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DETERMINATION OF PRIMARY METABOLITES IN CEREAL MILLING FRACTIONS

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ABSTRACT

The aim of this study was to determine of primary metabolites (content of starch, total dietary fibre, reducing sugars, proteins and amino acids) in four milling fractions of selected cereals (barley, wheat, oat, spelt, rye, triticale) grew in the year 2009. It was found that flour fractions (break flour and reduction flour) showed the lower content of primary metabolites than bran fractions (fine bran and coarse bran). The aim of this study was also to mention the potential use of bran parts of grain - substances from these parts can be isolated and after treatment, which causes their efficiently usable for human body, they can be used for fortification of wide range of food products.

Keywords: Cereals, primary metabolites, milling fractions

INTRODUCTION

Cereal-based foods have been staples for humans for millennia. Cereals are grown over 73 % of the total world harvested area and contribute over 60 % of the world food production **(Charalampopoulos *et al*. 2002)**. Cereal grains contain the macronutrients (protein, fat and carbohydrate) required by humans for growth and maintenance. They also supply important minerals, vitamins and other micronutrients essential for optimal health. However, it is becoming apparent that cereals in general have the potential for health enhancement beyond the simple provision of these nutrients and that their consumption can lower the risk of significant diet-related diseases quite substantially **(Jemal *et al*. 2005)**. In recent years, cereals have also been investigated regarding their potential use in developing functional foods **(Charalampopoulos *et al*. 2002)**.
Primary metabolites of cereals play significant role in human nutrition and also in food industry. The most abundant component of cereals is starch and is present in endosperm **(Lineback and Rasper, 1988)**. Starch contributes 50-70 % of the energy in the human diet providing a direct source of glucose, which is an essential substrate in brain and red blood cells for generating metabolic energy **(Perry *et al*. 2007)**. Starch is also important industrial material; roughly 60 % of starch from various cereal, tuber and root crops is used in foods (bakery products, ice cream, soups, sugar syrup, etc.) and 40 % in pharmaceuticals and non-edible purposes **(Burrell, 2003)**. Cereals are a major source of protein for humans but have low quality due to limitations in the amounts of essential amino acids, notably lysine **(Shewry, 2007)**. Proteins present in cereals play important role in food industry especially in milling and bakery. Cereal grains, mainly outer layers, are also rich on vitamins, minerals and dietary fibre.
The objective of this study was to determine of primary metabolites: starch, reducing sugar, dietary fibre, proteins and amino acids in milling fractions of selected cereals and their distribution into the dry milling fractions. The aim of this study was also to refer of unutilized potential of naturally occurring primary metabolites in cereals, which remains in the form of bran during the production of flour.

MATERIAL AND METHODS

Plant material

Cereals were grown in the year 2009 on a field nursery at Department of Environmental Protection and Organic Farming (DEPOF) Spišská Belá (Slovakia) and on a field at Plant Production Research Institute (PPRI) Piešťany (Slovakia). The used types and genotypes of cereals were: wheat (Torysa, PPRI), oat (Cacko, DEPOF), spelt (Roquir, PPRI), triticale (Kandar, PPRI), rye (Dankovské nové, PPRI), barley (Ezer, DEPOF). Before the measurement samples were milled by laboratory mill (Brabender Quadrumat Senior, Germany) into four milling fractions (MBF): break flour (MF I.; particle size < 155 µm), reduction flour (MF II.; particle size < 195 µm), fine bran (MF III.; particle size 195 – 265 µm), coarse bran (MF IV.; particle size > 530 µm), and stored at room temperature in closed containers.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia), Merck (Germany) and Sigma Aldrich (USA).

Starch content

The starch content was determined according to the polarimetric method of Ewers **(Muchová a Frančáková, 1992)**. The sample (1 g) was digested in a hot hydrochloric acid (10 mL of 1.125 % v/v) solution. After digestion (precipitation of nitrogen compounds with 0.2 mL of 15 % potassium ferrocyanide and 0.2 mL of 30 % zinc sulfate) and filtration, optical activity of the solution was measured by polarimetric method. The starch content in percent was determined multiplied by a specific factor. All analyses were performed in triplicates.

Total dietary fibre content

The total dietary fibre content was determined according methods AACC 32-05 and AOAC 985.29 (Megazyme).

Reducing sugars content

The reducing sugars content was determined by dinitrosalicylic colorimetric method according to the procedures described by **Wang, (2005)**. 0.5 g of sample was extracted with 80 % of ethanol for 24 hours. After filtration 100 µL of extract was mixed with 800 µL dinitrosalicylic acid and mixture was heated at 90° C for 5 minutes to develop the red-brown color. After cooling to room temperature 8 mL of distilled water was added and absorbance at 575 nm was measured (BioTek Microplate Reader ELx800, USA). Reducing sugars were expressed as mg Glucose equivalent per g of dry mass (mg GE /g DM). All analyses were performed in triplicates.

Extraction of proteins

Sample (0.25 g) was suspended in 5 mL of distilled water for 45 minutes and centrifuged at 2860 g for 20 minutes (Himac CT 6E, Japan). The extraction was repeated three times. The pellet was extracted with 5 mL of 10 % NaCl, stirred for 45 minutes and centrifuged at 2860 g for 20 minutes (Himac CT 6E, Japan). The extraction was repeated three times. The pellet remaining after the NaCl extraction was extracted with 5 mL of 70 % (v/v) ethanol to separate prolamins. The extraction was carried out three times for 45 minutes and extracts were centrifuged at 2860 g for 20 minutes. The pellet remaining after prolamins extraction was extracted with 5 mL of 0.2 % NaOH, stirred for 45 minutes and centrifuged. Alkaline extraction was repeated three times. The supernatants contained albumins, globulins, prolamins, and glutelins were analysed separately and from obtained values the averages were made **(Michalík, 2002)**.

