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Bioactives in organic and conventional milled cereal products from Croatian market

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Summary

A need for food that contributes to individual nutrition and health status, and also to the sustainability of the planet, is rising as a consequence of consumer's awareness of foods nutritional value and origin. This behaviour is mostly expressed through the preference toward organically grown and wholesome food. Science to some extent supports this preference, giving more evidence supporting the benefit of whole food, and indicating that organic might be more nutritious than conventional. However, the question that remains is - is it always better? Thus in this study organic and conventional cereal milling products from Croatian market were randomly selected (namely wheat bran, whole grain, semi-white and white wheat flour, and whole grain corn, oat and rye flour) in order to determine their antioxidant activity (AO), lignans, ferulic acid (FA), total phenolic (TPC) and dietary fibre (DF) content. The research aimed to point out how cereals contribute to the diet with their fibres and bioactives, and to see to what extent consumers can expect organic cereal products to be better source of bioactives than conventional ones when buying from the market.

Wheat bran distinguished among chosen products with its high FA, TPC, lignan content, and the strongest AO, especially in case of organic bran. Within whole grains organic corn flour exhibited the strongest AO and the highest TPC (2576 mg FAE kg-1), while organic oat flour was the most abundant in lignans (10064 µg kg-1). Higher content of FA, TPC, and lignans in organic wheat bran and established difference in bioactives between flours from the organic farming compared to conventional do not lead to firm conclusion that consumers can expect organically produced cereals to be superior in all nutrition aspects to conventional, as is often believed. Despite that, interestingly, among analysed nutritional parameters lignans were in all cases higher in organic products and less strong correlated to fibre content, indicating that they could be influenced by farming system. Obtained data highlight the benefit of consuming different wholegrain cereals as an important source of fibres and bioactive compounds, which, with the exception of lignans, are strongly positively correlated to the insoluble fibre content. .

Keywords: antioxidant activity; conventional and organic cereal milling product; fibre; lignans; phenolics

INTRODUCTION

Cereals are fundamental component of a balanced diet (Liu, 2007). In Europe the most consumed cereal is wheat, mostly found in the form of bakery products made from refined flour. Nowadays it is emphasized that whole grain cereals have a protective role in human health, due to high content of fibres and bioactive compounds such as phenolic acids and lignans (Liu, 2007; Fardet et al, 2008). Their influence on human health is expressed through different protective activities, e.g., soluble fibres reduce the risk of cardiovascular diseases and diabetes; both soluble and insoluble fibres promote positive status of digestive system; phenolic compounds are believed to possess antioxidant activity (Liu, 2007). Among other bioactives, cereal lignans distinguish with their structural similarity to endogenous oestrogens and oestrogenic and anti-oestrogenic effect they can exhibit. It is also believed that lignans possess antioxidant activity (Cotterchio et al 2008; Landete, 2012). However, in order to better understand lignan activity and their exposure through cereal intake there is a great need for a comprehensive lignan database (Durazzo et al, 2013). Many previous studies concluded that the amount of lignans and other bioactive compounds depends on different parameters, such as genotype, climate, soil, etc. (Smeds et al, 2009; Lv et al, 2013). Following, it can be believed that the type of farming system influences on a certain level the development of bioactives within the plant.

In the organic farming system the absence of synthetic protectors, such as fertilizers, could provoke plants to produce more bioactives in response to stronger environmental stress which occurs during organic production (Fares et al, 2012). Namely, bioactive compounds are secondary plant metabolites with protective role in plant (Liu, 2007). Consequently, consumption of organic cereals could result in an increased intake of diverse bioactive compounds, together with the reduced intake of pesticides and fertilizers, as recent literature review and data analysis by Barański et al (2014) indicated.

However, studies specifically dealing with bioactive compounds in cereals of different farming origin are scarce and inconclusive, whereas to our knowledge the influence of cereal farming system on lignan content has not been investigated. Langenkämper et al (2006) established no difference in antioxidant capacity and total phenolics level between organic and conventional farming systems after the long term wheat field experiment. Similarly, Dimberg et al (2005) analysed specific phenolic compounds in oat and found no differences as a consequence of the conventional or organic farming system used. As it goes for consumers, they tend to believe that organically produced food is more nutritious and pay higher price for the same (Barański et al, 2014).

Thus, the aim of this study was to examine lignan, total phenolics, ferulic acid and fibre content of different kind of do-

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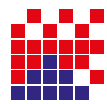
mestic cereal milling products together with their antioxidant activity. For the study white wheat, corn and rye flour as widely used in bread making were chosen, in comparison with less common whole grain wheat, oat flour and wheat bran. Samples were of both conventional and organic origin randomly selected from Croatian market as the interest was to see to what extent consumers can expect organic cereal products to be better source of bioactives than conventional ones.

MATERIALS AND METHODS

Chemicals and materials

Lignan standards of secoisolariciresinol, pinoresinol, and lariciresinol were a gift from Oy Separation Research Ab (Turku, Finland) and syringaresinol gift from School of Chemistry, University of St. Andrews, UK. Pentafluoropropionic anhydride (PFPA), styrene glycol, ferulic acid, gallic acid, enzyme *H. pomatia* β-glucuronidase/sulfatase type H-1, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tris(2-piridil)-s-triazin), and Folin-Ciocalteu's reagent were purchased from Sigma Aldrich (Taufkirchen, Germany).

