The effect of mechanical processing on avenanthramide and phenol levels in two organically grown Italian oat cultivars

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Abstract Avenanthramides (AVNs), free and bound phenols and their antioxidant capacities (ORAC) were evaluated in two *Avena sativa* L. cultivars, Donata and Flavia. The cultivars (cvs.) were grown in loamy and medium texture soils and assessed after industrial dehulling and milling. Total dietary fiber, β -glucan, starch and proteins were also evaluated. Cv. Donata showed 2.8 fold higher AVN storage as compared to cv. Flavia, which was linked with genotype. The accumulation of AVN content was also influenced by the texture of the soil. Dehulling resulted in a 75 and 37% AVN decrease in cv. Donata and Flavia, respectively. The dehulled grains of cv. Donata showed 40% reduction in free phenolic content, whereas the dehulled grains of both cvs. showed 67% reduction in bound phenols. Milling affected the bound phenolics and their antioxidant capacity. Cv. Flavia showed 1.3 fold higher β -glucan than that of cv. Donata. Total dietary fiber was reduced by 50 and 12% after dehulling and milling, respectively, while marginal changes in proteins were observed after milling. The results suggest that the choice of genotype and the kind of dehulling processes that are employed are essential considerations in the production of oat-based products with high AVN content and extra health benefits.

Keywords *Avena sativa* L. · Avenanthramides · β -Glucan · Dehulling · Milling · Polyphenols

Abbreviations

AVNs	Avenanthramides
BPs	Bound polyphenols
CAE	Caffeic acid equivalents
cvs.	Cultivars
d.w.	Dry weight
EFSA	European Food Safety Authority
FAE	Ferulic acid equivalents
FPs	Free polyphenols
ORAC	Oxygen radical absorbance capacity
SD	Standard deviation
TE	Trolox equivalents
TPs	Total polyphenols
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman 2-carboxylic acid
2c	<i>N</i> -(3,4-Dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid
2p	<i>N</i> -(4-Hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid
2f	<i>N</i> -(4-Hydroxy-3-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid

Introduction

Epidemiological investigations and dietary intervention trials have underscored the role of wholegrain consumption in decreasing the risk of major non-communicable diseases (Cho et al. 2013; Ye et al. 2012).

Oats (*Avena sativa* L., *Poaceae*) have an advantage over other cereals because they are often consumed as whole grains in oat-based products (van der Kamp et al. 2014).

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Moreover, from a nutritional standpoint, oats contain several nutrients, namely β -glucan, avenanthramides (AVNs), polyphenols, tocopherols and sterols, which have many potential health benefits (Clemens and van Klinken 2014; Singh et al. 2013).

Oat β -glucan has a pre-biotic role and improves the blood lipid and glycaemic profiles (Wang and Ellis 2014), whereas oat phenols act as antioxidants and cell signaling regulators (Masisi et al. 2016). Phenols are present in free (FPs) and bound (BPs) forms, and their antioxidant capacity can be evaluated by the Oxygen Radical Absorbance Capacity (ORAC) method (Antonini et al. 2016; Ninfali et al. 2005). Oat phenols are primarily located in the outer layers of the karyopsis and thus affected by processing (Panato et al. 2017; Peterson 2001).

Oat avenanthramides (AVNs) are a group of hydroxycinnamoylanthranilate alkaloids (Collins 1989) which possess antioxidant, anti-inflammatory, anticancer and anti-itching activities (Meydani 2009). Three main AVNs are generally assessed in oats: 2p, 2c, and 2f. They derive from 5-hydroxyanthranilic acid, which is linked to the *p*-coumaric (2p), caffeic (2c) and ferulic (2f) acids (Bratt et al. 2003). AVNs have been found in oat groats, hulls, bran and leaves (Yang et al. 2014), and environmental factors, genotype and their interactions strongly influence the AVN biosynthesis (Redaelli et al. 2016).

An important step in oat processing is dehulling, which is greatly influenced by pressure applied during dehulling and moisture content in grains (Kaur et al. 2014).

To our knowledge, little research has been done on the effect of industrial dehulling and milling on AVNs, FPs and BPs and their antioxidant capacities in different genotypes, grown in different soils (Rasane et al. 2015). Therefore, the aim of this study was to investigate the AVN content and ORAC in two husked oat cultivars (cvs.), grown in two different soils, from harvesting to milling. We also compared the effects of mechanized and manual dehulling and assessed the effect of industrial processing on β -glucan, total dietary fiber, protein and starch.