Protein quantification

The Bradford assay relies on the binding of the dye Coomassie Brilliant Blue G-250 to protein **(Bradford, 1976)**. Bradford reagent was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 mL of 95 % ethanol. The solution was then mixed with 100 mL of 85 % (w/v) phosphoric acid and made up to 1 L with distilled water **(Kruger, 2002)**. Towards 10 µL of protein fraction was added 300 µL of Bradford reagent. After 5 minutes, absorbance at 600 nm was measured (BioTek Microplate Reader ELx800, USA). Concentration of proteins was expressed as mg proteins per gram of dry mass (mg P/g DM). All analyses were performed in triplicates.

Amino acid analysis

Liquid-phase hydrolysis of powdered samples was performed in 6 M HCl containing 0.5% phenol at 110 ^{o}C for 24 hours under an argon atmosphere. The hydrolysates were lyophilized, dissolved in an appropriate volume of dilution buffer (sodium citrate buffer pH 2.2) and filtered through a 0.45 µm syringe filter before applying to the amino acid analyzer. Amino acids were determined by ion-exchange chromatography with strong cation ion-exchanger and sodium-citrate elution buffers system followed by post-column derivatization with ninhydrin and spectrophotometric detection, according to standard protocol of manufacturer of amino acid analyzer (Ingos, Czech Republic). For calibration of amino acid analyser the amino acid standard solution was used. Tryptophan was not determined as it is destroyed during acid hydrolysis, and asparagine and glutamine turn to aspartic acid and glutamic acid and in these forms are determined. All analyses were performed in triplicates.

RESULTS AND DISCUSSION

Starch content

Starch is the most important component of cereal grains, represents significant part of dry matter; consists of two polysaccharides – amylase and amylopectin. These polysaccharides have different characteristics, important in food industry. Structure of amylopectin chain is more ramified with compare to amylase, and amylopectin has also greater resistance to amylolytic enzymes than easy degradable amylase **(Raeker *et al.* 1998; Peng *et al.* 1999)**.
The content of starch in flour milling fractions of cereals (Tab.1) decreased in this order: wheat > triticale > barley > spelt > oat > rye; in bran milling fractions of cereals in this order: barley > rye > oat > spelt > triticale > wheat. The higher starch content was determined in wheat, spelt and triticale flour fractions. Wheat starch is very important, especially in bakery technology, because of ability to produce highly viscous solutions. The lower starch content was detected in rye, which compared to other cereals dominated non – starch polysaccharides having high ability to bind water. In bran milling fractions the highest starch content was determined in barley. It is expected, that barley due to its bioactive substance, including starch, will be widely used in food industry in the future.
In the summary, higher starch content was determined in flour milling fractions, whereas contain the highest proportion of endosperm. Our findings are consistent with the findings of other authors **(Cauvain, 2003; Tester *et al.* 2004; Borght, *et al.* 2005; Prugar *et al.* 2008; Mareček *et al.* 2011; Mareček *et al.* 2011)**.

Table 1 Starch content (%) in milling fractions of analysed cereal

Sample	I.	II.	III.	IV.
Barley (*Hord. vulgare*)	77.88 ± 1.66	61.69 ± 1.19	52.33 ± 0.57	36.77 ± 0.31
Wheat (*Trit. aestivum*)	75.35 ± 0.62	72.50 ± 0.72	29.04 ± 0.60	20.81 ± 0.60
Oat (*Avena nuda*)	72.20 ± 0.32	64.69 ± 0.69	33.88 ± 0.52	31.45 ± 0.50
Spelt (*Trit. spelta*)	73.79 ± 0.76	68.39 ± 0.41	33.65 ± 1.17	22.71 ± 0.35
Rye (*Sec.cereale*)	66.61 ± 0.23	60.69 ± 1.3	40.28 ± 0.86	39.21 ± 0.62
Triticale (*Triticosecale*)	77.31 ± 1.53	68.89 ± 1.21	30.56 ± 0.36	21.57 ± 0.57

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Total dietary fibre content

Dietary fibre is the rest of the cell walls of plant tissues, which in not subject to hydrolysis by digestive enzymes and passes unchanged through the digestive system. Consummation of foods with high content of dietary fibre mainly from cereal sources has many positives on the human body (reducing the risk of cancer, slow down the absorption of fats and carbohydrates etc.). Recommended daily intake of dietary fibre for children is 14g/kg, for adults 30 g/kg and for pensioners 20 g/kg **(Kajaba *et al.* 1997; Gray, 2006)**.
The determined total dietary fibre content (Tab. 2) in flour milling fractions of cereals decreased in following order: oat > barley > spelt > wheat > rye > triticale; and in bran milling fractions of cereals as follows: wheat > triticale > barley > rye> spelt > oat. The higher total dietary fibre content in flour milling fractions was determined in oat and barley mainly due to the content of β-glucans. Oat compared with other cereals, contains maximum level of β-glucans (3-7 %), which are concentrated in the internal aleurone and sub-aleurone layer surrounding the endosperm tissue (fraction II. – reduction flour). It has been shown that β-glucans have a positive effect on cholesterol, glucose and insulin level in the blood serum and also anticarcinogenic effect **(Havrlentová *et al*, 2008)**. For this reason, oat flour is used as nutrition additive to wheat flours. Very high total dietary fibre content was also determined in barley, although these values would be probably higher in food variety of barley. Barley analysed in this work belong to malt variety of barley, in which is reduced the value of β-glucans be breeding; but, for food industry are required varieties with higher content of desirable dietary fibre in endosperm. In other cereals (wheat, rye, spelt, triticale) was confirmed that higher content of dietary fibre is in outer layers of grain, rich for non-starch polysaccharides (arabinoxylans, cellulose) **(Topping a Clifton, 2001)**. **Ragaee *et al.* (2006)** determined total dietary fibre content in selected cereals and found that the higher dietary fibre content is located in the outer layers of grain, which is confirmed in our work.

