Optimisation of High Intensity Ultrasound Treatment of Proso Millet Bran to Improve Physical and Nutritional Quality

Running title: Improvement of Millet Bran using High Intensity Ultrasound

Nikolina Čukelj Mustač, Bojana Voučko*, Dubravka Novotni, Saša Drakula, Anamarija Gudelj, Filip Dujmić and Duška Ćurić

University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

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SUMMARY

Millet is an unexploited cereal with potential in the food industry due to its nutritional value and resistance to harsh climate conditions. Nutritious millet by-products have a potential to be exploited in the development of functional cereal products, but require processing in order to improve their physical and nutritional quality. Therefore, we investigated high intensity ultrasound as a pre-treatment for increasing the amount of freely available bioactives from proso millet bran. We also investigated high intensity ultrasound effect on enzymatic browning, water retention and protein digestibility, that are crucial for the utilization in the bakery and pasta industry. A 15 % millet bran suspension in water was treated with 400 W ultrasound probe during 5, 12.5, or 20 min, with the 60, 80, or 100 % of amplitude. High intensity ultrasound treatment with 80 % amplitude during 12.5 min showed the most significant improvement of the nutritive value; – the antioxidant activity measured by FRAP test increased by 15 % (p < 0.05), total phenolic content by 16 % (p < 0.05). Still, the impact on the dietary fibre soluble in water and not ethanol, was

*Corresponding author: Phone: 0038514605167; E-mail: bvoucko@pbf.hr

ORCID IDs: 0000-0002-5255-2910 (Čukelj Mustač), 0000-0001-8031-5253 (Voučko), 0000-0001-8760-6125 (Novotni), 0000-0002-4564-6724 (Drakula), 0000-0003-1237-0343 (Gudelj), 0000-0002-1615-3189 (Dujmić), 0000-0001-8970-9478 (Ćurić)

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evident after the 100 % amplitude and 20 min treatment, causing a rise by 38 %. High intensity ultrasound treatment of 100 % amplitude and 5 min caused largest improvements in water retention and limited browning of the sample. Polyphenol oxidase was activated by high intensity treatment, regardless of the applied heating of the sample. Ambiguous impact of high intensity ultrasound on proso millet bran characteristics required an optimization which showed that the optimal pre-treatment for a 15 % millet bran suspension in water is at 100 % amplitude during 9.3 min.