Fourteen cereal milling products, from both organic and conventional production were investigated: wheat bran and six flour samples of which four were labelled as whole grain, one as semi-white, and one as white. Organic wheat bran, corn, white wheat, semi-white wheat, whole grain wheat, whole grain oat, and whole grain rye flour were purchased from "O.P.G. Jazbec". Conventional corn flour was produced at "Poljodar tim". Conventional wheat bran, rye, white, semi-white and wholegrain wheat flour were purchased from "Granolio". "Advent Pula" provided whole grain oat from conventional production. Producers labelled flours as being white, semi-white or whole grain, while we determined the ash content of samples (Table 1). In tables and figures of this paper, if not stated differently, samples are whole grain. Prior to all analyses samples were sieved through 500 μm sieve.

Determination of ash, insoluble and soluble dietary fibre content

The ash content of flour was determined according to AOAC 923.03 method (AOAC, 2006). Total (TDF), insoluble (IDF) and soluble dietary fibre content (DSF) in flour was determined by the AOAC method 991.43 (AOAC, 2006) according to the Megazyme instructions (Bray, Ireland). All analyses were done in duplicate.

Determination of ferulic acid, total phenolic content and antioxidant activity

Total phenolic content (TPC) and ferulic acid (FA) was extracted after an alkaline hydrolysis according to modified *Healthgrain* method (Li et al, 2009). Flour (250 mg) was hydrolysed with 4 mL 2 mol L^{-1} NaOH for 4 h. After, the pH was adjusted to 2 with concentrated HCl, 4 mL of ethyl acetate was added, and sample was centrifuged 10 min at 4000 rpm. Extraction was repeated three more times with 2 mL ethyl acetate. Supernatants were pooled together and evaporated to dryness under N$_2$. Before FA, TPC and antioxidant activity analysis, samples were redissolved in methanol and filtered through cellulose 0.45 μm syringe filter (Chromafil RC, Macherey-Nagel, Germany). Extraction of each flour type was done in triplicate.

TPC was determined spectrophotometrically (UNICAM Heλios β, England) according to Yu et al (2002) with modifications. To a 0.2 mL aliquot of the methanol extract, 500 μL Folin-Ciocalteu's reagent and 1.5 mL 20% Na$_2$CO$_3$ was added, and volume adjusted to 10 mL with distilled water. The reaction mixture was kept in dark for 2 h. The absorbance was measured at 765 nm. Calibration curves were constructed from standards of ferulic and gallic acid. Results are expressed in

Table 1. *Ash, insoluble, soluble and total dietary fibre content of wheat bran and flour samples, expressed as g kg^{-1} dry basis, in ascending total dietary fibre order. Means in columns followed by different letters are significantly different (p ≤ 0.05)*

	ash	IDF	SDF	TDF
Wheat white - C	6.68 ± 0.04	16.00[a] ± 1.06	20.43[b] ± 3.19	36.42[a] ± 2.43
Wheat semi-white - C	7.79 ± 0.32	26.03[a] ± 3.39	20.95[b] ± 5.77	46.98[a] ± 8.22
Corn - C	9.91 ± 0.04	40.84[b] ± 2.86[b]	7.64[a] ± 1.82	48.48[a] ± 4.67
Wheat semi-white - O	9.43 ± 0.07	37.76[b] ± 1.79	20.07[b] ± 2.84	57.83[ab] ± 1.06
Wheat white - O	7.98 ± 0.07	39.73[b] ± 1.86	20.31[b] ± 4.67	60.04[ab] ± 5.41
Oat - O	13.49 ± 0.09	44.58[b] ± 6.66	29.24[bc] ± 2.25	73.82[b] ± 4.64
Corn - O	22.80 ± 0.06	68.20[c] ± 8.25	10.99[a] ± 1.13	79.20[b] ± 8.78
Oat - C	18.81 ± 0.04	66.90[c] ± 9.47	39.35[cd] ± 2.52	86.57[b] ± 13.33
Wheat - O	14.49 ± 0.08	96.02[d] ± 1.55	22.96[b] ± 2.61	118.98[c] ± 1.53
Rye - C	11.71 ± 0.11	80.95[d] ± 3.42	48.83[d] ± 2.42	129.78[c] ± 3.40
Rye - O	12.16 ± 0.21	85.49[d] ± 3.43	45.59[d] ± 5.80	131.08[c] ± 8.76
Wheat - C	21.15 ± 0.07	117.02[e] ± 4.05	21.33[b] ± 2.00	138.35[c] ± 1.45
Wheat bran - C	46.43 ± 1.09	305.34[f] ± 2.53	27.79[bc] ± 1.46	333.21[d] ± 3.68
Wheat bran - O	60.88 ± 1.21	363.83[g] ± 15.39	34.60[c] ± 1.63	403.43[e] ± 9.57

IDF - insoluble dietary fibre; SDF - soluble dietary fibre; TDF - total dietary fibre; O - organic; C - conventional

Table 2. *Total phenolic content of wheat bran and flour samples in ascending order, expressed as mg FAE kg^{-1} and mg GAE kg^{-1} dry basis. Values are means of three determinations. Means in columns followed by different letters are significantly different (p ≤ 0.05)*