Table 2 Total dietary fibre content (%) in milling fractions of analysed cereals

Sample	I.	II.	III.	IV.
Barley (*Hord. vulgare*)	1.62 ±0.21	10.54 ±1.52	12.58 ±1.79	47.24 ±2.47
Wheat (*Trit. aestivum*)	2.44 ±0.35	2.85 ±0.33	31.99 ±2.23	56.03 ±3.85
Oat (*Avena nuda*)	1.99 ±0.45	33.86 ±1.82	7.39 ±1.62	13.53 ±1.65
Spelt (*Trit. spelta*)	2.89 ±0.52	3.41 ±0.74	6.90 ±0.88	33.25 ±2.22
Rye (*Sec.cereale*)	1.84 ±0.15	2.66 ±0.22	24.12 ±2.56	24.27 ±1.72
Triticale (*Triticosecale*)	1.28 ±0.23	1.84 ±0.14	29.81 ±1.89	41.19 ±3.02

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Reducing sugars content

Reducing sugars are sugars which contain free aldehyde group in the molecule, which is also used for their determining. In cereal grains are represented mainly by glucose and fructose, but in small quantities, about 1 % of the total carbohydrate content.
The determined reducing sugars content (Tab. 3) in flour milling fractions of cereals decreased as follows: wheat > oat > barley > spelt > triticale > rye; and in bran milling fractions of cereals in following order: oat > wheat > barley > triticale > spelt > rye. The higher content of reducing sugars, with the exception of oat, was in the IV. milling fraction (coarse bran) of analysed cereals, whereas this fraction contain germ, which is typical for a high content of "prompt sugars". During milling of cereal grains part of the germ passes into III. milling fraction (fine bran), in which were determined higher reducing sugars content compared with flour milling fractions. The highest reducing sugars content was detected in

III. fraction of oat, due to the different structure of grain **(Prugar *et al.* 2008)**. In flour milling fraction lower reducing sugars content was detected, whereas in these fractions dominate polysaccharide – starch. Reducing sugars are very important in bakery industry in the production of yeast products as a source of "prompt sugars" for start yeast activity, until the starch degrades into simple sugars **(Cauvain, 2003)**.

Table 3 Reducing sugars (mg GE/g DM) in milling fractions of analysed cereals

Sample	I.	II.	III.	IV.
Barley (*Hord. vulgare*)	2.71 ± 0.03	2.31 ± 0.05	5.70 ± 0.11	6.27 ± 0.1
Wheat (*Trit. aestivum*)	4.01 ± 0.06	5.06 ± 0.08	4.40 ± 0.02	8.54 ± 0.07
Oat (*Avena nuda*)	3.84 ± 0.04	1.33 ± 0.07	8.29 ± 0.01	5.46 ± 0.09
Spelt (*Trit. spelta*)	3.76 ± 0.04	0.85 ± 0.03	5.30 ± 0.04	4.09 ± 0.02
Rye (*Sec.cereale*)	1.82 ± 0.02	1.33 ± 0.01	4.41 ± 0.03	5.38 ± 0.05
Triticale (*Triticosecale*)	2.06 ± 0.05	1.58 ± 0.07	4.33 ± 0.08	5.62 ± 0.07

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Protein quantifications

The amount of protein in cereal grains is very important in technological and nutritional aspect, whether in relation to human or animal nutrition value. Proteins of cereal grains are divided by solubility into four groups: albumins (water soluble), globulins (soluble in salt solutions), prolamins (soluble in alcohol), glutelins (soluble in solutions of acids and bases) **(Osborne, 1924).** Amount of proteins in cereal flours is affected by degree of milling – whole-grain flours are richer in proteins than white flours **(Posner, 2005; Haris, 2011)**.
The determined albumins content (Tab. 4) in flour milling fractions of cereals decreased in order: spelt > triticale > rye > wheat > oat > barley; in bran milling fractions of cereals in order: triticale > wheat > rye > oat > spelt > barley. The content of globulins (Tab. 4) in flour milling fractions decreased in order: oat > spelt > wheat > rye > triticale > barley; in bran milling fractions of cereals in order: oat> wheat > triticale> spelt > rye > barley. Albumins and globulins are essential in human nutrition due to favorable amino acid composition. In flour milling fraction the highest content of albumins and globulins was detected in spelt and oat. From literature data it is known, that oat contains greater portion of albumins and globulins (37-41 %) that was confirmed in our work. Similarly in this work was also confirmed that spelt contains higher content of these proteins in comparison to wheat **(Bonafaccia *et al.* 2000; Prugar *et al.* 2004)**. The lowest content of albumins and globulins in flour milling fractions was determined in rye and barley. **Velíšek, 1999; Michalík *et al.* 2004; Prugar *et al,* 2008** allege that rye in comparison to wheat contains higher content of these proteins; this information was not confirmed in our work. This may be due to variety and also agro-ecological conditions, which attributed to protein content by significant effect.
The determined prolamins content (Tab. 4) in flour milling fractions of cereals decreased in order: spelt > wheat > triticale > rye > barley > oat; in bran milling fractions of cereals in order: barley > spelt > rye > oat > triticale > wheat. The content of glutelins (Tab. 4) in flour milling fractions decreased in order: spelt > wheat > oat > triticale > rye > barley; in bran milling fractions of cereals in order: spelt> triticale > wheat> oat > rye > barley. Prolamins and glutelins belong to cereal storage proteins. The highest content of these proteins in flour milling fractions was determined in wheat species (*Triticum aestivum* L.; *Triticum spelta* L.). Prolamins in wheat species are called as gliadins, in barley as hordenins, in oat as avenins and in rya as secalins. Glutelins in wheat are signed as glutenins and together with gliadins as gluten proteins. Gluten proteins play significant technological roles in bakery industry.