**Key words:** antioxidant activity, enzymatic browning, high intensity ultrasound, millet bran, water retention

**INTRODUCTION**

Millet is an unexploited cereal with great potential in the agro-food industry due to its resistance to drought and harsh climate conditions (1), while bran is a by-product with potential health benefits due to its content of protein, essential fatty acids, minerals, vitamins, and components with antioxidant capabilities, such as polyphenols and phytosterols. Additional nutritional value of bran is its high content of dietary fibre (2) which leading authorities are constantly urging to be consumed daily due to their proven health benefits. According to the Academy of Nutrition and Dietetics, intake of 14 g of dietary fibre per 1000 kcal consumed can decrease the risk of obesity, cardiovascular disease and type 2 diabetes (3). In spite of that, a fibre-rich product like bran is underutilized and mostly used as animal feed (4). Unfortunately, application of bran to bakery products can have a deteriorating effect on its structure and sensory properties, causing a drop in volume and a bitter taste (2). Mechanism responsible for deleterious effect of bran on bakery products is not completely clear but is partially caused by higher water retention due to brans’ ability for dynamic hydration (2). Dynamic hydration is characteristic for dietary fibre from bran, and enables adsorption of water through two different mechanisms, one caused by external stress, and other caused by hydrogen bonds resulting in excess water intake. The absorbed water is available for starch gelatinization during baking which lowers starch gelatinization temperature and consequently shortens the time of loaf volume growth (5). In order to exploit the potential of bran as a raw material for production of cereal functional products, some novel processes are needed to modify its properties to suit them for the bakery industry.
Ultrasound is a robust non-thermal green technology which generates mechanical energy by sound waves, causing series of alternating contractions and expansions. The deteriorating effect of high intensity ultrasound on cell walls is caused by collapses of cavitation bubbles which cause strong shear forces that form micro fractures in plant tissues and help mass transfer, penetration of the solvent, diffusion and release of extractable compounds (6). Besides its effect on availability of compounds with high nutritive value, high intensity ultrasound can affect rheological properties of food and cause a change in appearance (colour) or applicability (water retention, protein digestibility) of a product. Change in colour, specifically occurrence of browning, that develops as a result of use of high intensity ultrasound, is possibly explained by activation of enzymes. Polyphenol oxidase (PPO) (1,2-benzenediol:oxygen oxidoreductase) is a generic name for a group of enzymes catalysing the oxidation of phenolic compounds which results in generation of melanin pigments (7). It is actively involved in plant defence and can be used as a marker of resistance to downy mildew infections in pearl millet (8). Because of its protective role, it is highly concentrated in the bran fraction of the grain (2). Browning of food products includes first a catalytic reaction of oxidation of mono- and di-phenolic compounds to quinones by PPO, and second a non-enzymatic condensation process of forming complex brown polymers – melanins (9). The catalytic reactions of PPO do not only affect the colour, but also the flavour, texture and nutritional value of the material (10). They result in oxidation of phenolics and also a decrease in available amino acids. Felton et al. (7) reported that presence of PPO caused a decrease of lysine content in casein.

With the aim of making bran an asset for production of cereal functional products, we investigated application of high intensity ultrasound to proso millet bran. The hypothesis of this research was that improvement in the nutritive value of millet bran could be achieved through a pre-treatment with high intensity ultrasound due to its potential of releasing dietary fibre and compounds with antioxidant activity form their insoluble/bound to soluble/free forms, while customizing proso millet bran physical characteristics for requirements of bakery industry by limiting enzymatic browning and water retention capacity.

MATERIALS AND METHODS

Proso millet bran
Millet bran was obtained after decortication of proso millet (*Panicum miliaceum*) Sonček variety grown in 2017, and given as a gift from Mlinopek (Murska Sobota, Slovenia). The bran was sieved through a 500 µm pore size sieve to achieve a product without foreign matter and of high nutritive value.

**Chemical composition analysis**

Chemical composition of the proso millet bran fraction smaller than 500 µm (PMBF) was determined according to the standard AACC Methods (11) and ICC Standard 136 (12). Protein content was determined by the AACC 46-12 Kjeldahl method (11) which included acid digestion of the sample, distillation of ammonia in 4 % boric acid (Gram-mol, Zagreb, Croatia) and titration of the distillate with 0.1 M HCl (Gram-mol, Zagreb, Croatia) until neutrality. Protein content was calculated using factor 6.25. Dry matter content was determined by AACC 44-19 method, by drying the samples in an air-oven (Instrumentaria, ST-01/02, Zagreb, Croatia) at 135 °C for 2 h (11). Total fat content was determined according to the ICC Standard 136 (12) which included the extraction with hexane (95%, J.T. Baker), removal of the solvent and weighing the obtained residue.

**Ultrasound treatment**

Forty-five grams of PMBF was suspended in 300 mL of distilled water in a 600 mL volume glass beaker. The sample was treated with the UP400S ultrasonic processor (Hielscher GmbH, Germany) at 400 W and 24 kHz, using a titanium 22 mm diameter probe (H22D, Hielscher GmbH, Germany). Treatments were conducted according to the central composite design generated with Design Expert 11.0.3 software (13) as shown in Table 1. Temperature change was monitored using a temperature probe which was immersed into the sample during high intensity ultrasound treatment. Predicted specific heat capacity of the sample, amounting to 3886 J/kg K was calculated from the determined chemical composition of the sample and values for specific heat capacity of individual food components according to Eq. 1 (14), according to Eq. 1:

\[ C_p = \sum Wi \cdot C_{pi} \]  

where \( C_{pi} \) is the specific heat capacity of a major food component and \( Wi \) is the mass fraction of a major food component. Together with the change in temperature during time, it was used to calculate the acoustic power \( P \) of high intensity ultrasound according to Eq. 2 (15,16):

\[ P = \frac{\sum Wi \cdot C_{pi} \cdot \Delta T \cdot \Delta t}{t} \]
\[ P = m \cdot C_p \cdot \left( \frac{dT}{dt} \right) /2 \]

where \( m \) is the weight of the sample (kg), \( C_p \) is the specific heat capacity of the sample (J/kg K), \( \frac{dT}{dt} \) is the change in temperature over time (K).

Control sample (0A20M) was the same PMBF-water suspension, but stirred for 20 min at room temperature on a magnetic stirrer (IKA C-MAG HS-7, Wilmington, USA) at 500 rpm. Afterwards, the samples were frozen to -80°C and freeze dried (Alpha 1-4 LSC Plus, Christ, Germany) during 42 h.

**Polyphenol oxidase activity**

The measurement of PPO was carried out using AACC 22-85.01 (17) method with some modifications. Sample mass was adjusted to 10 mg and the quantity of the L-DOPA (Sigma Aldrich, Steinheim, Germany) sample solution was adjusted to 0.5 mL and diluted with distilled water to a final volume of 1 mL. Afterwards, the samples were mixed on vortex (IKA MS 3, Basic KA Works INC, Wilmington, USA) for 55 minutes 1000 rpm and centrifuged (Micro CL 21 Thermo Scientific Fisher, Germany) for 5 minutes at at 6164×g, prior to spectrophotometric measurement (Specord 50 PLUS; Analytic Jena, Germany) at 475 nm. According to the applied method (17), activity of PPO is expressed as the absorbance.

**Browning index**

The colour of PMBF was measured using the colour space values (*L*, *a*, and *b*) of the CIELab system, using a colorimeter (Spectrophotometer CH-3500 D, Konica Minolta, UK). Browning index (BI)) was calculated according to (18) Eq. 3:

\[ BI = \frac{(a + 1.75 L)}{(5.645L + a - 0.3012b)} /3/ \]

**Dietary fibre**

Dietary fibre can be divided according to its solubility and molecular weight to: insoluble dietary fibre (IDF), higher molecular weight dietary fibre soluble in water that precipitates in 78% aqueous ethanol (SDFP), and lower molecular weight dietary fibre soluble in water that remains soluble in 78% aqueous ethanol (SDFS) were determined with the Integrated total dietary fibre assay kit (Megazyme, Ireland) according to AOAC 2011.25 method (19), using Shimadzu HPLC system.
with MetaCarb 67C column (Agilent, USA) and software LC solution v. 1.22 SP1 (Shimadzu, Japan) (19).

Water retention

Water retention capacity (WRC) of PMBF was determined according to the AACC 56-11-02 method (11) as modified by Jacobs et al. (20). Sample (2 g PMBF) was soaked in 20 mL of distilled water during 60 min, after which it was centrifuged at 2813 × g during 10 minutes (Rotina 35 D-78532, Hettich, Germany). The supernatant was discarded and the pellet drained during 15 min by placing the tubes at a 45° angle. The residue was weighed. The WRC capacity is expressed as percent of sample mass, on a dry basis.

Protein digestibility

In vitro protein digestibility (IVPD) was determined following the multi-enzyme method of Hsu et al. (21) modified according to Tinus et al. (22). Briefly, 500 mg of PMBF (weight equivalent to 62.5 mg protein) was rehydrated in 10 mL of distilled water at 37 °C for 1 hour, before adjusting the pH of the sample to 8.0. Drop in the pH value (ΔpH) of the sample 10 min after adding 1 mL of the multi-enzyme solution was measured with a pH meter (pH/mV temperature meter model 3510, Jenway, Cole-Parmer, Germany) and used to calculate the IVPD (%) according to the Eq. 3:

\[
IVPD = 65.66 + 18.1 \Delta p\text{H} / 3/
\]

The multi-enzyme solution consisted of 16 mg T0303 trypsin form porcine pancreas Type IX-S, 31 mg C4129 α-chymotrypsin from bovine pancreas Type II, and 13 mg P5147 from Streptomyces griseus Type XIV, all acquired from Sigma Aldrich (Merck, Steinheim, Germany).