	FAE	GAE
Wheat white - C	137.80^a ± 9.10	165.30^a ± 8.30
Wheat white - O	294.20^a ± 47.50	279.00^a ± 35.90
Wheat semi-white - O	293.20^a ± 41.70	279.90^a ± 30.40
Wheat semi-white - C	348.90^a ± 40.80	320.60^a ± 29.50
Corn - C	1061.00^b ± 44.90	845.40^b ± 34.70
Rye - O	1139.80^b ± 72.00	903.80^b ± 54.70
Rye - C	1210.60^b ± 108.80	955.60^b ± 82.20
Wheat - O	1274.60^b ± 300.90	1001.60^b ± 222.30
Oat - C	1806.80^c ± 58.50	1397.10^c ± 42.50
Wheat - C	2058.16^c ± 145.51	1453.85^c ± 93.71
Oat - O	2192.20^{cd} ± 247.00	1680.70^{cd} ± 1.82.50
Corn - O	2575.80^d ± 146.60	1966.90^d ± 110.50
Wheat bran - C	3672.50^e ± 141.40	2776.70^e ± 104.40
Wheat bran - O	5252.10^f ± 171.70	4003.70^f ± 128.30

FAE - ferulic acid equivalent; GAE - gallic acid equivalent; O - organic; C - conventional

two forms because ferulic acid is most abundant phenolic acid in cereals, while most of previous studies report TPC as gallic acid equivalents.

FA content was determined by HPLC-PDA (Agilent 1200 Series with G1315D photo diode array detector, USA), using modified IOOC method (2009). The column used was Nucleosil 5u C18, 100 A250 x 4.60 mm, 5 microns (Phenomenex, USA). Elution was performed with 0.2% H_3PO_4, methanol, and acetonitrile, with the flow 1.0 mL min^{-1}. Antioxidant activity (AO) was determined by DPPH radical-scavenging capacity and Ferric Reducing/Antioxidant Power (FRAP) assays as described by Belščak et al (2009), with some modifications. In order to assess the flour extracts DPPH radical-scavenging capacity 0.05 mL of extract was added to 1.9 mL of 0.06 mmol L^{-1} DPPH in methanol. The free radical scavenging capacity was evaluated by measuring the absorbance at 517 nm using a UV Vis spectrophotometer (UNICAM Heλios β, England) after 30 min reaction in dark. For FRAP assay, 0.025 mL of methanol extract was mixed with 2.5 mL of FRAP reagent. Absorbance was measured at 593 nm after incubation at room temperature for 4 min. In both assays AO was expressed as trolox equivalents (Belščak et al, 2009).

Determination of lignan content

Prior to lignan extraction, all flours were pulverised in a ball mill (MM 400, Retsch, Germany) to ensure proper homogenisation. Two hundred mg of flour was extracted with 5 mL of 70% methanol containing 0.3 mol L^{-1} NaOH at 60 °C. After 60 minutes the samples were centrifuged at 2500 rpm for 15 min. The extraction was repeated once more. Supernatants were pooled and pH was adjusted to ~5 using glacial acetic acid and evaporated to dryness under a N_2 stream. Enzymatic hydrolysis was performed with 2300 U of *H. pomatia* β glucuronidase/sulfatase dissolved in 5 mL of 0.05 mol L^{-1} Na-acetate buffer (pH 5). After incubation for 17 h at 37 °C the enzymatic hydrolysate was applied to SPE cartridge (Varian, Bond Elut - Certify II, 50 mg, 3 mL) and lignans were eluted with 3 mL of methanol. Lignans were quantified by GC-electron capture detection method (Čukelj et al, 2011). All analyses were done in triplicate.
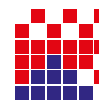
Statistical analysis

Data were subjected to factorial analysis of variance (ANOVA) for two independent variables: kind of milling product and farming system, by using Statistica 8 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Dietary fibre content

Table 1 shows IDF, SDF and TDF content of analysed samples. Organic, followed by conventional wheat bran, had the highest amount of TDF and IDF (> 300 g kg^{-1}), and in all samples the content of TDF and IDF was highly correlated to the ash content (r = 0.955 and 0.972 respectively, $p \le 0.001$). The highest amount of SDF was found in rye flour. On the opposite, the lowest amount of SDF was determined in corn flour, both organic and conventional. In cereals, IDF dominates over SDF (Vitaglione et al, 2008). SDF/IDF ratio in whole grain flours was similar to the ratio reported by Picolli da Silva and De Lourdes Santorio Ciocca (2005). In wheat samples soluble fibres made bigger fraction of TDF in white and semi-white flours (34 - 56%) than in the whole grain (19%) and in the bran, which contained more than 90% IDF. SDF were more abundant in rye and oat than in corn and wheat flour, which can be attributed to a higher content of arabinoxylans in rye, and beta-glucans in oat (Bunzel et al, 2001). Ragaee et al (2005) reported little lower SDF content in white wheat flour (16 - 18 g kg^{-1}) than in our study (20.1 - 20.3 g kg^{-1}) and IDF 19 - 30 g kg^{-1}, which is in accordance to our results for conventional samples. Similarly, Picolli da Silva and De Lourdes Santorio Ciocca (2005), and Ragaee et al (2005) determined lower ratio of SDF to IDF (37/141 g kg^{-1}) than we did, but they both ana-

lysed whole grain cereals, while we had flours with incomplete extraction rate.

