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Antioxidant and Physicochemical Properties of Hydrogen Peroxide-Treated Sugar Beet Dietary Fibre

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The aim of the present work was to examine if hydrogen peroxide treatment of sugar beet fibre that aimed at improving its physicochemical properties would impair its antioxidant potential. Three different sugar beet fibres were obtained from sugar beet – non-treated fibre (NTF) from sugar beet cossettes extracted with sulphurous acid, treated fibre (TF) from NTF treated with hydrogen peroxide in alkaline solution and commercially available Fibrex®. The antioxidant activity of extractable and non-extractable fibre fractions in ethanol/water mixture (80:20, v/v) of three fibre samples was estimated. Non-extractable fractions obtained after alkaline treatment of investigated fibres were much higher in phenolic compounds and possessed higher antioxidant potential than extractable fractions. Ferulic acid was proven to be the dominant phenolic acid. Regarding both extractable and non-extractable fractions, Fibrex® had the highest antioxidant activity in chemical tests, while NTF was superior in comparison with TF. Based on the results of Caco-2 cells-based test, all non-extractable fractions possessed potential for reactive oxygen species inhibition. Regarding the extractable fractions, only the TF manifested this effect. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: sugar beet fibre; antioxidant activity; non-treated fibre; treated fibre; Fibrex®; Caco-2 cell assay.

INTRODUCTION

Transformation of by-products of plant food processing into valuable functional ingredients may alleviate the problem of food waste. Sugar beet pulp (SBP) is a by-product left after the extraction of free sugar from commercially grown sugar beet. About 250 kg of pressed SBP containing 70–80% water is left over by the sugar industry after processing of 1 ton of sugar beet (Bogdanović *et al.*, 2013).

Recently, sugar beet fibre (SBF) obtained from SBP has been introduced in the food technology as a fibre source. The incorporation of SBF into cookie, bread, spaghetti and extrudate formulations was investigated and discussed in detail (Özboy and Köksel, 2000; Sakač *et al.*, 2011). It was reported that SBF can be used as a fat substitute in frankfurters offering both high dietary fibre and low-fat products (Vural *et al.*, 2004).

Sugar beet fibre possesses different physicochemical properties depending on the process applied in its production (Gyura *et al.*, 2009). Chemical treatments and drying conditions affecting physicochemical properties were found to influence the fermentability of SBF (Guillon and Champ, 2000).

On a dry weight basis, SBP contains 65–80% polysaccharides, consisting roughly of 40% cellulose, 30% hemicellulose and 30% pectins (Sun and Hughes, 1998). Ferulic acid represents the main phenolic acid in sugar beet; it exists in SBP in the concentration of 0.9%, mostly linked to sugar beet pectins (0.1–0.7%) (Yapo *et al.*, 2007). Literature data about its antioxidant effect indicate the possibility of multifunctional activity of SBF-containing products (Sakač *et al.*, 2011).

In our previous papers, in an attempt to produce SBFs suitable for application in bread and cookie formulations, physicochemical and antioxidant properties of SBFs obtained by different procedures were investigated (Gyura *et al.*, 2009; Sakač *et al.*, 2009). SBF treated with peroxide ions in alkaline medium [treated fibre (TF)] was found to possess better characteristics for application in bread and cookies than the non-treated fibre (NTF), because it becomes softer and more flexible; thus, it can prevent the degradation of gluten–starch matrix in a bakery product and avoid the negative effect on the product softness (Gyura *et al.*, 2009). On the other hand, TF was shown to possess the lowest antioxidant activity (AOA) of the extractable fraction (Sakač *et al.*, 2009).

Regarding the fact that insoluble polyphenols are considered to be the major contributors to total antioxidant capacity of cereals (Adom and Liu, 2002) and

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the fact that the major part of ferulic acid is present in its insoluble form bound to sugar beet pectins (Yapo *et al.*, 2007), the aim of this study was to estimate the AOA of extractable and non-extractable fractions of TF, NTF and commercially available SBF Fibrex® using both chemical and *in vitro* assays.

MATERIALS AND METHODS

Materials

Chemicals. All chemicals were of analytical grade and obtained from different producers. Water was purified Q4 using Millipore Elix 10 UV water purification system.