Materials and methods

Oat samples

Avena sativa L. (cv. Donata and Flavia) was provided by Terra Bio Soc., Schieti di Urbino (The Marches, Italy) and organically grown in two different soils: a loamy soil, located in Urbino (Italy, 43°43'34"N; 12°38'10"E) and a medium texture soil, located in Siena (Italy, 43°20'27"N; 11°2'38"E). The climatic conditions in the two experimental sites during the years 2012–2013 were similar: the mean temperature was 12.4 °C in Urbino versus 14 °C in

Siena; the mean rainfall was 880 mm in Urbino versus 900 mm in Siena.

The samples were labeled as follows: D1, cv. Donata in loamy soil; D2, cv. Donata in a medium texture soil; F1, cv. Flavia in loamy soil; F2, cv. Flavia in a medium texture soil.

D1 and F1 were sown in Urbino on September 25, 2012 and harvested at complete maturity, on July 10, 2013, with an average yield of 4.2 ± 0.2 t ha⁻¹; D2 and F2 were sown in Siena on October 20, 2012 and harvested at complete maturity, on August 30, 2013, with an average yield of 3.5 ± 0.1 t ha⁻¹. Harvesting was carried out by a threshing machine and the moisture content of raw grains at harvest was: $10.6 \pm 0.2\%$ for cv. Donata and $10 \pm 0.2\%$ for cv. Flavia. The agronomic characteristics of the soils are provided in Supplementary 1. Both soils were fertilized with the same amount of Guanito, a manure allowed by the organic regulatory system (European Commission 2007).

Dehulling and milling

Conditioned oat grains were dehulled with an industrial dehuller operating at an appropriate speed to yield an acceptable percentage of unbroken groats. The hulls accounted for $30 \pm 2\%$ of the groats. After industrial dehulling, the moisture content of the groats was: 10.0 ± 0.2 for the cv. Donata and 9.4 ± 0.1 for the cv. Flavia. The dehulled groats were sorted and packaged in 25 kg paper bags, then transferred to a stone mill, operating at 131 rpm, with a final flour yield of $75 \pm 1\%$ and a moisture content of $10.0 \pm 0.2\%$ (Prometeo S.r.l., Urbino, Italy).

Since the hulls were lost after the industrial dehulling process, we dehulled 20 g of D1 and F1 oat samples by hand to assess the total phenol (TP) and AVN content in both hulls and groats.

Chemicals

Folin–Ciocalteu's reagent, caffeic acid, ferulic acid, formic acid, sodium carbonate, 2,2'-azobis (2-amidinopropane) dihydrochloride, 6-hydroxy-2,5,7,8-tetramethylchroman 2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, USA). Ethanol, hexane, ethylacetate, acetonitrile were purchased from VWR International (Radnor, USA).

Chemical analyses

Starch, β -glucan and total dietary fiber were assayed using diagnostic kits from Megazyme International Ireland Ltd., following the procedures described by the American Association of Cereal Chemists (AACC), methods 70-13,

32-23, 32-05, respectively. Proteins were determined by analyzing the total nitrogen content using the Dumas method (AOAC, 999.13) and a LECO FP-428. The factor of 6.25 was used to convert total nitrogen to protein content. All chemical values were expressed as g 100 g⁻¹, on dry weight (d.w.). Moisture content was determined using the AACC method 44-16.

Extraction and detection of free (FPs) and bound (BPs) polyphenols

FPs were extracted following the procedure of Wise (2011), whereas BPs were extracted using the procedure described by Verardo et al. (2011). FPs and BPs were determined using the Folin–Ciocalteu method (Singleton et al. 1999). Based on the standard curve prepared with caffeic acid, the amount of FPs and BPs was calculated and expressed as mg of caffeic acid equivalents g⁻¹ d.w.

ORAC analysis

Antioxidant activity was assessed using the ORAC assay (Oxygen Radical Absorbance Capacity), as described by Ninfali et al. (2005) using a Fluostar Optima plate reader fluorimeter (BMG Labtech, Offenburgh, Germany), equipped with a temperature-controlled incubation chamber and automatic injection pump. A calibration curve was constructed each time with the standard Trolox; hence, the ORAC values were expressed as μmol of Trolox Equivalents (TE) g⁻¹ d.w.

