a column are not statistically different ($p > 0.05$) using Tukey's test.

TEAC – Trolox equivalent antioxidant capacity (expressed per kilogram of dry matter), *TEAC*_{DPPH} – *TEAC* determined using 2,2-diphenyl-1-picrylhydrazil radical, *TEAC*_{ABTS} – *TEAC* determined using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid cation radical, SOD - superoxide dismutase (activity expressed per gram of dry matter).

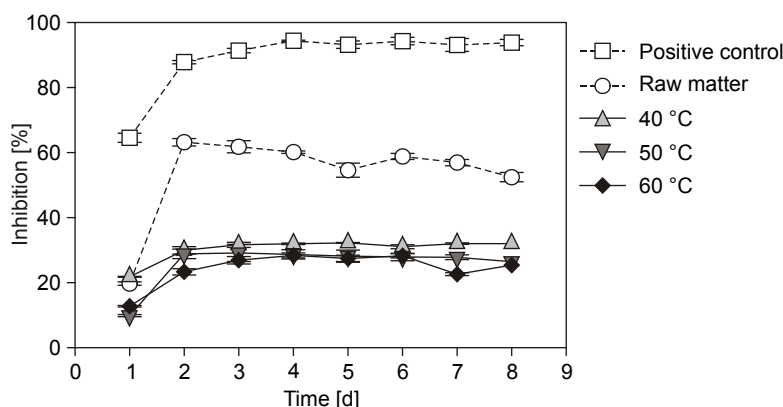


Fig. 1. Inhibition of linoleic acid peroxidation at different drying temperatures.

Raw material also included. BHT served as a positive control. Vertical bars mean standard deviations.

BROŽKOVÁ et al. [20], who determined significantly higher lipid peroxidation inhibition in samples dried at 60 °C in comparison with those at 40 °C. This discrepancy can be attributed to the different composition of samples. Significant negative correlation was found between linoleic acid peroxidation inhibition and peroxide values ($r = -0.648$, $p = 0.043$).

Effect of dehydration temperature on oxidative stability

The ingredients used for the preparation of MPRF samples in this study were described as rich sources of unsaturated fatty acids (linoleic, alpha-linolenic), which may undergo oxidative damage during storage and processing [12]. Hydroperoxides of fatty acids are formed during the initial stage of oxidation (measured by peroxide value) followed by the formation of conjugated dienes as intermediates, to aldehyde compounds (measured by TBARS value) as an indicator of rancidity of oil and fat [32]. The effect of drying temperature on oxidative stability of MPRF samples is described in Tab. 3. Peroxide

values were higher for MPRF samples dried at 50 °C and 60 °C in comparison with samples dried at 40 °C, with p -values of 0.026 and 0.038, respectively. However, the overall effect of drying temperature on peroxide value was found to be negligible ($p = 0.058$). Although it was previously described that formation of hydroperoxides from lipids increased in the range of storage temperatures of interest (i.e. 30–50 °C), time of temperature duration and relative humidity of drying air have to be taken into account [11]. Despite the increase of peroxide values with the increase of drying temperature, these are far below the limit ($< 10.0 \text{ meq}\cdot\text{kg}^{-1}$) recommended by International Food Standard provided by Codex Alimentarius [33]. Conjugated dienes content was similar for raw matter and MPRF samples dried at 40 °C ($p = 0.753$), whereas a significant decrease was observed when dried at 50 °C ($p = 6.412 \times 10^{-7}$) and 60 °C ($p = 1.884 \times 10^{-5}$). According to ANOVA procedure, drying temperature was found to be a significant factor influencing the formation of conjugated dienes ($p = 2.560 \times 10^{-6}$), whereas TBARS values did not show any trend with the in-

Tab. 3. Oxidative stability of minimally processed “raw food” samples after drying at different temperatures.

	Peroxide value [$\text{meq}\cdot\text{kg}^{-1}$]	TBARS [$\mu\text{mol}\cdot\text{kg}^{-1}$]	Conjugated dienes [$\text{g}\cdot\text{kg}^{-1}$]
Raw matter	0.77 ± 0.10^a	4.85 ± 0.09^a	0.95 ± 0.01^a
Drying temperature			
40 °C	0.62 ± 0.02^b	0.68 ± 0.01^d	0.96 ± 0.02^a
50 °C	0.86 ± 0.07^a	3.02 ± 0.13^b	0.70 ± 0.01^b
60 °C	0.86 ± 0.01^a	1.66 ± 0.03^c	0.78 ± 0.01^c

Results expressed as the mean \pm standard deviation of three independent experiments.

Values followed by the same letter in superscript within a column are not statistically different ($p > 0.05$) using Tukey's test. Peroxide value is expressed as milliequivalents of peroxide per kilogram of oil, TBARS – thiobarbituric acid-reactive substances (expressed as micromoles of malondialdehyde equivalents per kilogram of dry matter).

crease in drying temperature in this study. Significant correlations were observed between antioxidant properties of samples (measured by mean of ABTS^{•+}-scavenging activity) and peroxide value ($r = -0.594$, $p = 0.041$), TBARS value ($r = -0.878$, $p = 0.002$) and conjugated dienes ($r = 0.567$, $p = 0.014$).

CONCLUSIONS

The effect of dehydration temperature on microbiological quality, antioxidant activity and lipid stability of minimally processed “raw food” samples containing buckwheat flakes, brown flaxseeds, chia seeds, hazelnuts, cashew nuts and dried goji berries was established. MPRF samples were prepared according to the recipe frequently used by vegetarians and vegans. Despite the fact that fungi were present in all the ingredients in the range from 2.65 log CFU·g⁻¹ to 4.31 log CFU·g⁻¹, the dehydration process caused a decrease in the content of fungi to below the limit of detection. On the other hand, aerobic and facultative anaerobic bacteria (measured as TVC), as well as coliform bacteria persisted in MPRF samples dehydrated at 40 °C at high levels (> 7.48 log CFU·g⁻¹ and 7.89 log CFU·g⁻¹, respectively). Significant improvement of microbiological quality of final product was attained after dehydration at 60 °C. Antioxidant activity as determined by •DPPH- and ABTS^{•+}-scavenging assays, and by lipid peroxidation inhibition assay, slightly decreased or remained constant at higher dehydration temperatures, respectively. While small (even significant) changes in antioxidant activity and lipid stability were observed in buckwheat-based MPRF samples, microbiological quality was improved with the increase of the dehydration temperature.

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