Cell culture. Human intestinal epithelial Caco-2 cells (American Type Culture Collection, LGC Promochen, Italy) were used between passages 30 and 50. The cells were routinely maintained at 37 °C in a 5% CO_2 atmosphere in 75 cm^2 polystyrene flasks in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 1 M Hepes [4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid], 2.5% non-essential amino acid 1X and 2 mM L-glutamine. The medium was changed every 48 h in conformity with the manufacturer's protocols.

Plant material and production procedures. Fibrex®, article 595 with the particle size smaller than 125 µm, is a commercially available fibre produced by Nordic Sugar, Sweden.

Cossettes were obtained from mechanically cleaned and washed sugar beet, which was later sliced to dimensions of approximately 1 × 2 × 60 mm. These sugar beet cosettes were used for the production of NTF. Sugar beet cossettes were extracted with sulphurous acid at 75 °C and pH = 5.7 during 60 min. After the treatment, the NTF was pressed to remove the excess water, dried in vacuum kiln at 30 °C, ground in a laboratory mill (LM 3100, Falling Number, Perten Instruments, Sweden) and sieved through a laboratory sieve (SZ-1, ZBPP, Bydgoszoz, Poland) to obtain a fraction with a particle size of less than 150 µm, which was used for further analysis.

After pressing, the NTF was treated with hydrogen peroxide solution up to blend concentration of 20 g/L H_2O_2 at pH = 11, which was adjusted with 10 mol/L NaOH. After the treatment of 24 h, the blend was neutralized with concentrated HCl until the pH reached the value of 6–7. Chlorine ions were washed out with distilled water to reach negative reaction to Cl^- ions. The obtained TF were pressed to remove the excess water and after drying at 80 °C, the fibres went through the same process as it is described earlier in the case of NTF.

Methods

Physicochemical characteristics of dietary fibres. Dry matter and mineral matter were determined according to the methods specified by Reinefeld and Schneider (1983). Protein content was determined according to AOAC Official Method 985.29: Protein and Ash Contents in Food and Food Products, Final Action 1990. Insoluble fibre content was determined according to AOAC Official Method 991.42: Insoluble Dietary Fibres in Foods and Food Products, Final Action 1994. Soluble fibre content was determined according to AOAC Official Method 993.19: Soluble Dietary Fibres in Foods and Food Products, Final Action 1996. Total fibre content was calculated by adding together the contents of insoluble and soluble fibres.

Water holding capacity was determined by the modified Stauffer method (1993). Reflectance was determinated by the method of Thibault *et al.* (1994) using the tristimulus photoelectric colorimeter (Chroma meter CR-400, Minolta Co., Ltd, Japan).

Preparation of the free and bound phenolic extracts. Free phenolic compounds (extractable fraction) were obtained by maceration of the samples with ethanol/water mixture (80:20, v/v), with the ratio of raw material to ethanol solution of 1:10, for 24 h at room temperature and subsequently extracted in an ultrasonic bath at room temperature (23 ± 1 °C) for 10 min. The extract was filtered through a filter paper (Whatman, Grade 4 Chr, UK) and dried using vacuum-evaporator at 40 °C. The yield was calculated based on the dry mass of the samples. The dried extract was dissolved in ethanol/water (80:20, v/v) to 10 mL volume and used for further investigation of AOA.

Residues obtained after the extraction of free phenolic compounds were submitted to alkali hydrolysis to obtain non-extractable fraction. Alkali hydrolysis was performed as follows: dry residues (10 g) were refluxed with 10 mL 50% KOH (in water) and 50 mL methanol for 30 min under nitrogen gas at the temperature of boiling. The alkali hydrolysate was neutralized with an appropriate amount of acid. Hydrolysed antioxidants were separated by extraction with ethyl acetate. After the evaporation to dryness, the obtained residues were dissolved in ethanol/water (80:20, v/v) to 10 mL volume and used for further analyses.

Total phenolic content. Total phenolic content (TPC) was determined spectrophotometrically at 750 nm (6405 UV/VIS, Jenway, Stone, Staffordshire, UK) using Folin–Ciocalteu reagent (Singleton *et al.*, 1999). Gallic acid was used as a standard, and results were expressed as gallic acid equivalents (microgram gallic acid equivalents per gram of sample on dry mass basis).