When the same types of organic and conventional flour were compared, all organic flours, with the exception of oat flour, had higher levels of TDF and IDF fibre. The existing difference could be most likely result of the differences in the milling process, harvest year and genotype (Hansen et al, 2003). Langenkämper et al (2006) in field experiments on wheat varieties noticed some significant increase of dietary fibre in bio-dynamic system, but finally concluded that the relationship between dietary fibre levels and organic versus conventional farming system cannot be established.

Total phenolic content

Table 2 shows TPC of different cereal products from organic and conventional cultivation. Values expressed as GAE are in general lower that the ones expressed as FAE, but the correlation between assays is strong (r = 0.999, $p \leq 0.001$). Phenolic compounds are mostly located in outer layers of grain (Fardet **et al, 2008)** so it was of no surprise that wheat bran showed to be the richest in TPC. Organic wheat bran had higher content of total phenolics compared to the conventional wheat bran, which could be also attributed to the higher TDF and IDF content. The detected total phenolic amount in wheat bran in general is similar to the one published in study by Verma et al (2008) where it was found TPC in bran to be in range 3406 - 6702 mg GAE kg^{-1}.

Among flours, organic corn flour was richest in TPC, the same as in the work by Adom and Liu (2002) who compared phenolic content of corn, wheat, oat and rice. However, in our study, conventionally produced corn flour had significantly lower ($p \leq 0.05$) content of phenolics compared to its organic counterpart, which could be explained by lower extraction rate during processing. In opposite to Adom and Liu (2002) organic oat, and not wheat flour, followed corn flour in TPC. Nevertheless, results of TPC are in accordance with Ragaee et al (2006)

who determined TPC in wheat to be 501 - 562, and in rye 1026 mg GAE kg^{-1}.

It is known that phenolic compounds are located in the grain outer layers, especially aleurone layer (Fardet et al, 2008). Following current understanding of phenolics-fibre relationship, in this study very strong positive correlation between the TPC and amount of IDF (r = 0.896, $p \leq 0.001$) and TDF (r = 0.882, $p \leq 0.001$) and no correlation with SDF was found. This comes as no surprise since it is known know that the content of analysed TPC extract contained phenolic acids with ferulic acid - the structural element of insoluble fibre component (Bunzel et al, 2001; Vitaglione et al, 2008) - being the dominant one. Unanswered still remains the proportion of other types of cereal phenolics, such as carotenoids and alkyl-resorcinols, in TPC extract; and how their content would relate to the dietary fibre.

Ferulic Acid Content

In this work focus was on FA as being the main phenolic acid in cereals (Adom and Liu, 2002; Żuchowski et al, 2009). Indeed, FA and TPC showed a strong positive correlation (r = 0.903, $p \leq 0.001$), and it was shown that both gallic and ferulic acid can be used as a calibration standard in total phenolic assays. Content of FA is shown in Figure 1. Similarly to TPC, content of FA in analysed milling products was the highest in organic wheat bran (3015 mg kg^{-1}), and among whole grain flour types, organic corn flour was the richest in FA. Similarly, previous studies showed corn to contain more FA than other cereals (Adom and Liu, 2002). Matilla et al (2005) reported that ferulic acid and dehydrodimers accounted for 63 and 15%, respectively, of phenolic acids in corn flour. Bound ferulic acid makes up the greatest percentage (98%) of the ferulic acid in corn (Matilla et al, 2005).

The lowest content of FA was determined in white and semi-white wheat flour (< 200 mg kg^{-1}). The detected amounts are in accordance with the study of Matilla et al (2005) who
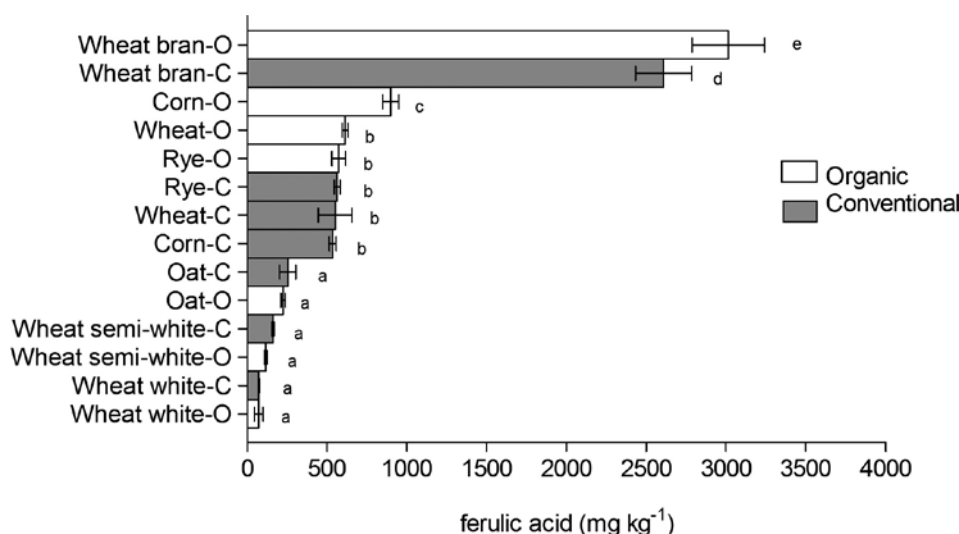