Extraction of phenolic compounds

Freeze-dried samples were used for the extraction of free phenolic compounds, according to modified health grain method (23). Precisely, 250 mg of sample was weighed into a 2 mL Eppendorf safe lock tube. To each sample, 1 mL of 80% ethanol (V/V, HPLC grade; Fisher Chemical, Fisher Scientific, Hampton, NH, USA) was added, vortexed for 0.5 min at 1500 rpm and placed into ultrasonic bath (Bandelin electronic RK 100 H, Sonorex, Berlin, Germany) for 10 min, after which the samples were centrifuged (Micro CL 21 Thermo Scientific Fisher, Germany) at 6164 × g for 15 min, and the supernatant was decanted into new tubes. Extraction process was repeated two more times and the combined supernatants were evaporated under a nitrogen
stream. Samples were stored at -20 °C. Prior to further analysis of total phenolic content (TPC) and antioxidant activity determination, the samples were dissolved in 0.5 mL of methanol (ultra gradient HPLC grade, J.T. Baker), centrifuged and transferred into new tubes.

Total phenolic content

Total phenolic content was determined according to Yu et al. (24) with modifications. Distilled water (0.4 mL), extract (0.015 mL) and Folin-Ciocalteu reagent (Sigma Aldrich, Steinheim, Germany) (0.1 mL) were pipetted into a cuvette and put aside for 3 min after which 20 % Na$_2$CO$_3$ (Sigma Aldrich, Steinheim, Germany) (0.3 mL) and distilled water (1.185 mL) were added. The solution was mixed and incubated in the dark for 2 h. The absorbance was measured at 765 nm (Specord 50 PLUS; Analytic Jena, Germany). Measurements were done in triplicate and the results expressed as mg gallic acid equivalent (GAE) per gram of sample, using a calibration curve constructed from the standard of gallic acid (Sigma Aldrich, Merck).

Antioxidant activity

Antioxidant activity was determined by DPPH radical-scavenging capacity, ferric reducing antioxidant power (FRAP) and ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay as described by Belščak et al. (25) with some modifications. For the DPPH assay, 0.02 mL of the extract was added to 0.95 mL or 0.06 mM DPPH (2,2-diphenyl-picrylhidrazyl; Sigma Aldrich, Merck) in methanol (ultra gradient HPLC grade, J.T. Baker). The free radical scavenging capacity was evaluated by measuring the absorbance at 517 nm, after 30 min reaction in the dark. For FRAP assay, 0.01 mL of methanol extract was mixed with 1 mL of FRAP reagent. Absorbance was measured at 593 nm after incubation for 4 min. For the ABTS assay, 0.02 mL of extract was mixed with 2 mL of ABTS reagent (Sigma Aldrich, Merck), mixed, and the absorbance measured at 734 nm after 6 min incubation in the dark. Measurements were done in triplicate and the results were expressed as µmol Trolox equivalent (TE) per gram of sample, using a calibration curve constructed from the standard solution of Trolox (>97 %, Sigma Aldrich, Merck).

Statistical analysis

One-way ANOVA with post-hoc Tukey's test was applied in order to determine significant differences between means, followed by correlation analysis, using GraphPad Prism 5 (26). All analyses were performed at 95 % confidence level. The optimization of high intensity ultrasound treatment of PMBF was done with Design Expert (13), utilizing the parameters with a significant
change in the output values after the treatment. The required parameters were set as following: maximization of TPC, FRAP, SDFS, and minimization of WRC, PPO and BI. Power of high intensity ultrasound, DPPH, ABTS, IDF, SDFP, IVPD were not included in the optimization since they only showed significant change compared to the control sample (0A20M), but were not affected by the amplitude and time of high intensity ultrasound treatment (there was no significant change between the high intensity ultrasound treated samples).