High-performance liquid chromatography analysis of phenolics. High-performance liquid chromatography analysis was performed by using a liquid chromatograph (Agilent Technologies 1200, Santa Clara, CA, USA). Separation of components was performed using an Agilent LiChrospher 100 RP 18e (5 µm), 250 × 4 mm i.d. column, with a flow rate of 1 mL/min and mobile phase consisted of A [500 mL of H_2O plus 9.8 mL of 85% H_3PO_4 (w/w)] and B (acetonitrile) with the following gradient elution: 90–75% A, 0–25 min; isocratic 75% A, 25–30 min; 75–55% A, 30–46 min. The injected volume was 4 µL, and it was carried out automatically. The sample was prepared by dissolving 50.00 mg ± 5% of dry extract in 10 mL of methanol and filtering through

0.2 µm PTFE filters (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany). Standard solutions of phenolic acids were prepared at a final concentration of 0.01 mg/mL (protocatechuic, *p*-hydroxibenzoic, chlorogenic, vanilic, gentizinic, syringic, *p*-coumaric, ferulic and sinapic acids), and 0.025 mg/mL (caffeic acid and chlorogenic acid) in methanol. Detection was performed using diode array detector, and the chromatograms were recorded at $\lambda = 280$ nm (for protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, gentizinic, syringic, *p*-coumaric and sinapic acid) and 325 nm (for ferulic acid). The identification was carried out referring to retention time and spectra matching of sample and the standard solution. Quantification was performed by external calibration.

DPPH radical scavenging activity. The extract was diluted with ethanol/water (80:20, v/v) in an appropriate manner to obtain a series of dilutions. Effect of the examined extract on the content of 1,1-diphenyl-2-picryl-hydrazyl radical ($DPPH^{\bullet}$) was estimated according to the modified method of Hatano *et al.* (1988).

The IC_{50} value (g/mL) was defined as the concentration of an antioxidant extract, which was required to quench 50% of the initial amount of $DPPH^{\bullet}$ under the experimental conditions given. It was obtained by interpolation from linear regression analysis.

Reducing power. The extract was diluted with ethanol/water (80:20, v/v) in an appropriate manner to obtain a series of dilutions. The reducing power was determined by measuring the formation of Perls' Prussian blue [potassium iron (III) hexacyanoferrate (II)] at 700 nm (Oyaizu, 1986). The IC_{50} value (mg/mL) was defined as the concentration of the extract that causes a decrease in the absorbance of reaction mixture up to 0.5. It was obtained by interpolation from linear regression analysis.

Antioxidant activity by β-carotene bleaching method. The extract was diluted with ethanol/water (80:20, v/v) in an appropriate manner to obtain a series of dilutions. Oxidative loss of β-carotene in a β-carotene/linoleic acid emulsion was used to assess the AOA of the examined extract according to the method of Moure *et al.* (2001). Degradation rate of the extracts was calculated according to the first-order kinetics, and the AOA was expressed as inhibition (in %) relative to the control (Al-Saikhan *et al.*, 1995).

The IC_{50} value (g/mL) was defined as the concentration of the extract at which the AOA was 50% under the experimental conditions. It was obtained by interpolation from linear regression analysis.

Chelating activity on Fe^{2+}. Chelating activity on Fe^{2+} was measured according to the method of Decker and Welch (1990). The IC_{50} value (mg/mL) was defined as the concentration of an antioxidant extract, which chelates 50% of the present Fe^{2+} under the experimental conditions. It was obtained by interpolation from linear regression analysis.

Cell viability. Cellular viability was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Aviello *et al.* (2011). Caco-2 cells, plated in a 96 multiwell plate at a density of 1×10^4 cells/well, were treated with vehicle (0.1% ethanol, v/v) or extracts (0.3–10 µg/mL). After 24 h, the cells were incubated with the MTT solution (0.25 mg/mL) at 37 °C for 1 h. The supernatant was removed, and the formed formazan crystals were dissolved in dimethylsulfoxide (100 µL/well) at room temperature for 10 min. The absorbance was measured at 490 nm in a multiwell plate reader (iMark™ microplate absorbance reader, BioRad, Milan, Italy). The mean absorbance, taken from cells grown in the absence of the extracts (vehicle alone), was taken as 100% cell survival (control).

