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Preparation of non-traditional Dickkopf and Richard wheat flakes: Phenolic and vitamin profiles and antioxidant activity

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ABSTRACT

Produced non-traditional, conventional and commercial wheat flakes were analysed for vitamin B-complex, α-tocopherol, total phenolics and HPLC profile and antioxidant activity in soluble free, soluble conjugated and insoluble bound phenolic fractions. Moreover, dietary intakes of RDI (Recommended Daily Intake) or AI (Adequate Intake) of vitamins were evaluated. Non-traditional wheat flakes were significantly higher contributors of vitamin B to RDI or AI, also, the highest polyphenol contents (283–362 mg GAE/100 g) were determined in Dickkopf and Richard wheat flakes. Additionally, predominant phenolics were identified. To understand the contribution of individual phenolics to an antioxidant activity their mutual correlations were evaluated. Regarding free phenolics, the main contributors to an antioxidant activity ($r > 0.6961$) were catechin, syringic and caffeic acids; in conjugated fraction epigallocatechin, gallic and ferulic acids ($r > 0.8088$); while chlorogenic, ferulic, protocatechuic and *p*-hydroxybenzoic acids were the main contributors in insoluble bound fractions ($r > 0.6417$).

1. Introduction

Wheat (*Triticum* spp.) plays an important role as a food ingredient in Europe (Yilmaz, Brandolini, & Hidalgo, 2015). It is known that sufficient intake of whole non-traditional wheat grains with dietary fibre, vitamins and antioxidants may contribute to low the risks of cancer, cardiovascular diseases, type II diabetes, age-related ailments and to enhance BMI value (Andersson, Dimberg, Åman, & Landberg, 2014; De Brier et al., 2015). These health benefits are partly attributed to the excellent radical scavenging activities of phenolics and tocols detected in wheat grains (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010).

There has been an increasing trend in the production of whole grain products, such as flakes from non-traditional wheats as whole food item to enhance benefits for human health (Luthria, Lu, & John, 2015). Flaking is relatively a simple process that incorporates heat treatments necessary for the stabilisation of all nutrient components. Commercial thermal processing affects the endosperm structure of grains from the drying step. Hydrothermal treatment is used to stabilise the products by inactivating enzymes, especially lipase and peroxidase. In addition steaming modifies the hydration of macromolecular components of flakes, such as starch, β-glucans and proteins (Prückler et al., 2014). Nevertheless, more data is needed regarding the nutritional composition and nutritional intakes of non-traditional wheat flakes. Together with an increased attention to the nutritional aspect of food, it has been observed that non-traditional wheat grains have a higher phenolic and fibre content (Hidalgo & Brandolini, 2014). Regarding a higher antioxidant activity and nutritional values seems to be promising to use flakes produced from Dickkopf white (*T. aestivum* x *T. spelta* Schlegel cross) and Richard red wheats (*T. aestivum* var. *milturum*) which are newly cross-bred. Dickkopf was enrolled into *Rote Liste der gefährdeten einheimischen Nutzpflanzen* in Germany and was recognized as *Passagier arche guter geschmack Slow-Food* in Germany as well (Sumczynski, Bubelová, Sneyd, Erb-Weber, & Mlček, 2015). This may lead to a higher commercial production of value-added wheat varieties rich in health-beneficial components.

The aim of this study was to prepare non-traditional wheat flakes from Dickkopf and Richard wheats using hydrothermal treatment and rolling, and to determine vitamin B-complex and α-tocopherol by using HPLC-DAD to evaluate their appropriate contributions to RDI (Recommended Daily Intake) or AI (Adequate Intake) compared with commercial flakes. Also, total phenolic content and phenolics profile were determined by using HPLC-DAD and antioxidant activity in free, conjugated and insoluble bound phenolic fractions. Besides, the

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appropriate correlations between antioxidant activity and phenolics were evaluated.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, methanol, NaOH, HCl, $NaCO_3$ and trifluoracetic acid were obtained from Penta (Prague, Czech Republic). Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, purity ≥ 97%) (all Sigma Aldrich, Prague, Czech Republic) were applied for phenolics and antioxidant activity analysis. Further, individual phenolic and vitamin standards were as follows: catechin, epicatechin, epigallocatechin, kaempferol and ellagic, vanillic, caffeic, *p*-hydroxybenzoic, chlorogenic, *o*-coumaric and protocatechuic acids (Sigma Aldrich, Prague, Czech Republic); ferulic and cinnamic acids and quercetin (Merck, Darmstadt, Germany); syringic and *p*-coumaric acids (Tokyo Chemical Industry, Tokyo, Japan); thiamine hydrochloride, riboflavin, nicotinic acid, calcium-D(+)-pantothenate, pyridoxine-hydrochloride, folic acid and D,L-α-tocopherol (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). All phenolic and vitamin standards and solvents were of HPLC-grade.

2.2. Production of non-traditional wheat flakes

Two commercial white and two commercial red wheat flakes (samples marked as I and II) were purchased in markets (Zlín, Czech Republic and Vienna, Austria, respectively) and three conventional wheat grains (Bakfis, Bodyček and Federer) were provided by VP Agro (Prague Czech Republic). Next, two different types of non-traditional wheat grains were used: two samples of Dickkopf white and two samples of Richard red grains (both marked as I and II). All samples were obtained between 2015 and 2016. Non-traditional and conventional wheat grains were not ground, but directly cooked in amount of 5 × 0.5 kg of each sample in water bath equipped with a thermometer (95 °C): Dickkopf, Bekfis, Bodyček and Federer wheats for 15 min and Richard red wheat for 10 min (the cooking time corresponded to the condition in which the grain is suitable for consumption). Afterwards, cereals were dried at room temperature for 30 min and non-traditional and conventional wheat flakes were prepared using a Combi-Star mill grinder (Waldner Biotech, Lienz, Austria) equipped with a flake roller to a thickness of 0.75 mm. Flakes were re-dried for 1 h at 80 °C in laboratory oven till dry matter reach up 900 mg/kg (AOAC, 2007). Non-traditional and conventional wheat flakes were stored in non-transparent plastic bottles at 20 °C, the storage period was limited to 1 week.

