Wheat chaff utilization: Evaluation of antioxidant capacity of waste streams generated by different pretreatments

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A B S T R A C T

Wheat chaff was prepared for the enzymatic conversion by ultrasound, alkali or hydrothermal pretreatment while obtained waste liquid fractions were evaluated for their antioxidant activity. Waste streams were assayed by standard antioxidant methods to evaluate their potential as sources of valuable products. Results showed that all waste streams expressed high antioxidative activities with sample after alkali pretreatment being even superior in comparison to standard antioxidants. The most of the antioxidative activities of assayed waste fractions showed strong correlation with concentration of reducing sugars, which can be attributed to the identified presence of the xylooligosaccharides. Results indicated potential use of waste streams from investigated pretreatments for co-production of valuable products with high antioxidative capacities.

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1. Introduction

Every year, huge amounts of agro-industrial wastes are generated without any substantial use, thus implying an increase in the economic and environmental costs of their management (Aires et al., 2016). On the other side, lignocellulose biomass could be a source for the production of high-value products (Kim et al., 2016). Wheat chaff is raw lignocellulose material with the compact structure composed from cellulose (36–39%), hemicelluloses (18–21%), and lignin (16%) (Griffin et al., 2011; Bledzki et al., 2010) and because of high carbohydrate content can potentially be used as feedstock for the production of valuable products of biotechnology. However, this low-cost material is insufficiently researched so far and under-utilized although it is abundant in regions with intensive wheat production. Only in Serbia, where annual production of wheat is approximately 2.4×10^6 t (Statistical Office of the Republic of Serbia, 2015) the amount of wheat chaff can be estimated to 10^6 t/year on the base of relations given in literature (Kernan et al., 1984). So far, wheat chaff has been investigated as a source of polymers for bio-composites (Mamun and Al Bledzki, 2014), sugars for bioethanol production (Duguid et al., 2007) and material for heavy metal adsorption (Sud et al., 2008).

The main limitation in enzymatic conversion of lignocellulose materials to sugars is their resistance to enzymes action, so, pre-treatment is the first and necessary step to fractionate lignin and prepare target components for the hydrolysis (Weng et al., 2008). In addition, pretreatment has strong influence on down-stream costs involving enzyme loading as well as waste treatment demands (Wyman et al., 2005) because it generates waste stream in the form of liquid fraction which has no further use for the carbohydrates (cellulose) hydrolysis. Those waste flows can be investigated to evaluate potential for their utilization as a source of high-value products thus increasing the economic and environmental feasibility of overall process.

The aim of the present study was to evaluate waste streams obtained after hydrothermal, alkali and ultrasound pretreatment of wheat chaff on the base of their antioxidative capacity. The basis for the selection of pretreatments was the fact that all of these applied methods have been reported to efficiently improve yield of forthcoming enzymatic hydrolysis of lignocellulose biomass (Kim et al., 2016; Singh et al., 2016; Zhuang et al., 2016). Hydrothermal pretreatment does not substantially affect cellulose and lignin allowing several advantages associated with this process such as water used as reaction medium and reduced sugar loss which is very important from an economic point of view (Singh et al., 2016). The alkaline treatment procedure results in xylan rich waste liquid fraction (Zhu et al., 2013). This method utilizes sodium hydroxide, which is non-corrosive and non-polluting chemical while pretreatment itself is carried out under mild conditions (Kim et al., 2016). Ultrasound used for lignocellulose pretreatment affects hemicellulose in biomass (Ebringerova and Hromadkova, 2002) while recent

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reports have shown that when applied without chemicals it is a green alternative to conventional techniques (Chemat et al., 2017).

To evaluate antioxidant potential of waste, different methods corresponding to different levels of antioxidative actions in vitro were conducted – DPPH, ABTS scavenging activity, reducing antioxidant power assay, hydroxyl radical scavenging activity and chelating ability of ferrous ions (Fe^{2+}) were determined. The results are additionally expressed as middle effective concentrations for their comparison with standard antioxidants. In addition, correlations between the antioxidative activities determined by different methods and the concentration of the carbohydrate components of waste streams were calculated.

2. Materials and methods

2.1. Wheat chaff

Wheat chaff used as raw material in this study was kindly supplied by A.D. "Mlin" (Žabalj, Serbia). Raw material was grounded to pass 0.8 mm sieve and stored at −20 °C.

2.2. Pretreatment of wheat chaff

In order to prepare material for the forthcoming step of cellulose hydrolyses (which was the part of the other study) wheat chaff was subjected to different pretreatments. In all experiments, concentration of suspension was 10% (w/w) based on dry weight and pretreated substrates were further enzymatically hydrolyzed for the production of glucose as a part of the other study.

2.2.1. Ultrasound (US) pretreatment

Titanium cylindrical horn of ultrasonic homogenizer (JY96-IIN, Ningbo Scientz Biotechnology) was immersed in the suspension of wheat chaff prepared in bi-distilled water, at frequency 25 kHz for 10 min. Frequency at 25 kHz was used because the mechanical effect of ultrasound is expressed at low frequencies (Mason et al., 2011) and this was important for pretreatment of wheat chaff. Pretreated solid material was separated by filtration through laboratory filter paper (Macherey-Nagel MN 651/120) and obtained filtrate as waste stream was further analyzed.