Figure 1. *Ferulic acid content of organic (O) and conventional (C) wheat bran and flour samples, in ascending order, expressed as mg kg^{-1} dry basis. Bars represent means of three determinations ± SD. Different letters indicate significant difference between samples (p ≤ 0.05).*

Table 3. *Lignan composition of wheat bran and flour samples expressed as µg kg⁻¹ dry basis, in ascending total lignan order. Values are means of six determinations. Means in columns followed by different letters are significantly different (p ≤ 0.05)*

	SECO	LARI	PINO	SYR	TOTAL
Wheat white - C	25.10ᵃ ± 6.36	30.80ᵃ ± 17.29	36.37ᵃ ± 10.58	235.42ᵃ ± 118.23	327.70ᵃ ± 141.21
Wheat white - O	62.75ᵃ ± 3.19	201.40ᵃ ± 24.04	47.39ᵃ ± 14.95	132.98ᵃ ± 52.29	444.52ᵃ ± 53.46
Wheat semi-white - C	33.81ᵃ ± 10.27	78.36ᵃ ± 45.35	59.33ᵃ ± 38.53	500.31ᵃᵇ ± 118.87	671.80ᵃ ± 210.15
Wheat semi-white - O	75.27ᵃ ± 8.51	213.45ᵃ ± 31.00	203.34ᵃ ± 44.17	212.13ᵃ ± 22.66	704.39ᵃ ± 63.92
Corn - C	207.79ᵇ ± 15.72	nd	74.42ᵃ ± 29.82	453.60ᵃᵇ ± 60.12	735.81ᵃ ± 97.80
Corn - O	232.53ᵇᶜ ± 70.33	nd	129.15ᵃ ± 86.30	841.48ᵇ ± 97.58	1203.17ᵃ ± 163.34
Wheat - C	583.57ᵈ ± 10.44	858.39ᶜ ± 38.91	981.28ᵇᶜ ± 226.45	1130.71ᶜ ± 143.00	3553.96 ᵇ ± 252.98
Wheat - O	287.80ᶜ ± 39.21	1440.05ᵈ ± 160.21	1010.39ᵇᶜ ± 163.36	1144.40ᶜ ± 194.74	3882.64ᵇ ± 358.00
Rye - C	nd	576.95ᵇ ± 91.14	692.66ᵇᶜ ± 183.41	2576.52ᵈ ± 434.85	3846.14ᵇ ± 500.31
Oat - C	nd	918.58ᶜ ± 208.10	3271.10ᵈ ± 553.31	983.86ᵇᶜ ± 169.33	5173.54ᶜ ± 908.32
Wheat bran - C	284.35ᶜ ± 23.83	1883.61ᵉ ± 266.91	1267.80ᶜ ± 99.39	3543.95ᵉ ± 322.00	6979.71ᵈ ± 507.33
Rye - O	nd	1832.57ᵉ ± 174.22	2970.74ᵈ ± 154.09	3526.57ᵉ ± 389.94	8329.88ᵉ ± 681.15
Oat - O	nd	2639.56ᶠ ± 127.76	6845.43ᵉ ± 157.72	579.02ᵇ ± 42.00	10064.01ᶠ ± 281.67
Wheat bran - O	1051.58ᵉ ± 40.13	6416.88ᵍ ± 226.74	2952.03ᵈ ± 205.59	3255.35ᵉ ± 237.26	13675.84ᵍ ± 572.99

SECO - secoisolariciresinol; LARI - lariciresinol; PINO - pinoresinol; SYR - syringaresinol; nd - not detected
O - organic; C - conventional

reported FA (mg kg⁻¹): 860, 890, 100 - 120; 3000; 250; 380 for rye flour; wheat whole grain, wheat white flour; wheat bran; oat and corn flour respectively; as well as with other studies (Vitaglione et al, 2008).

Organic wheat bran (3015.2 mg kg⁻¹) had a significantly higher ($p ≤ 0.05$) content of FA compared to its conventional counterpart (2609.5 mg kg⁻¹), while the great difference between organic and conventional corn flour was probably related to different dietary fibre content. Gasztonyi et al (2011) determined that FA content of wheat varieties was in range 230 - 550 mg kg⁻¹. Without fungicide treatment those levels were little lower, but they finally concluded that climate factors could have stronger impact on phytochemical concentration than the production method itself. Similarly, Żuchowski et al (2009) examined phenolic acid content in winter wheat and concluded that organic agriculture leads just to a small insignificant increase in phenolic acids content.

FA content, similarly to TPC, strongly positively correlated with the amount of TDF ($r = 0.965$, $p ≤ 0.001$) and IDF ($r = 0.977$, $p ≤ 0.001$) while no correlation was established with soluble fibre. This is in accordance with Bunzel et al (2001) who investigated dimers of ferulic acid in cereals and concluded that they are more abundant in IDF, that is, that they contribute to the cross-linking of polysaccharides which probably results with production of insoluble fibres.

Antioxidant Activity

Phenolics such as ferulic acid exhibit antioxidant activity both *in vitro* and *in vivo* (Fardet et al, 2008). Figure 2 shows AO of extracted phenolics of different cereal samples analysed with DPPH and FRAP method. Although both methods measure AO, results are probably not consistent due to interference of extracts with reaction systems and different action mechanism; similarly as shown in previous study (Yu et al, 2002).