RESULTS AND DISCUSSION

Millet bran fraction containing only particles smaller than 500 µm was primarily chosen in order to remove the larger impurities that are a part of the proso millet bran raw material due to an underdeveloped technology for small grain purification. Other than that, a fraction of smaller particles was separated due to preliminary analysis results that showed that this fraction is richer in protein (results not shown). Indeed, the chosen fraction had a high content of protein (13.9 g/100 g) which is an advantage considering that the chosen PMBF has a mediocre protein digestibility (Fig. 1). Bran fractionation also resulted in a product that was high in fibre content (35.8 g/100 g) especially insoluble dietary fibre (Table 2). Higher fat content of the PMBF (9.98 g/100 g) is considered an advantage considering that proso millet contains a high amount of unsaturated fatty acids, particularly linoleic fatty acid (27) which has potential health benefits.

Treatment with high intensity ultrasound is mostly utilized for extraction procedures and further development of its application as a pre-treatment of raw material in the food industry is needed. In addition, although the positive effect of high intensity ultrasound treatment of wheat bran on extraction of compounds with antioxidant activity has already been shown and the mechanism explained (28,29), effect of high intensity ultrasound is specifically dependent of the material and therefore needs to be optimized for millet bran.

Acoustic power and temperature change are useful descriptive properties of the high intensity ultrasound treatment which simplify understanding the effect of cavitation and temperature during the treatment. Power of high intensity ultrasound was proportional to the temperature gradient ($r=0.811$, $p<0.001$) which was the in the first few minutes of treatment and therefore, the shortest treatments had the highest high intensity ultrasound power (Fig. 2). Time of treatment is the determining factor for the total change in temperature ($r=0.923$, $p<0.001$). In all treatments lasting
5 min final sample temperature did not rise above 40 °C, indicating that in these treatments the cavitation effect was not prevailed by the temperature increase, while in other treatments (12.5 and 20 min) the rise of temperature possibly smothered the effect of cavitation (30).

High intensity ultrasound can have both an activating and inactivating effect on enzymes (31,32), depending on its power, but also on the properties of the product (pH, water activity, etc.) (29), as well as on the enzyme type, its amino acid composition, and conformation structure (33). In this research, activity of PPO was higher in all treated samples in comparison to the control (0A20M) (Fig. 3). This was surprising for treatments that resulted in sample temperatures above 60 °C (Fig. 2) (60A20M; 80A20M; 100A12.5M; 100A20M). The longer treatments with a higher final temperature (Fig. 2 and Fig. 3) caused the largest rise in PPO activity (30-83 % augmentation compared to the control (0A20M). Indeed, activity of PPO positively correlated with the time of treatment ($r=0.770$, $p=0.001$) and with the temperature change ($r=0.849$, $p<0.0001$). It is possible that the energy pumped into the system with the use of high intensity ultrasound, accelerated the reaction of PPO by promoting the collision of enzyme and substrate (32). Also, longer exposure to high intensity ultrasound could have resulted in rupture of the cell wall which could consequently cause an improved accessibility of the membrane bound PPO (34). Browning of the samples correlated well with the activity of PPO ($r=0.853$, $p<0.0001$) (Fig. 3). Enzymatic browning occurs in two reactions of which first is dependent on PPO activity and results in formation of quinones. The second is a non-enzymatic reaction of quinones with amino acids and phenols in which the reddish brown coloured melanin is formed (10). Therefore, the potential mechanism in which high intensity ultrasound enhances browning of the sample is not only by accelerating the reactions of PPO, but also, by making a larger amount of the second substrate (phenols) available for the reaction, by freeing them from their insoluble form.

