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Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli

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ABSTRACT

The five different types of muesli composed of non-traditional wheat flakes were prepared and analysed. Dickkopf wheat, red wheat, kamut and spelt were compared with commercial wheat flakes. Wheat flakes and muesli were assessed for basic analyses (dry matter, ash, protein, starch and fat content), total phenolic and flavonoid content, antioxidant activity (ABTS and DPPH assays), crude fibre content and *in vitro* digestibility. Furthermore, sensory evaluation of muesli involving scale and ranking preference tests was provided. Flakes and muesli made from Dickkopf wheat and red wheat showed the highest total phenolic and flavonoid content and, consequently, the highest antioxidant activity. Moreover, these cereals were high in crude fibre and thus were less digestible. On the other hand, the lowest total phenolic and flavonoid contents and antioxidant activity were determined in commercial flakes and muesli produced from these flakes. The flakes made from non-traditional wheat were sensorially comparable to commercial products.

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1. Introduction

Many cereals and muesli products are dry foods containing protein, fat and saccharides and minor nutritional parameters are minerals and vitamins. Wheat cereals have been the most widely studied as a fibre source, recently also as a source of polyphenols and flavonoids. A number of *in vitro* studies have shown that the function of phenolic compounds is as free-radical scavengers, and quenchers of singlet oxygen formation (Zilic et al., 2011). The primary components of dietary fibre of wheat are non-starch polysaccharides including insoluble dietary fibre (cellulose, hemicellulose and lignin) and soluble dietary fibre (gums and mucilages) (Ho, Azis, & Azahari, 2013). The crude fibre (CF) is composed of cellulose and lignin which are parts of the unhulled grains.

Wheat (*Triticum aestivum* L.) is one of the oldest food crops, which has achieved a central role as a staple food. Non-traditional wheat grains are well known as food with low glycemic index resulting in 11% reduction each in total lipids, triglycerides and LDL-cholesterol concentration. Moreover, low *in vitro* digestibility response was also observed. These health benefits are in part attributed to the antioxidant capacity of phenolic and flavonoid compounds in cereals detected in crude fibre fractions (Guo & Beta, 2013). The measurements of both *in vitro* dry matter digestibility (DMD) and organic matter digestibility (OMD) have been widely used to evaluate the digestibility of food to simulate human digestion (Altangerel, Sengee, Kramarova, Rop, & Hoza, 2011; Xia et al., 2012).

There is a lot of information published about oat and rye flakes. However, articles dealing with the nutritional composition of wheat flakes are very scarce (Ewaidah & Alkahtani, 1992) and none of them is concerned in flakes made from non-traditional wheat types. Due to this fact more data are needed regarding the nutritional composition of non-traditional wheat flakes.

Since non-traditional types of cereal have a higher polyphenol content and therefore higher antioxidant activity it can be assumed that flakes and muesli made from non-traditional cereals will have the following positive characteristics. It seems to be highly interesting to use Dickkopf wheat, which is newly cross-bred. In the year 2011, Dickkopf wheat was written into Rote liste von kommerziellen anlage beeinträchtigt in Germany and was recognised as Passagier arche guter geschmack slow-food in 2013 in Germany as well. This could lead to new opportunities for breeding

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and possible commercial production of value-added wheat varieties rich in health-beneficial components for making other functional foods.

For this reason the objective of the present study was to (i) prepare non-traditional wheat flakes and muesli products, (ii) determine dry matter, ash, crude protein, starch, fat, total phenolic content, total flavonoid content, antioxidant activity, crude fibre content and *in vitro* digestibility and (iii) implement sensory evaluation of wheat muesli containing non-traditional wheat flakes.

2. Materials and methods

2.1. Equipment and chemicals

The non-traditional wheat flakes were prepared using a Combi-Star mill grinder equipped with a flake roller (Waldner Biotech, Austria). A Venticell 111 laboratory oven (BMT Medical Technology, the Czech Republic) and a muffle furnace (Veb Elektro Bad, Germany) were used to determine the dry matter and ash content. Digestibility was performed using a Daisy^{II} incubator and an Ankom^{220} fibre analyser (both Ankom Technology, the USA) was used to determine crude fibre. Furthermore, common laboratory equipment was used; a water bath (Memmert, Germany), filter bags (type F57, Ankom Technology, the USA), a P 3000 polarimeter (Krüss Optronic, Germany), Pro-nitro 1430 (JP Selecta, Spain) and spectrophotometer Lambda 25 (Perkin Elmer, the USA).

The following enzymes were used in the digestibility study: pepsin (E.C. 3.4.23.1) with an activity of 0.7 FIG-U/mg and a mixture of pancreatin enzymes with an activity of 350 FIG-U/g protease, 6000 FIG-U/g lipase and 7500 FIG-U/g amylase (all Merck, Germany). The phenolic acid standard (Gallic acid; Sigma Aldrich, Germany) and the flavonoid standard (Rutin trihydrate; Roth, Germany) were used for the total phenolic and flavonoid quantification. Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (trolox) and 2,2′-azinobis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) (all Sigma Aldrich, Germany) were applied for antioxidant potential evaluation. Acetone, methanol, ethanol, HCl, KH_2PO_4 and $Na_2HPO_4{\cdot}12\ H_2O$, $K_2S_2O_8$ were obtained from Penta (the Czech Republic). H_2SO_4, NaOH and trichloracetic acid were obtained from Merck (Germany). $NaNO_2$, $NaCO_3$, $AlCl_3{\cdot}6\ H_2O$, CH_3COOH and CH_3COONa were purchased from Sigma Aldrich (Germany).