2.2.2. Alkali (AL) and hydrothermal (HT) pretreatments

The suspension of wheat chaff in 0.1 mol/L NaOH or in bi-distilled water for hydrothermal and alkali pretreatment, respectively, was autoclaved for 30 min at 220 °C, at pressure 4 bar. Pretreated material was centrifuged at 5000 rpm during 10 min at laboratory centrifuge (Sorvall RC-5B Refrigerated Super Speed Centrifuge) and obtained supernatant as waste stream was used for further evaluation.

2.3. Sugar analysis

Concentration of reducing sugars in samples was determined by DNS method (Miller, 1959) with xylose as standard.

In additions, sugars in waste fractions were analyzed by HPLC system (Waters, US) with RI detector using Zorbax column in isocratic mode at flow rate of mobile phase 1 mL/min (water: acetonitrile = 60:40 (v/v)). For sugars identification analytical standards for glucose and xylose (Supelco, Sigma), xylotriose, xylotetraose and xylopentose (Megazyme, Ireland) were used.

2.4. Analysis of antioxidative activities

2.4.1. Reducing power activity

The reducing power activity (RP) of the sample was measured using the method of Yen and Chen (1995) with slight modifica-

tions. Samples or ascorbate was dissolved in 200 mmol/L phosphate buffer, pH 6.6. To 70 μL sample, an aliquot 35 μL 1% (w/v) potassium ferricyanide was added and incubation was carried out for 20 min at 50 °C. After that, 135 μL ultrapure water and 33 μL 10% (w/v) trichloroacetic acid were added and reaction mixture was centrifuged at 5000 rpm (Mini Spin, Eppendorf) for 10 min. Finally, 27 μL 0.1% (w/v) ferric chloride (FeCl$_3$) was mixed with the reaction medium and incubated for 10 min at room temperature. Then, absorbance was measured at 700 nm and results of reducing power activities are expressed as OD. For the correlation analysis, reducing capacity was expressed in percentage (%) and calculated according to the following equation:

$$Reducing \quad power \ activity \ (\%) = 100 - \left(\frac{A_{control} - A_{sample}}{A_{control}} \times 100 \right) \tag{1}$$

where $A_{control}$ is control absorbance i.e. absorbance of a reaction medium with ascorbate (0.1 mg/mL) and A_{sample} is sample absorbance i.e. absorbance of a reaction medium with sample. For standard curve, ascorbic acid was used in concentration range 0–0.1 mg/mL.

2.4.2. ABTS radical-scavenging activity assay

The ABTS radical-scavenging activities of samples were determined according to the method of Re et al. (1999) with slight modifications. A stock ABTS radical solution was prepared by mixing ABTS aqueous solution (final concentration 7 mmol/L) with potassium persulphate (final concentration 2.45 mmol/L). This mixture was incubated for 16 h at room temperature in the dark. After incubation, the bi-distilled water was mixed with the stock solution of ABTS until it displayed an absorbance of 0.70 ± 0.02 at 734 nm. Samples were dissolved in phosphate saline buffer (PBS), pH 7.0, to form sample solution in different concentration. A 50 μL sample was mixed with 950 μL of the diluted ABTS radical solution. The solution was mixed on the vortex for 30 s and incubated in a dark environment for 6 min at room temperature. Then, the absorbance was measured at 734 nm. Control solution was prepared using 50 μL of PBS instead of the sample while PBS was used as blank. The scavenging effect was calculated according to the following equation:

$$ABTS \quad radical \quad scavenging \quad activity \ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \times 100 \right) \tag{2}$$

where $A_{control}$ is the absorbance of the control solution and A_{sample} represents the absorbance of the ABTS radical with tested samples, respectively. The standard curve was prepared with ascorbic acid and was linear between 0 and 0.08 mg/mL.

2.4.3. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined using Fentons reaction method with slight modification (Zhang et al., 2015). The reaction mixture generating hydroxyl radicals contained 0.1 mL H$_2$O$_2$ (20 mmol/L), 0.1 mL FeSO$_4$ (9 mmol/L), 0.1 mL salicylic acid ethanolic solution (9 mmol/L), 2 mL bi-distillated water and 1 mL of the sample. The reaction mixture was incubated for 30 min at 37 °C. A control solution, using bi-distilled water instead of the sample, was prepared in the same manner. The absorbance of the reaction mixture was measured at 510 nm. Hydroxyl radical scavenging activity (%) was calculated according to the following equation:

$$Hydroxyl \quad radical \quad scavenging \quad activity \ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \times 100 \right) \tag{3}$$

where $A_{control}$ was the absorbance of the control and A_{sample} was the absorbance of the sample mixed with reaction solution, respectively. Ascorbic acid was used as standard.