The most successful treatments of PMBF with high intensity ultrasound, as measured by FRAP and TPC, were the ones that lasted 12.5 min (Table 3). Best results were achieved with 80 % amplitude and 12.5 min of treatment which resulted in 15.2 % higher TPC than in the control sample and 16.3 % improved antioxidant activity measured by FRAP test (Table 3). The link between the growing antioxidant activity and the amount of free phenolics was further confirmed by the positive correlation between FRAP and TPC test ($r=0.884$, $p<0.001$). Heating of samples by high intensity ultrasound treatment could have caused bioactive component degradation.

According to Chandrasekara et al. (35) heating of phenolic compounds can cause degradation of
formation of complexes with macromolecules, and result in reduced TPC. In this research, temperature rise of the samples during treatment did not show a negative correlation with the TPC. Our results are in agreement with the ones of Luo et al. (36). Additionally, it is possible that the effect of high intensity ultrasound on TPC is concealed by the activating effect which high intensity ultrasound has on PPO. Consequently, the freed phenolics could have been used up in the PPO oxidation reactions, making them inaccessible again. Except for that, it is possible that high intensity ultrasound enhanced the activity of another phenolics degrading enzyme, e.g. peroxidase (7) which would contribute to this effect. Although change in the FRAP antioxidant activity of PMBF was positive, DPPH and ABTS showed no change, except for treatment with 80 % amplitude during 5 min. Different results between various antioxidant tests are caused by the difference in reagents used in each of them and therefore, reaction with compounds of various chemical composition.

Health benefits of daily consumption of whole grain cereals are mostly attributed to a higher dietary fibre intake (3). Soluble fibre seems to have a more significant effect on health than insoluble dietary fibre (37). Since high intensity ultrasound has the potential to cause physical changes and increase diffusion of soluble compounds (38), it could have an impact on quantity of soluble fibre by making them more available to the solvent. Indeed, Zhang et al. (39) showed that high intensity ultrasound impacts the degree of polymerization resulting in better solubility of fibre. Generally, high intensity ultrasound treatment did not significantly affect IDF, except for the treatment at 60 % amplitude during 5 minutes, which caused a significant 14.2 % increase of IDF compared to the control (Table 3). High intensity ultrasound treatment did not significantly affect the SDFP soluble fibres either, but changed the amount of SDFS. Treatments with 60 % or 80 % amplitude caused a drop in the concentration of SDFS, while only 100 % amplitude during 20 min caused a 23.6 % increase of SDFS compared to the control. Despite the increase of SDFS, the concentration of IDF remained unchanged.

Compared to the control, only 100 % high intensity ultrasound treatment resulted in a decrease of WRC. All other treatments increased WRC which is undesirable for bran intended for bakery products. Possible explanation for the isolated effect of 100 % amplitude of high intensity ultrasound is the high concentration of starch (26 % dry mass) of PBMF. To a certain point, high intensity ultrasound enhances starch water swelling ability (40), but as the temperature and the intensity of the treatment increase, the treatment damages and brakes-down the starch granules.
causing gelatinisation and a lesser WRC capacity. Further on, high fibre content of PBMF certainly influenced the WRC. Elleuch et al. (41) explained that mixing of a sample could result in opening up of the fibre structure which would in turn make hydroxyl groups of cellulose more accessible to interactions with water. Since vigorous mixing happens as a result of high intensity ultrasound application, this is a possible reason for enlargement of WRC in the high intensity ultrasound treated samples.

Although not statistically significant, protein digestibility decreased during the high intensity ultrasound treatment in comparison to the control (Fig. 1). It is possible that this effect is a consequence of a higher polyphenol level after treatment since some polyphenols can bind to proteins making them inaccessible (42). The effect could also be caused by the temperature increase during high intensity ultrasound treatment. Our results agree with the finding that digestibility of proso millet proteins decreases upon cooking due to the formation of hydrophobic aggregates (43). This effect is specific for proso millet proteins as high intensity ultrasound treatment usually leads to partial defolding of protein structure (44).