In the summary the highest content of proteins was determined in bran milling fractions. These fractions contain the most valuable component of grain – aleurone, which is botanically part of endosperm, but during wetting of grains before milling it has tendency to adhere to the bran.

Table 4 Protein fractions content (mg /g DM) in milling fractions of analysed cereals

Sample		Albumins	Globulins	Prolamins	Glutelins
Barley *(Hord. vulgare)*	**I.**	11.71 ± 0.87	10.06 ± 3.67	28.44 ± 5.87	93.22 ± 4.32
	II.	30.99 ± 4.76	17.44 ± 1.72	55.53 ± 0.87	108.11 ± 176
	III.	47.55 ± 1.74	43.46 ± 1.84	54.71 ± 1.94	108.54 ± 1.04
	IV.	52.08 ± 2.52	47.82 ± 1.69	52.75 ± 3.56	156.29 ± 2.87
Wheat *(Trit. aestivum)*	**I.**	17.11 ± 4.65	15.13 ± 0.81	43.77 ± 3.65	104.97 ± 3.91
	II.	33.66 ± 3.77	20.91 ± 3.17	49.13 ± 0.89	140.28 ± 3.04
	III.	63.82 ± 1.95	76.93 ± 2.94	26.20 ± 3.95	138.47 ± 0.95
	IV.	56.60 ± 4.51	65.24 ± 0.43	30.47 ± 3.76	139.57 ± 0.51
Oat *(Avena nuda)*	**I.**	12.95 ± 1.63	66.82 ± 0.83	30.62 ± 1.93	107.61 ± 1.93
	II.	37.37 ± 2.16	34.48 ± 4.72	40.84 ± 5.76	128.56 ± 2.76
	III.	55.68 ± 1.77	100.97 ± 5.07	47.11 ± 4.77	168.71± 0.97
	IV.	59.28 ± 4.67	109.97 ± 3.62	63.20 ± 1.16	196.49 ± 4.97
Spelt *(Trit. spelta)*	**I.**	28.20 ± 3.73	25.20 ± 5.87	78.75 ± 0.73	129.07 ± 0.73
	II.	34.35 ± 1.18	24.55 ± 5.18	81.77 ± 1.28	189.67 ± 0.18
	III.	47.97 ± 1.91	49.55 ± 3.91	61.35 ± 0.91	166.74 ± 5.03
	IV.	46.28 ± 2.93	68.37 ± 2.88	79.20 ± 6.04	202.08 ± 0.87
Rye *(Sec. cereale)*	**I.**	21.71 ± 1.25	12.55 ± 3.27	14.55 ± 2.25	78.29 ± 1.95
	II.	37.97 ± 3.78	21.68 ± 6.03	28.08 ± 4.28	116.52± 6.23
	III.	62.11 ± 2.33	51.80 ± 5.33	30.97 ± 5.55	94.76 ± 2.41
	IV.	57.73 ± 5.08	52.73 ± 1.51	43.55 ± 3.97	147.16 ± 4.08
Triticale *(Triticosecale)*	**I.**	26.99 ± 2.77	13.07 ± 0.96	41.66 ± 5.56	105.73± 5.11
	II.	33.55 ± 0.95	18.53 ± 2.75	51.82 ± 4.95	144.17 ± 4.95
	III.	65.55 ± 1.71	71.37 ± 5.76	37.33 ± 0.83	125.04 ± 0.71
	IV.	61.11 ± 0.56	66.71 ± 3.93	38.51 ± 4.56	141.82 ± 3.76