Nonetheless, with both methods the strongest AO activity for wheat bran was observed. White wheat flour, mostly used in an average everyday diet, had the lowest AO activity. Adom and Liu (2002) determined AO activity of bound grain phenolics to be highest in corn flour and decline in following order: corn > wheat > oat. Similar order for organic whole grain flours was established, but conventionally produced whole grain wheat had stronger AO compared with the corn from the same farming system.

In this work two different assays to evaluate AO activity were used. FRAP method showed organic wheat bran to possess stronger AO activity than conventionally produced ones, whereas DPPH method showed the opposite - conventionally produced bran had slightly but insignificantly higher AO activity. The biggest difference in results between these two methods was found for organic oat flour. The reason for this could derive from specific antioxidants in oat, such as avenanthramides and their reaction with antioxidant assay reagents. In general, AO assays work on different chemical principles and it is recommended to conduct multiple assays in order to cover a wider range of action of present food antioxidants (Apak et al, 2013), as well as not to make misleading conclusions since antioxidant activity depends on the conditions of the used test. Here, although strong correlation ($r > 0.85$, $p ≤ 0.001$) between used antioxidant assays was determined, FRAP determined AO activity had a stronger correlation with TPC and FA ($r > 0.97$, $p ≤ 0.05$), compared to of DPPH test ($r > 0.85$, $p ≤ 0.001$). Similarly, FRAP results had a stronger correlation with insoluble fibre and ash content than DPPH results. It was shown that antioxidant activity assay based on the scavenging of free radical such as DPPH• does not use biologically relevant radical (Apak et al, 2013), while FRAP uses chelated ferric ion as oxidising agent.
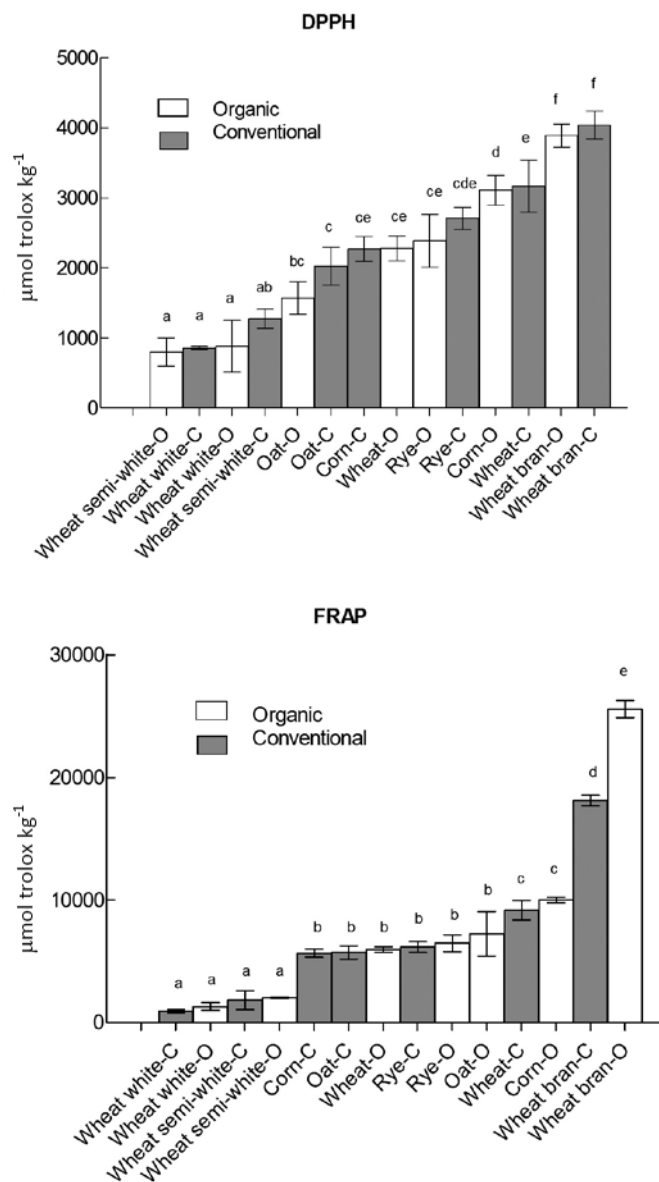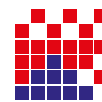
Figure 2. *Antioxidant activity of organic (O) and conventional (C) wheat bran and flour samples determined by DPPH and FRAP method, in ascending order, expressed as μmol trolox kg⁻¹ dry basis. Bars represent means of three determinations ± SD. Different letters indicate significant difference between samples (p ≤ 0.05).*

Lignan Content

Analytical data on lignan content in food is crucial to determine its exposure through diet. Table 3 shows lignan content of analysed cereal samples. Detected lignans were secoisolariciresinol (SECO), lariciresinol (LARI), pinoresinol (PINO) and syringaresinol (SYR) in different amounts, depending on the sample analysed. Generally, SECO contributed the least to overall lignan content. The highest amount of SECO and LARI was found in wheat bran, both organic and conventional. Oat flour was richest in PINO but did not contain SECO. SYR was dominant lignan in rye flour.