Detection of reactive oxygen species generation. Generation of intracellular reactive oxygen species (ROS) was estimated by a fluorescent probe, 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (Aviello *et al.*, 2011). Cells, plated in a 96 multiwell plate at the density of 1×10^4 cells/well, were incubated with the extracts (1–30 µg/mL) at 37 °C for 24 h. Afterwards, the cells were rinsed and incubated with 100 µM DCFH-DA in Hanks' balanced salt solution containing 1% fetal bovine serum for 1 h. Finally, cells were rinsed and incubated with the Fenton's reagent (H_2O_2/Fe^{2+} 2 mM) at 37 °C for 3 h. The DCF fluorescence intensity, which correlates with the amount of ROS formed intracellularly, was detected using a fluorescent microplate reader (Perkin-Elmer Instruments), with the excitation wavelength of 485 nm and the emission wavelength of 538 nm.

Statistical analysis. Results were expressed as the mean values of three replications ± standard deviation or standard error. To determine statistical significance, Student's *t*-test was used for comparing a single treatment mean with a control mean, and a one-way analysis of variance followed by Duncan's multiple range tests was used for analysis of multiple treatment means. Statistical data analysis software system STATISTICA [StatSoft, Inc. (2008). DATA ANALYSIS SOFTWARE SYSTEM, version 9.0. www.statsoft.com] and GRAPHPAD PRISM 5.0 (GraphPad Software, San Diego, CA, USA) were used for analysis. p values <0.05 were regarded as significant.

RESULTS AND DISCUSSION

Physicochemical characteristics of the investigated sugar beet dietary fibres (NTF, TF and commercially available Fibrex®) are shown in Table 1. The purpose of TF production was to improve ~~hydratation~~ properties of the fibre and its sensory characteristics, thus making it suitable for bread formulation. Alkaline hydrogen peroxide treatment is well known for those purposes (Gould, 1987, 1989); therefore, it was applied in TF production. TF had the highest total fibre content, that is, the highest soluble fibre content as a result of the alkaline degradation of fibre macrostructure and microstructure (Table 1). The treatment with hydrogen peroxide in alkaline media also contributed to the significant increase ($p < 0.05$) in water holding capacity of TF, which was proven to provide better sensory

Table 1. Physicochemical characteristics of sugar beet dietary fibres – NTF, TF and Fibrex®

Parameters	NTF	TF	Fibrex®
Dry matter content (%)	91.6 ± 0.36[a]	97.9 ± 0.26[b]	91.4 ± 0.21[a]
Crude protein (% in DM)	9.90 ± 0.14[c]	4.38 ± 0.17[a]	9.30 ± 0.22[b]
Non-soluble fibres (% in DM)	66.3 ± 0.36[b]	61.4 ± 0.40[a]	68.6 ± 0.40[c]
Soluble fibres (% in DM)	13.6 ± 0.40[b]	33.9 ± 0.30[c]	6.08 ± 0.16[a]
Total fibres (% in DM)	79.9 ± 0.39[b]	95.3 ± 0.44[c]	74.7 ± 0.32[a]
Water-holding capacity (g H_2O/100 g)	649 ± 19.0[b]	1549 ± 41.5[c]	418 ± 13.9[a]
Minerals (% in DM)	9.20 ± 0.05[b]	9.14 ± 0.09[b]	3.80 ± 0.05[a]
Reflectance	57.9 ± 0.61[a]	76.0 ± 1.30[c]	68.8 ± 0.67[b]

Values are means of three determinations ± standard deviation.
Values of the same row with the same letter in superscript are not statistically different ($p < 0.05$).
NTF, non-treated; TF, treated fibre; DM, dry matter.

characteristics of bakery products compared with other investigated fibres (Filipovic *et al.*, 2007). Šereš *et al.* (2005) reported that the addition of TF in bread formulation caused significant improvement of some parameters, that is, softness and elasticity of dough.