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Amino acid composition

Essential amino acid content

Essential amino acids are not synthesized in body of higher animals with simple stomach (monogastric animals) and so they must be received into the body by foods **(Velíšek *et al.* 2002)**. The essential amino acids are: valine, leucine, isoleucine, lysine, threonine, phenylalanine, tryptophan and methionine. In this study was found, that the most abundant essential amino acid in analysed cereals was leucine; low abundant essential amino acid was lysine. Lysine is limiting essential amino acid in cereal grains and cereal products, because lysine is minimally represented in cereal proteins. This amino acid is very important in human body for resorption of calcium, for activity of muscle, production of hormones, enzymes and antibodies. The recommended daily intake of lysine for adults is 30 mg/kg, and it can be ensured by higher consumption of legumes and pseudocereals (in case of plant foods).
The highest content of essential amino acids in analysed flour milling fractions (Tab. 5) was in oat, rye and triticale; in bran milling fractions in spelt and triticale, which is associated with a high content of albumnis and globulins in these fractions, whereas these proteins are rich in essential amino acids. Similarly **Fernandez-Figares *et al.* (2000)**, reported that grain of triticale contains higher content of lysine compared to other cereals. Currently triticale grain is used mainly for feeding, but in the future will be necessary to increase its use in food industry, whereas contains essential compounds for human body. **Shewry, (2007)** determined the content of essential amino acids in samples of wheat, oat, rye and barley and found that the most abundant was leucine, and low abundant essential amino acid was lysine. Further, he reported that oat had the highest content of essential amino acids in comparison to other cereals. Our findings are consistent with the findings of other authors **(Sharpe a Schaeffer, 1993; Shewry, 2007; Prugar *et al.* 2008)**, which reported that bran milling fractions (III. fraction – fine bran) contain high essential amino acids content.

Table 5 Essential amino acids content (mg/g DM) in milling fractions of analysed cereals

Sample		VAL	LEU	ILE	LYS	THR	PHE
Barley *(Hord. vulgare)*	**I.**	2.92 ±0.24	4.13 ±0.34	2.17 ±0.19	1.98 ±0.21	1.92 ±0.21	3.24 ±0.27
	II.	4.87 ±0.21	6.73 ±0.27	3.57 ±0.16	3.58 ±0.16	3.27 ±0.15	5.34 ±0.19
	III.	6.32 ±0.27	8.54 ±0.39	3.57 ±0.19	4.62 ±0.19	4.41 ±0.21	6.82 ±0.31
	IV.	5.76 ±0.35	7.77 ±0.41	4.15 ±0.23	3.91 ±0.34	3.95 ±0.23	6.26 ±0.34
Wheat *(Trit.aestivum)*	**I.**	3.43 ±0.39	5.72 ±0.68	2.87 ±0.33	1.92 ±0.23	2.18 ±0.28	3.99 ±0.47
	II.	3.50 ±0.29	5.66 ±0.51	2.84 ±0.25	2.09 ±0.19	2.24 ±0.21	3.89 ±0.37
	III.	6.35 ±0.53	8.09 ±0.65	4.12 ±0.35	5.61 ±0.45	4.45 ±0.52	5.25 ±0.44
	IV.	6.01 ±0.45	8.02 ±0.58	4.02 ±0.25	4.71 ±0.22	4.00 ±0.22	5.36 ±0.38
Oat *(Avena nuda)*	**I.**	4.11 ±0.31	6.55 ±0.41	3.41 ±0.23	3.60 ±0.25	2.94 ±0.21	4.49 ±0.35
	II.	7.00 ±0.23	5.85 ±0.32	3.11 ±0.12	3.11 ±0.32	2.61 ±0.36	4.16 ±0.12
	III.	6.20 ±0.32	9.30 ±0.43	4.84 ±0.21	5.88 ±0.33	4.73 ±0.24	6.30 ±0.32
	IV.	2.92 ±0.45	8.43 ±.0.35	4.29 ±0.33	4.74 ±0.14	3.74 ±0.23	5.82 ±0.37

Spelt *(Trit.spelta)*	I.	4.63 ±0.44	7.45 ±0,65	3.84 ±0.41	2.35 ±0.27	2.83 ±0.31	5.13 ±0.54
	II.	4.74 ±0.51	7.42 ±0.75	3.86 ±0.34	2.78 ±0.31	3.03 ±0.34	5.11 ±0.53
	III.	7.55 ±0.22	9.75 ±0.23	5.07 ±0.12	6.72 ±0.22	5.25 ±0.15	6.30 ±0.13
	IV.	6.79 ±0.82	9.04 ±1.19	4.70 ±0.05	5.25 ±0.63	4.39 ±0.58	5.97 ±0.66
Rye *(Sec.cereale)*	I.	2.95 ±0.15	4.11 ±0.21	2.22 ±0.11	2.23 ±0.13	2.04 ±0.01	2.98 ±0.11
	II.	4.43 ±0.39	6.34 ±0.57	3.34 ±0.29	3.21 ±0.31	2.98 ±0.28	4.48 ±0.41
	III.	4.91 ±0.39	6.37 ±0.51	3.45 ±0.27	4.13 ±0.31	3.46 ±0.26	4.51 ±0.36
	IV.	6.02 ±0.57	7.60 ±0.69	4.10 ±0.37	4.70 ±0.41	4.23 ±0.49	5.47 ±0.51
Triticale *(Triticosecale)*	I.	3.56 ±0.11	5.34 ±0.06	2.82 ±0.16	2.42 ±0.11	2.35 ±0.06	3.78 ±0.21
	II.	4.77 ±0.47	7.07 ±0.24	3.69 ±0.24	3.43 ±0.32	3.16 ±0.32	4.87 ±0.19
	III.	7.28 ±0.11	9.10 ±0.12	4.77 ±0.12	6.93 ±0.11	5.09 ±0.11	5.90 ±0.05
	IV.	6.33 ±0.21	7.93 ±0.11	4.25 ±0.19	5.36 ±0.19	4.29 ±0.11	5.21 ±0.09

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Semi-essential amino acids content

Semi-essential amino acids are essential for children and broods, because their biosynthesis is insufficient to support the growth of young organisms **(Velíšek *et al.* 2002)**. Semi-essential amino acids are tyrosine, histidine and arginine.