2.2. Production of non-traditional wheat flakes and muesli

Five different types of wheat were used: Dickkopf wheat (hybrid of *T. aestivum* (Dickkopf) and *Triticum spelta* (Schlegel) (Hochschule für Wirtschaft und Umwelt Nürtingen-Geislingen, Germany), red wheat (*T. aestivum*, Hlavac organic farming-house, the Czech Republic), spelt (*T. spelta*, the Czech Republic), kamut (*Triticum turgidum* subsp. *turanicum*, Canada) and commercial flakes from white wheat (*T. aestivum*, the Czech Republic).

Non-traditional wheat grains were cooked in water: Dickkopf wheat, red wheat and spelt for 10 min, kamut for 15 min. Afterwards, non-traditional wheat flakes were prepared using a Combi-Star mill grinder equipped with a flake roller. Flakes were re-dried for 1 h at 80 °C in a laboratory oven. The fruit/nut part of the model muesli products was prepared from cut dried fruits/nuts, particularly cranberries:apples:apricots:almonds in the ratio 1:1:1:1 to make a total fruit/nut amount of 30% in muesli. The products (non-traditional wheat flakes and muesli) were stored in non-transparent bottles at 25 °C, the storage period was limited to 10 days. Non-traditional wheat flakes alone and muesli containing non-traditional wheat flakes were analysed separately.

2.3. Basic chemical analyses

The dry matter was carried out as described in the Association of Official Analytical Chemists, method number 925.45 (AOAC, 1990). The ash content was determined by using modification of the 08-01 method recommended by the American Association of Cereal Chemists (AACC, 1995). The content of total nitrogen was determined according to Kjeldahl. The content of nitrogen was multiplied by the coefficient 5.83, in the case of muesli 6.25, and expressed as a crude protein. The crude fat content was assessed using the Soxhlet method. Ewers' polarimetric method was employed to determine the starch content after the acid hydrolysis (ISO 10520:1997).

2.4. Sample extraction

For the detection of total phenolic and flavonoid contents, extracts were prepared by continuous shaking of 2.5 g of ground samples with 10 mL of methanol for 1 h in sonic bath at 40 °C according to Kim, Tsao, Yang, and Cui (2006). After that, 4 M NaOH was added to hydrolyse the solid residue by shaking for 2 h in the dark at room temperature to involve bounded phenols. After centrifugation (45 min, 3421 g), the supernatant was used for experiments.

For the antioxidant activity determination, extracts were prepared by continuous shaking of 2 g of ground samples with 20 mL of methanol in sonic bath for 1 h at 35 °C. In order to assess the antioxidant capacity, 4 M NaOH was added to hydrolyse the sample by shaking for 2 h in the dark at room temperature (Serpen, Gökmen, Pellegrini, & Fogliano, 2008). Afterwards, 0.5 mL of 80% trichloroacetic acid was added and centrifuged for 30 min at 3421 g. The supernatant was used for experiments.

2.5. Determination of the total phenolic content

The total phenolic content (TPC) was determined by Folin–Ciocalteu method with a modification (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, 0.2 mL of the sample extract was added to 5 mL of redistilled water and 0.5 mL of Folin–Ciocalteu reagent was added. After a 5-min equilibration, the mixture was neutralized with 1.5 mL of 20% $NaCO_3$, mixed well by a vortex. After a 30-min reaction, the absorbance of the mixture was measured at 765 nm with a UV–VIS spectrophotometer. Gallic acid (0–800 mg/L) was used as a reference standard, and the results were expressed as mg of gallic acid equivalents (GAE) per kg of the sample (mg GAE/kg sample).

2.6. Determination of the total flavonoid content

The total flavonoid content (TFC) was determined according to Dewanto, Wu, Adom, and Liu (2002) with a modification. Briefly, 8.5 mL of 20% ethanol was mixed with 0.85 mL of the extract and 0.375 mL of 0.5 M $NaNO_2$. After 5 min, 0.375 mL of 0.3 M $AlCl_3{\cdot}6\ H_2O$ solution was added and the mixture was allowed to stand for 5 min. Then, 2.5 mL of 1 M NaOH was added. The absorbance was measured after 10 min at 506 nm. Rutin (0–1400 μg/L) was used as a standard and the results were expressed as mg of rutin equivalent (RE) per kg of the sample (mg RE/kg sample).

2.7. Determination of total antioxidant activity by the DPPH method

Antioxidant activity of samples was evaluated by DPPH radical scavenging activity assay with a modification (Ferri, Gianotti, & Tassoni, 2013). Briefly, an aliquot of the sample extract (450 μL) was combined with 8.55 mL of freshly made DPPH radical solution in methanol (0.17 M). After standing for 60 min in the dark, the

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