2.3. Extraction of vitamin B-complex and chromatographic conditions for vitamin B-complex determination

Milled sample (1.0 g) was weighted and dissolved in 0.1 mol/LHCl (8 mL) to extract vitamins B in ultrasonic bath for 1 h at 30 °C. Then, the mixture was centrifuged (Velocity 13μ, Dynamica Scientific Ltd., Newport Pagnell, UK) at 12300x*g* for 20 min, thereafter the supernatant was injected through a nylon filter (0.2 μm) and immediately applied to HPLC (Lebiedzińska & Szefer, 2006).

The individual vitamins B were determined by using HPLC system (Thermo Scientific Dionex Ultimate 3000; Waltham, MA, USA) consisting of Thermo Scientific Dionex UltiMate 3000 Diode Array Detector type DAD-3000RS, UltiMate 3000 rapid separation autosampler and a binary pump HPG-3x00RS. The vitamin B profile was measured according to Ciulu et al. (2011) with a minor modification. Vitamins were separated using Zorbax Eclipse XDB C18 (150 × 4.6 mm; 3.5 μm) column (Agilent Technologies, CA, USA). Data signals were acquired and processed on PC running the LC Chromeleon™ 7.2 Chromatography Data System (Thermo Scientific, MA, USA). Fifty μL of the sample were introduced onto the column and eluted under gradient conditions performed with 0.25 mL/L trifluoracetic acid (A) and acetonitrile (B). The solvent gradient was set as follows: 0% B at 0–10 min, increasing from 10 to 20 min to 25–45% B, 19–20 min 40% B, 20–21 min 40 − 0% B, 21–25 min 0% B. The mobile phase flow rate was 1 mL/min, column temperature was set at 25 °C and the chromatogram was recorded at 270 nm for thiamine and riboflavin, 210 nm for nicotinic and pantothenic acids and pyridoxine and 290 nm for folic acid. The DAD response was linear for all vitamins B within the calibration range of 0.1–10.0 μg/mL with the correlation coefficients exceeding 0.9995. The appropriate vitamins B were identified by using the retention time and the method of standard addition for the particular vitamin.

2.4. Extraction of α-tocopherol and chromatographic conditions for α-tocopherol determination

Flakes (1.0 g) were transferred into volumetric flasks with 5 mL of methanol. The flasks were placed in the water bath at 40 °C for 3 h (the samples were shaken). Afterwards, extract solution was filtered through the 0.2 μm nylon filter before the injection to HPLC (Khan et al., 2010).

Chromatographic analysis was performed using the HPLC system Thermo Scientific Dionex Ultimate 3000 (Waltham, MA, USA) consisting of a Thermo Scientific Dionex UltiMate 3000 Diode Array Detector type DAD-3000RS. α-Tocopherol content was measured according to Khan et al. (2010) with a minor modification. Discovery C18 (250 × 4.6 mm; 5 μm) column (Supelco, USA) was used. Twenty μL of the sample were introduced onto the column and eluted under the isocratic elution condition performed with the mobile phase consisting of methanol (95 mL) and redistilled water (5 mL). The mobile phase flow rate was set at 1 mL/min, column temperature at 30 °C and chromatogram was recorded at 205 nm. D,L-α-tocopherol was used as a standard within the calibration range of 1.0–50.0 μg/mL, with the correlation coefficients exceeding 0.9996. The appropriate α-tocopherol was then identified by using the retention time and the method of standard addition.

2.5. Evaluation of vitamins contribution from wheat flakes to RDI or AI

Dietary intake levels for essential vitamins from wheat flakes were estimated; and they were consequently compared to RDI or AI (where RDI has not been established) as suggested by the FAO/WHO (2004). There is no recommendation for daily intake of flakes, therefore the serving size of flakes was set to 100 g. Estimates were made for adults aged 31–50, both male and female.

2.6. Extraction of soluble free, soluble conjugated and insoluble bound phenolics

Soluble free phenolics were extracted using the method reported by Qiu, Liu, and Beta (2010). Briefly, ground flakes (1.5 g) were added to 15 mL of methanol solution (prepared by mixing 80 mL of methanol and 20 mL of redistilled water) and extracted twice in an ultrasonic bath for 1 h at room temperature. After centrifuging (Velocity 13μ, Dynamica Scientific Ltd., UK) at 12300x*g* for 20 min, supernatants were combined and evaporated to dryness. Then, dried methanol extract was re-dissolved in 10 mL of the mixture of methanol (5 mL) and redistilled water (5 mL) and used as a crude extract containing free phenolics and as an intermediate product for soluble bound phenolic extraction.

Crude methanol extract and residue obtained after the crude extraction (re-washed with 10 mL of redistilled water) were separately hydrolysed using 10 and 20 mL of 4 mol/L NaOH in an ultrasonic bath under nitrogen gas for 4 h, respectively, to release ester-or ether-linked phenolics in an ultrasonic bath. After alkaline hydrolysis, the solution was adjusted to a pH 3–4 with 6 mol/L HCl and re-extracted using 50 mL of ethyl acetate twice. After the centrifugation at 12300x*g* for