In this work was found that the most abundant semi-essential amino acid (Tab. 6) is arginine, which is necessary for activity of hypophysis, synthesis of proteins, collagens and neurotransmitter nitric oxide. Histidine, important in human body for synthesis of histamine was low abundant semi-essential amino acid in analysed cereal. Absence of this amino acid in human body causes the illness – histidinemia. For this reason is important, especially for children adequate intake of histidine by consumption of cereals, legumes and pseudocereals. Similar findings reported **Fernandez-Figares *et al.* (2000)** and **Etonihu *et al.* (2009)**, which determined semi-essential amino acids in the sample of triticale and wheat. In flour milling fractions the highest content of semi-essential amino acids was detected in oat and spelt; in bran milling fractions in spelt and triticale. Similarly than essential amino acids, bran milling fractions (III. fraction – fine bran and IV. fraction– coarse bran) showed higher content of these amino acids with compare to flour milling fractions.

Table 6 Semi-essential amino acids content (mg/g DM) in milling fractions of analysed cereals

Sample		TYR	HIS	ARG
Barley *(Hord. vulgare)*	I.	2.01 ±0.24	1.43 ±0.08	2.70 ±0.61
	II.	3.36 ±0.19	2.44 ±0.11	4.95 ±0.26
	III	4.16 ±0.17	3.11 ±0.16	6.66 ±0.26
	IV.	3.69 ±0.24	3.11 ±0.19	5.93 ±0.39
Wheat *(Trit.aestivum)*	I.	2.48 ±0.32	1.95 ±0.21	3.29 ±0.45
	II.	2.47 ±0.27	1.98 ±0.15	3.44 ±0.23
	III.	3.94 ±0.32	4.26 ±0.34	9.34 ±0.53
	IV.	3.83 ±0.23	4.18 ±0.15	8.73 ±0.14
Oat *(Avena nuda)*	I.	3.53 ±0.21	3.08 ±0.02	5.98 ±0.45
	II.	2.80 ±0.11	2.24 ±0.16	4.49 ±0.23
	III	4.76 ±0.21	3.74 ±0.22	8.76 ±0.53
	IV.	4.54 ±0.25	2.97 ±0.19	8.87 ±0.14
Spelt *(Trit.spelta)*	I.	3.43 ±0.31	2.65 ±0.39	4.38 ±0.01
	II.	3.40 ±0.24	2.69 ±0.29	4.56 ±0.47
	III.	4.79 ±0.23	4.96 ±0.19	10.42±0.4
	IV.	4.33 ±0.37	4.64 ±0.48	9.23 ±0.11
Rye *(Sec.cereale)*	I.	1.72 ±0.09	1.52 ±0.07	2.91 ±0.22
	II.	2.68 ±0.25	2.29 ±0.19	4.50 ±0.42
	III.	3.01 ±0.27	2.87 ±0.22	5.78 ±0.43
	IV.	3.70 ±0.37	3.81 ±0.38	7.43 ±0.72
Triticale *(Triticosecale)*	I.	2.52 ±0.07	2.05 ±0.08	3.48 ±0.14
	II.	3.23 ±0.24	2.52 ±0.17	4.90 ±0.55
	III.	4.42 ±0.12	4.80 ±0.11	9.82 ±0.08
	IV.	4.03 ±0.06	4.47 ±0.08	8.77 ±0.24

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

Non-essential amino acids content

Non-essential amino acids are: asparagic acid, glutamic acid, serine, proline, glycine and alanine. These amino acids are synthesis in our body, but certain conditions (physical and psychological stress, irregular diet) can cause their lack in human body. In analysed cereals the highest content of non-essential amino acids was detected in spelt, which confirmed the arguments of another authors, that spelt is rich for amino acids in comparison to other cereals **(Moudrý a Dvořáček, 1999; Stehno a Vlasák, 1999; Bonafaccia *et al.* 2000)**.

The most abundant non-essential amino acids in analysed cereals were glutamic acid and proline, their content was the highest mainly in flour milling fractions, which is associated with a high content of storage proteins (prolamins and glutelins) in these fractions. Similar findings reported **Shewry, (2007)** and **Etonihu *et al.* (2009)** who determined non-essential amino acids in the samples of oat, rye, barley and wheat. The results of bran milling fractions of analysed cereals showed (with exception of glutamic acid and proline), that these fractions contain higher content of non-essential amino acids in comparison to flour milling fractions, what is associated with the content of aleurone and germ in these fractions.

Table 7 Non-essential amino acids content (mg/g DM) in milling fractions of analysed cereals