CONCLUSIONS

Enhancement of antioxidant activity and total phenolic content of PMBF was most successful with application of 80 % amplitude for 12.5, min while improvement of physical properties, browning, water retention were most pronounced at 100 % amplitude and 5 min. Besides that, dietary fibre solubility was significantly higher after the 100 % amplitude and 20 min treatment. PPO activity was enhanced with high intensity ultrasound treatment regardless of the temperature of the sample, but the effect was not statistically significant at shorter treatment times. Therefore, utilization of high intensity ultrasound as an asset for improving millet bran intended for the bakery industry requires optimization. According to our results, best pre-treatment (desirability 0.62) for acquiring improved PMBF, is a 15 % water solution of PMBF that is treated with a 400 W high intensity ultrasound processor and a 22 mm probe, at 100 % amplitude, during 9.3 minutes. Taking into consideration its effect on both physical and nutritive characteristics, further research should be focused on possibility of application of high intensity ultrasound on deactivation or activation of specific enzymes of PMBF, such as PPO.
ACKNOWLEDGEMENTS

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26. Prism version 7.04, GraphPad Software, La Jolla California, USA, 2017; available at: www.graphpad.com


Fig. 1. Water retention capacity (WRC) and protein digestibility (IVPD) of the high intensity ultrasound treated proso millet bran fraction smaller than 500 µm samples (mean value±standard deviation) in comparison with the control (0A20M). Means with different superscript differ significantly for WRC at p<0.05

Fig. 2. Final temperature, temperature gradient and power of high intensity ultrasound of proso millet bran fraction smaller than 500 µm samples
Fig. 3 Browning index (BI) and polyphenol oxidase activity (PPO) of high intensity ultrasound treated proso millet bran fraction smaller than 500 µm samples (mean value±standard deviation), in comparison with the control (0A20M). Means with different superscript differ significantly for PPO at p<0.05.
Table 1. Central composite design of high intensity ultrasound treatments of proso millet bran fraction smaller than 500 µm samples

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Table 2. Mass fractions (g/100 g dm) of insoluble (IDF), and soluble (SDFP and SDFS) dietary fiber of the high intensity ultrasound treated proso millet bran fraction smaller than 500 µm samples, in comparison to the control

<table>
<thead>
<tr>
<th>Sample</th>
<th>$w$(IDF)/%</th>
<th>$w$(SDFP)/%</th>
<th>$w$(SDFS)/%</th>
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<td>0A20M (control)</td>
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<td>(35.70±0.42)$^{ac}$</td>
<td>(1.45±0.00)$^a$</td>
<td>(0.331±0.005)$^b$</td>
</tr>
<tr>
<td>100A5M</td>
<td>(32.52±0.96)$^a$</td>
<td>(1.50±0.11)$^a$</td>
<td>(0.451±0.001)$^c$</td>
</tr>
<tr>
<td>100A12.5M</td>
<td>(34.11±0.89)$^a$</td>
<td>(1.34±0.00)$^a$</td>
<td>(0.353±0.002)$^b$</td>
</tr>
<tr>
<td>100A20M</td>
<td>(34.58±0.10)$^a$</td>
<td>(1.55±0.10)$^a$</td>
<td>(0.752±0.001)$^d$</td>
</tr>
</tbody>
</table>

Results are presented as mean value±standard deviation (p˂0.05). Means with different superscript within same column differ significantly at p<0.05. IDF=insoluble dietary fibre, SDFP=dietary fibre soluble in water that precipitates in 78 % aqueous ethanol, SDFS=soluble dietary fibre in water and 78 % aqueous ethanol.
Table 3. Change in antioxidant capacity measured by FRAP, DPPH, ABTS and total phenolic content of high intensity ultrasound treated proso millet bran fraction smaller than 500 µm samples, in comparison to the control