White wheat flour had a very low total lignan content compared to eight times higher amount in whole grain wheat flour (Table 3), which is in accordance with previous studies (Durazzo et al, 2013). The highest amount of total lignans in flour was detected in organic oat and rye flour (Table 3). Detected amount of lignans is similar to results of previous studies (Durazzo et al, 201; Smeds et al, 2009; Smeds et al, 2012) with some discrepancy most probably caused as a consequence of different varieties in agricultural and growth conditions (Smeds et al, 2009). Most previous studies examined lignan content in grains (Durazzo et al, 2013) while in this study lignan content of different flours was determined, and as that the data can be used to extend lignan database. It is interesting to notice that all organics had higher total lignan content compared to their conventional counterpart (Table 3). This was significant ($p \leq 0.05$) for wheat bran, rye and oat flour. These results are worth to be further examined since there are no studies comparing lignan content in different organic and conventional grains (Barański et al, 2014).

Total lignans correlated significantly positively with FRAP antioxidant activity but weaker than other bioactives. Total lignan content was weakly correlated to TDF (r = 0.738, $p \leq 0.01$) and IDF (r = 0. 0.703, $p \leq 0.01$). On the other hand, among all tested bioactive compounds only lignans, specifically SYR, showed some positive correlation with SDF content. To our knowledge there are no studies investigating direct association between dietary fibre and lignans but it has been shown that lignin, component of dietary fibre, could be precursor of dietary lignans. Nevertheless, gravimetric method for dietary fibre analysis determines only fractions of lignin. Studies have observed positive correlations between TDF and lignan excretion in organism (Bartkiene et al, 2011), although some of them showed weak correlation coefficients (0.46) between intake of lignans and DF (Mileder et al, 2005). This study indicates that lignans could have different relationship patterns to DF compared to more investigated FA and TPC, which further raises questions of their occurrence and metabolism in digestive tract. Also it confirms conclusions of previous study (Bartkiene et al, 2011) on the importance of knowledge on possible correlations between lignans and DF composition in cereal products.

CONCLUSION

In this study antioxidant activity, lignans, total phenolics, ferulic acid and fibre content of different milling products were quantified, aiming to contribute to the cereal bioactives databases. Focus of the research was also to see to what extent consumers can expect organic cereal products to have higher content of bioactives compared to conventional ones. Finally the study tried to establish the relationship between determined bioactives of milling products. Content of bioactive compounds in whole grain flours or bran itself is higher than in refined ones. Some differences in bioactives amount are most likely related to the cereal specie and production process. Chosen milling products cannot be with significance compared from the point of farming system, as samples were randomly taken from the market, but it is worth mentioning that consistent difference favouring organic production was established for lignans. It should be further investigated if lignan content could be significantly related to the farming system, as there

are no studies related to this topic. Other examined parameters were higher only in some cases in organic milling products.

Antioxidant activity of milling products showed to be directly related to the contents of phenolic acids while lignans weaker contribute to antioxidant activity, and are not strongly related to fibre content as other phenolic compounds. Data related to ferulic acid, antioxidant activity, and total phenolic content were logical continuation of previous studies, showing cereals to be rich source of phenolics and antioxidants, ant that was especially shown for corn flour which is frequently used for bread making in Croatia. Still, wheat flour is the most popular cereal milling product, and the advantage should be given to types with higher extraction rate. Whole grain oat and rye flour usage should be recommended for their lignan and soluble fibre content providing further health benefits, while more advantage should be taken from bran, which is mostly considered as a milling by-product.

Results from this study should give a push to further research, as data on cereal bioactives from organic and conventional production is still lacking, especially the one related to lignans and their relation to dietary fibre.

ACKNOWLEDGMENT

LITERATURE

Adom K.K., Liu R.H. (2002) Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, 50, 6182-6187.

AOAC. (2006) "Official methods of analysis of AOAC". AOAC International, Gaithersburg

Apak R., Gorinstein S., Böhm V., Schaich K.M., Özyürek M., Güçlü K. (2013) Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure and Applied Chemistry*, 85(5), 957-998.

Barański M., Srednicka-Tober D., Volakakis N., Seal C., Sanderson R., Stewart G.B., Benbrook C., Biavati B., Markellou E., Giotis C., Gromadzka-Ostrowska J., Rembiałowska E., Skwarło-Sońita K., Tahvonen R., Janovská D., Niggli U., Nicot P., Leifert C. (2014) Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *British Journal of Nutrition*, 112(05), 794-811.

Bartkiene E., Juodeikiene G., Basinskiene L., Liukkonen K.H., Adlercreutz H., Kluge H. (2011) Enterolignans enterolactone and enterodiol formation from their precursors by the action of intestinal microflora and their relationship with non-starch polysaccharides in various berries and vegetables. *LWT-Food Science and Technology,* 44(1), 48-53.

Belšćak A., Komes D., Horžić D., Ganić K.K., Karlović D. (2009) Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Research International*, 42(5), 707-716.

Bunzel M., Ralph J., Marita J.M., Hatfield R.D., Steinhart H. (2001) Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture*, 81(7), 653-660.

Cotterchio M., Boucher B.A., Kreiger N., Mills C.A., Thompson L.U. (2008) Dietary phytoestrogen intake - lignans and isoflavones - and breast cancer risk (Canada). *Cancer Causes & Control*, 19(3), 259-272.