Dietary fibres originating from different sources have been shown to possess AOA, which is considered beneficial in the prevention of some diseases (Bensadón *et al.*, 2010; Milala *et al.*, 2013; García-Lomillo *et al.*, 2014). Because phenolic compounds significantly contribute to the SBF overall AOA, the amount of total phenolics and phenolic composition were estimated (Table 2). Significant differences in TPC between extractable fractions were not found ($p < 0.05$). TPC of non-extractable fractions was much higher. These results support the fact that the majority of phenolic compounds exist in insoluble form bound to sugar beet pectins (Yapo *et al.*, 2007). Ferulic acid was shown to be the predominant phenolic acid in investigated fibres (Table 2), and this observation was in accordance with previously published results (Micard *et al.*, 1994; AOAC, 1996). Besides ferulic acid, *p*-coumaric, vanilic, syringic and *p*-hydroxybenzoic acid have been identified (Table 2). Fibrex® was the richest source of phenolic compounds, while NTF had approximately two times higher TPC compared with TF. Therefore, it can be concluded that TF production leads to the highest total fibre content (Table 1) but the lowest TPC (Table 2), which should be taken into consideration during creation of new added-value products.

Antioxidant activity of sugar beet dietary fibres was tested using chemical and Caco-2 cells-based (ROS) assays. Chemical assays included determination of DPPH radical scavenging activity, AOA, chelating activity on Fe^{2+} and Fe^{3+}/Fe^{2+} reducing power (Table 3). As expected, the AOA of non-extractable fractions was significantly higher than that of extractable fractions (with the exception of the chelating activity). Also, TF exhibited much lower AOA (indicated by higher IC_{50} values) than the other investigated samples (Table 3),

Table 2. Total phenolic content and content of phenolic acids (mg/100 g sample) determined in extractable (E) and non-extractable (N) fractions of non-treated fibre (NTF), treated fibre (TF) and Fibrex®

	NTF-E	NTF-N	TF-E	TF-N	Fibrex®-E	Fibrex®-N
Total phenolic content	27.2 ± 0.95[a]	265 ± 50.3[c]	8.45 ± 0.53[a]	109 ± 3.83[b]	21.9 ± 0.71[a]	391 ± 59.2[d]
p-Hydoxybenzoic acid	0.11 ± 0.01[a]	0.19 ± 0.01[a]	0.12 ± 0.02[a]	—	—	0.49 ± 0.02[b]
Vanillic acid	0.44 ± 0.01[a]	0.57 ± 0.01[a]	0.43 ± 0.02[a]	0.46 ± 0.02[a]	—	8.36 ± 0.42[b]
Syringic acid	—	—	0.06 ± 0.01	—	—	—
p-Coumaric acid	0.11 ± 0.02[a]	0.95 ± 0.01[b]	0.06 ± 0.01[a]	0.91 ± 0.02[b]	—	—
Ferulic acid	1.02 ± 0.24[a]	255 ± 58.2[c]	0.30 ± 0.10[a]	102 ± 26.8[b]	0.11 ± 0.03[a]	305 ± 2.42[d]

Values are means of three determinations ± standard deviation.
Values of the same row with the same letter in superscript are not statistically different ($p < 0.05$).

Table 3. Antioxidant activity of extractable (E) and non-extractable (N) fractions of non-treated fibre (NTF), treated fibre (TF) and Fibrex®, expressed as IC_{50} (g sample/mL)

	NTF-E	NTF-N	TF-E	TF-N	Fibrex®-E	Fibrex®-N
$DPPH^{\bullet}$ scavenging activity	1.13 ± 0.19[d]	0.07 ± 0.01[ab]	1.93 ± 0.52[e]	0.14 ± 0.01[b]	0.89 ± 0.11[c]	0.02 ± 0.01[a]
Antioxidant activity	3.21 ± 0.36[d]	0.34 ± 0.07[a]	4.84 ± 0.69[e]	0.59 ± 0.06[ab]	2.45 ± 0.15[c]	0.81 ± 0.32[b]
Reducing power	0.48 ± 0.01[d]	0.06 ± 0.01[a]	1.01 ± 0.06[e]	0.13 ± 0.01[b]	0.27 ± 0.01[c]	0.03 ± 0.01[a]
Chelating activity on Fe^{2+}	1.24 ± 0.19[c]	1.13 ± 0.09[c]	0.47 ± 0.20[b]	0.18 ± 0.06[a]	0.22 ± 0.07[a]	0.30 ± 0.05[a]

Values are means of three determinations ± standard deviation.
Values of the same row with the same letter in superscript are not statistically different ($p < 0.05$).