Sample		ASP	GLU	SER	PRO	GLY	ALA
Barley *(Hord. vulgare)*	I.	3.55 ±0.32	16.43 ±1.34	2.47 ±0.21	7.29 ±0.46	2.17 ±0.16	2.25 ±0.18
	II.	6.04 ±0.26	25.16 ±0.53	4.05 ±0.14	10.90 ±0.18	3.77 ±0.11	3.93 ±0.13
	III.	8.35 ±0.32	34.22 ±1.46	5.30 ±0.23	14.56 ±0.78	5.14 ±0.19	5.37 ±0.21
	IV.	7.55 ±0.63	31.28 ±0.26	4.78 ±0.36	13.45 ±0.12	4.62 ±0.33	4.80 ±0.35
Wheat *(Trit.aestivum)*	I.	3.52 ±0.52	29.14 ±0.25	3.76 ±0.41	9.19 ±0.76	2.99 ±0.25	2.56 ±0.25
	II.	3.63 ±0.45	28.08 ±0.31	3.75 ±0.28	9.08 ± 0.79	3.15 ±0.18	2.74 ±0.19
	III.	9.92 ±1.11	15.51 ±0.31	5.50 ±0.57	7.73 ±0.84	7.20 ±0.67	6.50 ±0.63
	IV.	8.95 ±0.59	28.26 ±2.57	5.31 ±0.36	7.97 ±0.19	6.90 ±0.53	5.83 ±0.41
Oat *(Avena nuda)*	I.	7.03 ±0.51	19.79 ±0.93	4.12 ±0.25	4.78 ±0.34	4.33 ±0.26	4.01 ±0.24
	II.	5.43 ±0.41	22.11 ±1.59	3.71 ±0.32	8.39 ±0.08	3.64 ±0.22	3.53 ±0.21
	III.	11.34 ±0.57	28.02 ±1.35	6.36 ±0.26	8.48 ±0.05	7.26 ±0.34	6.71 ±0.34
	IV.	9.37 ±0.43	23.52 ±0.54	5.51 ±0.31	6.62 ±0.48	6.10 ±0.31	5.54 ±0.28

Spelt *(Trit.spelta)*	**I.**	4.48 ±0.05	37.25 ±1.31	4.79 ±0.41	1.,6 ±0.17	3.68 ±0.35	3.21 ±0.35
	II.	4.86 ±0.35	34.72 ±3.82	4.83 ±0.54	11.71 ±1.24	3.94 ±0.43	3.54 ±0.38
	III.	11.42 ±0.21	30.05 ±0.22	6.49 ±0.19	9.80 ±0.11	8.23 ±0.21	7.64 ±0.21
	IV	9.06 ±1.31	32.28 ±0.76	6.06 ±0.79	10.32 ±1.16	7.36 ±0.98	6.29 ±0.81
Rye *(Sec.cereale)*	**I.**	4.23 ±0.34	16.30 ±0.93	2.68 ±0.25	6.24 ±0.34	2.48 ±0.26	2.54 ±0.24
	II.	5.88 ±0.57	26.66 ±1.59	4.06 ±0.32	9.56 ±0.08	3.77 ±0.22	3.72 ±0.21
	III.	7.87 ±0.59	22.29 ±1.35	4.24 ±0.26	8.28 ±0.05	4.87 ±0.34	4.66 ±0.34
	IV.	10.12 ±0.59	28.40 ±0.54	5.23 ±0.31	10.21±0.48	6.53 ±0.31	5.66 ±0.28
Triticale *(Triticosecale)*	**I.**	4.44 ±0.09	23.9 ±0.64	3.56 ±0.06	8.82 ±0.31	3.13 ±0.09	2.88 ±0.09
	II.	5.84 ±0.73	30.95 ±3.43	4.69 ±0.44	9.70 ±0.13	4.24 ±0.41	3.98 ±0.38
	III.	11.69 ±0.18	26.39 ±0.83	6.14 ±0.15	8.58 ±0.22	7.73 ±0.19	7.51 ±0.16
	IV.	9.99 ±0.31	26.18 ±0.41	5.55 ±0.28	8.33 ±0.22	7.03 ±0.19	6.27 ±0.21

I. – break flour., II. – reduction flour, III. – fine bran, IV. – coarse bran

CONCLUSION

In this study, we prepared and evaluated milling fractions from selected varieties of cereals. Flour fractions (break flour and reduction flour) characterize the lowest content of the primary metabolites. Bran fractions (fine bran and coarse bran) showed higher content of primary metabolites, but 30 – 80 % of these fractions are unused in food industry, and they are used mainly as animal feed. Bran fractions are rare utilizated in food industry, because they are very often contaminated with heavy metals and microscopic fungi. Fortunately, current technology can eliminate these problems to a minimum, which is argument for a higher consumption of whole-grain cereal products.

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REFERENCES

BONAFACCACIA, G., GALLI, V., FRANCISCI, R., MAIR, V., SKRANJA, V., KREFT, I. 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-444.

BORGHT, A., GOESAERT, H., VERAVERBEKE, W. S., DELCOUR, J. A. 2005. Fractionation of wheat and wheat flour into starch and gluten: overview of the main process and the factors involved. *Journal of Cereal Science*, 41, 221-237.

BRADFORD, M. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein – dye binding. *Analytical Biochemistry*, 72, 248-254.

BURRELL, M. M. 2003. Starch: the need for improved quality and quantity – an overview. *Journal of Experimental Botany*, 54, 451–456.

CAUVAIN, S. P. 2003. *Bread making: Improving Quality*. USA: CRC Press, 589 p, 2003. ISBN 0-8493-1762-2.

CHARALAMPOPOULOS, D.,WANG, R., PANDIELLA, S. S., WEBB, C. 2002. Application of cereals and cereal components in functional foods: A review. *International Journal of Food Microbiology*, 79, 131-141.

ETONIHU, A. C., AYODEL. J. T., IBRAHIM. M. 2009. Proximate and amino acid composition of wheat species of pigeon pea grown in Kogi state, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology*, 24, 32-36.

FERNANDEZ-FIGARES, I., MARINETTO, J., ROYO, C., RAMOS, J. M., GARCIA DEL MORAL, L.F. 2000. Amino-acid composition and protein and carbohydrate accumuliation in the grain of tritical grown under terminal water stress simulated by a senescing agent. *Journal of Ceral Science*, 32, 249-258.

GRAY, J. 2006. Dietary fibre, definition, analysis, physiology and health. Brussels: ILSI, 36 p. ISBN 90-78637-03-X.