Čukelj N., Jakasa I., Sarajlija H., Novotni D., Ćurić D. (2011) Identification and quantification of lignans in wheat bran by gas chromatography-electron capture detection. *Talanta*, 84, 127-132.

Dimberg L.H., Gissén C., Nilsson J. (2005) Phenolic compounds in oat grains (Avena sativa L.) grown in conventional and organic systems. *AMBIO*. 34(4): 331-337.

Durazzo A., Zaccaria M., Polito A., Maiani G., Carcea M. (2013) Lignan Content in Cereals, Buckwheat and Derived Foods. *Foods,* 2(1), 53-63.

Fardet A., Edmond Rock, E., Rémésy, C. (2008) Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected in vivo. *Journal of Cereal Science,* 48, 258-276.

Fares C, Codianni P., Menga V. (2012) Effects of organic fertilization on quality and antioxidant properties of hulled wheats. *Italian Journal of Food Science*, 24(2), 188-193.

Gasztonyi M.N., Farkas R.T., Berki M., Petróczi I.M., Daood H.G. (2011) Content of phenols in wheat as affected by varietal and agricultural factors. *Journal of Food Composition and Analysis*, 24(6), 785-789.

Hansen H.B., Rasmussen C.V., Bach Knudsen K.E., Hansen, Å. (2003) Effects of genotype and harvest year on content and composition of dietary fibre in rye (Secale cereale L) grain. *Journal of the Science of Food and Agriculture*, 83(1), 76-85.

IOOC - International Olive Oil Council. 2009 COI/T.20/Doc.29 - Determination of biophenols in olive oils by HPLC.

Landete J.M. (2012) Plant and mammalian lignans: A review of source, intake, metabolism, intestinal bacteria and health. *Food Research International,* 46(1), 410-424.

Langenkämper G., Zörb C., Seifert M., Mäder P., Fretzdorff B., Betsche T. (2006) Nutritional quality of organic and conventional wheat. *Journal of Applied Botany and Food Quality*, 80(2), 150-154.

Li L., Harflett C., Beale, M.H., Ward J.L. (2009) Phenolic acids in Analysis of bioactive components in small grain cereals. In: Shewry P.R., Ward J.L. (Ed) Healthgrain methods, p. 41-52. Harpenden, UK.

Liu R.H. 2007. Whole grain phytochemicals and health. *Journal of Cereal Science,* 46, 207-219.

Lv J., Lu Y., Niu Y., Whent M., Ramadan M.F., Costa J., Yu L.L. (2013) Effect of genotype, environment, and their interaction on phytochemical compositions and antioxidant properties of soft winter wheat flour. *Food Chemistry*, 138(1), 454-462.

Mäder P., Hahn D., Dubois D., Gunst L., Alföldi T., Bergmann H., Oehme M., Amadò R., Schneider H., Graf U., Velimirov A., Fließbach A., Niggli U. (2007) Wheat quality in organic and conventional farming: results of a 21 year field experiment. *Journal of the Science of Food and Agriculture*, 87(10), 1826-1835.

Mattila P., Pihlava J.M., Hellström J. (2005) Contents of phenolic acids, alkyl-and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, 53(21), 8290-8295.
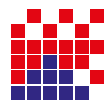
Milder I.E., Feskens E.J., Arts I.C., de Mesquita H.B.B., Hollman, P.C., Kromhout D. (2005) Intake of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in Dutch men and women. *The Journal of Nutrition*, 135(5), 1202-1207.

Picolli da Silva, L., de Lourdes Santorio Ciocca, M. (2005) Total, insoluble and soluble dietary fiber values measured by enzymatic-gravimetric method in cereal grains. *Journal of Food Composition and Analysis*, 18(1), 113-120.

Ragaee S., Abdel-Aal E.S.M., Noaman, M. (2006) Antioxidant activity and nutrient composition of selected cereals for food use. Food Chemistry, 98(1), 32-38.

Saura-Calixto F. (2010) Dietary fibre as a carrier of dietary antioxidants: an essential physiological function. *Journal of Agricultural and Food Chemistry*, 59(1), 43-49.

Smeds A.I., Jauhiainen L., Tuomola, E., Peltonen-Sainio P. (2009) Characterization of variation in the lignan content and composition of winter rye, spring wheat, and spring oat. *Journal of Agricultural and Food Chemistry*, 57(13), 5837-5842.

Smeds A.I., Eklund P.C., Willför S.M. (2012) Content, composition, and stereochemical characterisation of lignans in berries and seeds. *Food Chemistry*, 134, 1991-1998.

Verma B., Hucl P., Chibbar R.N. (2008) Phenolic content and antioxidant properties of bran in 51 wheat cultivars. *Cereal Chemistry*, 85(4), 544-549.

Vitaglione P., Napolitano A., Fogliano V. (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends* in *Food Science & Technology,* 19(9), 451-463.

Yu L., Perret J., Davy B., Wilson J., Melby C.L. (2002) Antioxidant properties of cereal products. *Journal of Food Science*, 67(7), 2600-2603.

Żuchowski J., Kapusta I., Szajwaj B., Jończyk K., Oleszek W. (2009) Phenolic acid content of organic and conventionally grown winter wheat. *Cereal Research Communications*, 37(2), 189-197.