which is in a correlation with lower TPC of TF sample (Table 2). This fact could be explained by the loss of ferulic acid during the production of TF. It is known that ferulic acid, the main phenolic compound in SBP, is associated with the peptic polysaccharides and that is found ester linked to either the C-2 hydroxyl group ofarabinofuranose or C-6 hydroxyl group of galactopyranose residues of the peptic side chains. Under the production conditions of TF, process of saponification occurs, leading to breaking of ester bonds. A great part of released ferulic acid was probably lost with rinsing after the saponification. The other explanation would be further polymerization of ferulic acid, leading to the formation of ferulic acid dehydrodimers as the cross-linkage bridges in peptic polysaccharides (Mathew and Abraham, 2004).

A biological assay was applied to evaluate the capacity of fibres to inhibit ROS production induced by the Fenton's reagent (H_2O_2 and Fe^{2+}) in a human intestinal cell line, that is, Caco-2 cells (Fig. 1). The AOA of non-extractable fractions was significantly higher than that of extractable fractions, and this finding correlates with the results obtained by the chemical assays. However, the non-extractable fraction of Fibrex® (Fibrex®-N), despite its highest TPC (Table 2), did not exert significantly higher AOA compared with other investigated non-extractable fractions. The non-extractable fraction of TF (TF-N), which possessed the lowest TPC (Table 2), exerted the AOA with a potency and efficacy similar to that of Fibrex®-N and NTF-N [EC_{50} (μg/mL): TF-N 1.04 ± 1.04; NTF-N 0.87 ± 1.27; Fibrex®-N 1.46 ± 1.02. E_{max} (% of inhibition): TF-N 44.0 ± 0.37; NTF-N 42.4 ± 2.46; Fibrex-N 35.7 ± 0.27]. Moreover, only the extractable fraction of TF (TF-E) has been found to reduce the production of ROS induced by the Fenton's reagent in a concentration-dependent manner (0.3–10 μg/mL) [EC_{50} (μg/mL): TF-E 3.49 ± 2.78. E_{max} (% of inhibition): TF-E 41.6 ± 2.42]. It can be assumed that not only polyphenols but also other substances, as well as the synergistic effects were responsible for determined AOA of TF-E. It was excluded that the inhibitory effect of investigated dietary fibres on ROS production was due to cytotoxic action, because all SBF extracts did not reduce cell viability in Caco-2 cells [cell viability (%): control 100.2 ± 1.2; Fibrex®-E 0.3 μg/mL 102.1 ± 3.2; Fibrex®-E 1 μg/mL 99.8 ± 4.6; Fibrex®-E 3 μg/mL 100.9 ± 6.2; Fibrex®-E 10 μg/mL 98 ± 5.9; TF-E 0.3 μg/mL 99.7 ± 6.5; TF-E 1 μg/mL 101 ± 4.8; TF-E 3 μg/mL 101.5 ± 6.5; TF-E 10 μg/mL 99.4 ± 2.8; NTF-E 0.3 μg/mL 104.2 ± 5.7; NTF-E 1 μg/mL 101.7 ± 4.2; NTF-E 3 μg/mL 99.6 ± 4.3; NTF-E 10 μg/mL 101.9 ± 6.5; Fibrex®-N 0.3 μg/mL 97.9 ± 6.2; Fibrex®-N 1 μg/mL 102.6 ± 4.8; Fibrex®-N 3 μg/mL 105.9 ± 5.2; Fibrex®-N 10 μg/mL 99.5 ± 6.8; TF-N 0.3 μg/mL 99.2 ± 5.6; TF-N 1 μg/mL 101.3 ± 4.2; TF-N 3 μg/mL 102.5 ± 5.7; TF-N 10 μg/mL 103.8 ± 4.6; NTF-N 0.3 μg/mL 100.9 ± 4.4; NTF-N 1 μg/mL 99.7 ± 5.3; NTF-N 3 μg/mL 104.3 ± 6.2; NTF-N 10 μg/mL 101.7 ± 6.2. $n = 3$ experiments including eight to ten replicates for each treatment]. Dimethylsulfoxide (20%, v/v) used as a positive control significantly reduced cell viability (cell viability (%): 37.9 ± 1.9. $p < 0.001$ versus control).