HARIS, L. 2011. Optimalizácia triedenia a skladovania potravinárskej pšenice z pohľadu jej mlynsko – pekárskeho využitia. Dissertation work, Nitra: SPU, 139 p.

HAVRLENTOVÁ, M., BIELIKOVÁ, M., MENDEL, Ľ., KRAIC, J., HOZLÁR, P. 2008. Korelácia (1-3) (1-4)-β-D-glukánu s vybranými kvalitatívnymi parametrami v zrne ovsa siateho. *Agriculture*, 54, 65-71.

JEMAL, A., WARD, E., HAO, Y., THUN, M. 2005. Trends in the leading causes of death in the United States 1970 – 2002. *American Medical Association*, 294, 1255-1259.

KAJABA, I., ŠIMONČIČ, R., GINTER, E., ONDREJKA, J., KALAČ, J., TRUSKOVÁ, I., BZDÚCH, U. Odporúčané výživové dávky pre obyvateľstvo SR. *Vestník MZ SR*, 45, p 7-8, from day 28.4. 1997.

KRUGER, N.J. 2002. The protein protocols Handbook. Totowa: Humana Press Inc.,1172 p. ISBN 0-89603-940-4.

LINEBACK, D. R., RASPER, V. F. 1988. Wheat carbohydrates. In Pomeranz, Y. (Ed.) third ed. Wheat, Chemistry and Technology St. Paul: MN, p. 277-372. ISBN 9781891127557.

MAREČEK, J., LÍŠKOVÁ, M., FRANČÁKOVÁ, H., MOCKO, K. 2011. The Slovak and Serbian varieties *Triticum aestivum* L. and *Triticum durum* L. and their qualitative parameters evaluation. *Journal on Processing and Energy in Agriculture*, 15, 143-145.

MAREČEK, J., MOCKO, K., FRANČÁKOVÁ, H., MENDELOVÁ, A., LÍŠKOVÁ, M. 2011. Assessing the quality of Serbian varieties *Triticum aestivum* L. and *Triticum durum* L. *Potravinárstvo*, 5, 290-293.

MICHALÍK, I., URMINSKA, J., PETR, J., GÁLOVÁ, Z. , KNOBLOCHOVÁ, H. 2004. Variabilita frakčnej skladby zásobných bielkovín zrna cereálií a pseudocereálií. *Sborník z medzinárodní konference „Proteiny 2004"*, MZLU Brno, p. 17-20. ISBN 80-7157-779-0.

MOUDRÝ, J., DVOŘÁČEK, V. 1999. Chemical composition of grain of different spelt (*Tr. Spelta*, L.) varieties. *Rostlinná výroba*, 45, 533-538.

MUCHOVÁ, Z., RANČÁKOVÁ, H. 1992. Hodnotenie a využitie rastlinných produktov. Nitra: SPU, 165 p. ISBN 80-7137-039-8.

OSBORNE, T. B. 1924. The vegetable proteins. London: Green&Co, 154 p. ISBN 9780548477564.

PENG, M., GAO, M., ABDEL-AAL, E. S. M., HUCL, P., CHIBBAR, R. N. 1999. Separation and characterization of A- and B-type starch of granules in wheat endosperm. *Cereal Chemistry*, 76, 375-379.

PERRY, G. H., DOMINY, N. J., CLAW, K. G., LEE, A. S., FIEGLER, H., REDON, R. 2007. Diet and evolution of human amylase gene copy number variation. *Nature Genetics*, 39, 1256-1260.

POSNER, E. S. 2005. Wheat flour milling. Minnesota: AACC, 489 p. ISBN 1577685083.

PRUGAR, J. a kol. 2008. Kvalita rostlinných prodoktů na prahu 3. tisíclett. Praha: VÚPS, 327 p. ISBN 978-80-86576-28-2.

RAEKER, M. O., GAINES, C. S., FINNEY, P. L., DONELSON, T. 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. *Cereal Chemistry*, 75, 721-728.

RAGAEE, S., ABDEL-AAL, E. S., NOAMAN, M. 2006. Antioxidant activity and nutrient composition of selected cerals for food use. *Food Chemistry*, 98, 32-38.

SHARPE, F. T., SCHAEFFER, G. W. 1993. Distribution of amino acids in bran embryo and milled endosperm and shifts in storage protein subunits of in vitro-selected and lysine-enhanced mutant and wild type rice. *Plant Science*, 90, 145-154.

SHEWRY, P. R. 2007. Improving the protein content and composition of cereal grain. *Journal of Cereal Science*, 46, 239-250.

STEHNO, Z., VLASÁK, M. 1999. Možnosti pěstovaní a využití pšenice špaldy. *Úroda*, 47, 6-7.

TESTER, R., KARKALAS, J., QI, X. 2004. Starch – composition, fine structure and architecture. *Journal of Cereal Science*, 39, 151-165.

TOPPING, D. L., CLIFTON, P. M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Review*, 81, 1031-1064.

VELÍŠEK, J. 1999. Chemie potravin *I*. Praha: OSSIS Tábor, 352 p. ISBN 80-902391-3-7.

VELÍŠEK, J. et al. 2002. Chemie potravin. Praha: OSSIS Tábor, 331 p. ISBN 80-86659-00-3.

WANG, N. S. 2005. Experiment no. 4A, Glucose assay by dinitrosalicylic colorimetric method. Retrieved 14/5-2005 from the University of Maryland, Department of Chemical Engineering.