<table>
<thead>
<tr>
<th>Sample</th>
<th>FRAP as TE/(µmol/g)</th>
<th>DPPH as TE/(µmol/g)</th>
<th>ABTS as TE/(µmol/g)</th>
<th>TPC as GAE/(µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0A20M (control)</td>
<td>(2.33±0.09)ac</td>
<td>(1.61±0.12)ac</td>
<td>(2.61±0.1)ab</td>
<td>(1.59±0.18)ac</td>
</tr>
<tr>
<td>60A5M</td>
<td>(2.55±0.31)c</td>
<td>(1.60±0.04)ac</td>
<td>(2.94±0.12)a</td>
<td>(1.69±0.08)abc</td>
</tr>
<tr>
<td>60A12.5M</td>
<td>(2.62±0.03)c</td>
<td>(1.67±0.07)c</td>
<td>(2.56±0.04)ab</td>
<td>(1.73±0.06)abc</td>
</tr>
<tr>
<td>60A20M</td>
<td>(2.64±0.02)c</td>
<td>(1.57±0.06)ac</td>
<td>(2.90±0.12)a</td>
<td>(1.79±0.08)ab</td>
</tr>
<tr>
<td>80A5M</td>
<td>(2.01±0.05)a</td>
<td>(1.36±0.03)b</td>
<td>(2.32±0.01)b</td>
<td>(1.51±0.06)c</td>
</tr>
<tr>
<td>80A12.5M</td>
<td>(2.78±0.10)bd</td>
<td>(1.59±0.05)ac</td>
<td>(2.73±0.12)ab</td>
<td>(1.87±0.05)b</td>
</tr>
<tr>
<td>80A20M</td>
<td>(2.52±0.16)cdde</td>
<td>(1.44±0.07)ab</td>
<td>(2.86±0.03)ab</td>
<td>(1.66±0.07)abc</td>
</tr>
<tr>
<td>100A5M</td>
<td>(2.25±2.59)ce</td>
<td>(1.61±0.06)ac</td>
<td>(2.39±0.24)ab</td>
<td>(1.69±0.09)abc</td>
</tr>
<tr>
<td>100A12.5M</td>
<td>(2.59±0.04)cd</td>
<td>(1.54±0.03)ac</td>
<td>(2.37±0.17)bc</td>
<td>(1.71±0.05)abc</td>
</tr>
<tr>
<td>100A20M</td>
<td>(2.13±0.16)ae</td>
<td>(1.50±0.07)abc</td>
<td>(2.62±0.11)ab</td>
<td>(1.54±0.06)c</td>
</tr>
</tbody>
</table>

Results are presented as mean value±standard deviation (p˂0.05). Means with different superscript within same column differ significantly at p<0.05. FRAP= ferric reducing antioxidant power, DPPH= radical-scavenging capacity, ABTS=((2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay, TPC=total phenolic content, TE=Trolox equivalent, GAE=galic acid equivalent.
Table 4. Significant coefficient (p<0.05) of researched parameters during high intensity ultrasound treatment of proso millet bran fraction smaller than 500 µm

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>A (amplitude)</th>
<th>B (time)</th>
<th>AB</th>
<th>B²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>1.8277</td>
<td>0.0176</td>
<td></td>
<td>-0.1803</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>2.7325</td>
<td>0.0796</td>
<td></td>
<td>-0.3839</td>
<td></td>
</tr>
<tr>
<td>WRC</td>
<td>262.6</td>
<td>-6.0439</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI</td>
<td>6.8</td>
<td>0.7215</td>
<td>0.64729</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td>0.4866</td>
<td>0.0656</td>
<td>0.1116</td>
<td>0.049601</td>
<td>0.0629</td>
</tr>
</tbody>
</table>

TPC=total phenolic content, FRAP=ferric reducing antioxidant power, WRC=water retention capacity, BI=browning index, PPO=polypehnel oxidase activity