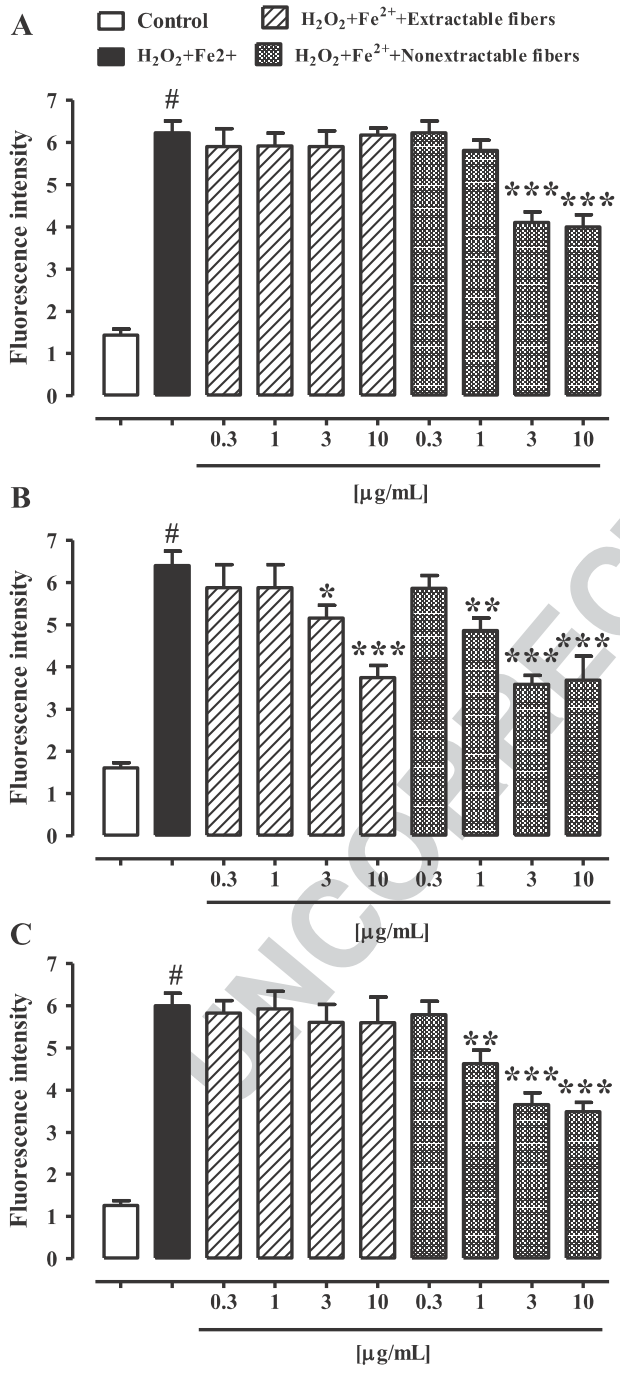

Figure 1. Reactive oxygen species production induced by Fenton's reagent (2 mM H_2O_2/Fe^{2+}) in human intestinal epithelial Caco-2 cells after 24-h exposure to the extractable and non-extractable fraction of Fibrex® (A), treated fibre (B) and non-treated fibre (C) at the concentration range of 0.3–10 μg/mL. Results are means ± standard error ($n = 5$). # $p < 0.001$ versus control; ** $p < 0.01$ and *** $p < 0.001$ versus H_2O_2/Fe^{2+} alone.

CONCLUSIONS

Three types of SBF, possessing different physicochemical properties and aimed for different purposes had different TPC. Non-extractable fractions of the investigated fibres were much higher in TPC and had higher AOA than the extractable fractions, because majority of phenolic compounds in fibres exist in bound forms. Ferulic acid was proven to be the dominant phenolic acid in all investigated fibres. Fibrex® had the highest AOA, while NTF was superior in comparison with TF.

Based on the results of the capacity of fibres to inhibit ROS production, all non-extractable fractions possessed potential for ROS inhibition. The extractable fraction of TF was the only of all investigated extractable fractions that manifested this effect.

These findings are of interest because SBFs could be used not only in the creation of food products but also could offer important benefits for human health.

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Conflict of Interest

The authors declare no conflict of interest.

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