HPLC–UV–MS analysis of AVNs

The ethanolic extract containing the FPs was filtered before analysis (Iso-Disc Filters, PFTE-4-4; 4 mm $\times$ 0.45 μm ; Supelco, Bellefonte, USA) and directly analyzed in a Water instrument equipped with Alliance HT 2795 High Performance Liquid Chromatography (HPLC), 2996 Photo Diode Array (PDA) and Micromass LC/MS ZQ 2000 detector, following the known procedure with some modifications (Jastrebova et al. 2006; Wise 2011). A C18 column, LiChroCART[®] (250 $\times$ 4 mm), with a particle size of 5 μm , was used. The mobile phase that was used consisted of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B). The gradient was changed as follows: 0–5 min 2.4% A (isocratic), 5–20 min to 24% A, 20–38 min to 40% A, 38–50 min to 75% A. The total running time was 50 min. The injected sample volume was 50 μL , and the flow rate was 0.8 mL min⁻¹. UV spectra were recorded from 220 to 420 nm, whereas the chromatograms were registered at 330 nm. Electrospray ionization (ESI) was operated in positive and negative ion mode in a range of 150–370 amu. Capillary voltage was set at 3 kV, source

temperature at 100 °C and desolvation temperature at 300 °C. The cone and desolvation nitrogen gas flows were 50 and 500 L h⁻¹, respectively. Data were processed using MassLynx 4.1 (Waters, Milford, MA). To identify AVNs in the HPLC chromatograms, retention time, UV spectra, MS ESI(+) and ESI(–) spectra were compared with data in literature (Jastrebova et al. 2006). Quantification was performed by external standard calibration and concentrations were expressed as μg of ferulic acid equivalents g⁻¹ d.w. (Antonini et al. 2016).

Microscopic analysis

The dehulled groats and hulls were analyzed by means of an optical stereomicroscope at 10 $\times$ and reproductions were performed by computer analysis.

Statistical analysis

All chemical analyses were performed in triplicate, and the results were reported as the mean value $\pm$ standard deviation (SD). Polyphenol and AVN concentrations were measured in triplicate, and the results were reported as mean $\pm$ SD. Antioxidant capacity was established by eight independent determinations for each sample; each value was the mean $\pm$ SD. Linear regression analysis was performed using Microsoft Excel[®] software; statistical significance was tested using the Student's *t* test with a $p \leq 0.05$; two-way ANOVA was carried out to study the effect of genotype, soil and their interaction on antioxidant and macronutrient levels, using the SPSS[®] 17.0 software (SPSS Inc., IBM, Chicago, USA).

Results

Effect of genotype and soil type on antioxidants level

The effect of different genotypes and soils on the total AVNs, total phenols and total antioxidant capacity (ORAC) of the four husked raw grains is shown in Fig. 1a–c, respectively.

The total AVN content in Donata raw grains was about 2.8 fold higher than that of cv. Flavia (Fig. 1a). Figure 1a shows that cv. Donata, sown in the loamy soil (D1), had a significantly higher AVN concentration (+17%) than the same cv. sown in the medium texture soil (D2), whereas cv. Flavia had a significantly higher AVN concentration in the medium texture soil (F2 > F1; +30%).

Total phenols did not show any statistically significant variations among the soils or cultivars (Fig. 1b). On the contrary, the ORAC values of cv. Flavia were 30% lower from cv. Donata (Fig. 1c). Within the same cv., no

Fig. 1 Effect of different genotype and soil on total AVNs (a), total phenols (b) and total ORAC (c) of raw grains.^{a, d} Different letters indicate significant differences ($p \leq 0.05$) due to genotype (D1 vs. F1; D2 vs. F2) or soil type (D1 vs. D2; F1 vs. F2)

statistically significant difference was observed for the ORAC values in the two soils (Fig. 1c).

To better describe the difference in AVN concentrations in the two cvs. we assayed the AVNs by distinguishing the three main forms: 2c, 2p and 2f (Table 1).

Donata grains showed the relative percentages of 2c, 2p, and 2f AVNs of 26, 37, 37%, respectively (Table 1); whereas, Flavia grains showed the storage of 2c, 2p and 2f AVNs of 22, 40, 38%, respectively.

In the raw Donata grains, FPs and BPs were in equal concentration. On the contrary, in the raw Flavia grains, BPs were significantly higher than FPs. Nevertheless, the

ORAC values of FPs were significantly higher than those of BPs in all samples (Table 1).

Supplementary 2 shows results of analysis of variance (F values) for the raw grains of the two oat cvs. The genotype effect was highly significant ($p \leq 0.001$) for most of the antioxidants i.e., individual (2c, 2p, 2f) and total AVNs, FPs, BPs, ORAC of FPs, and total ORAC (FPs + BPs). In addition, the soil effect was significant ($p \leq 0.05$) for the accumulation of FPs and for their ORAC. The effect of the interaction, soil $\times$ genotype, was highly significant ($p \leq 0.001$) for individual (2c, 2p, 2f) and total AVNs and for the accumulation of BPs (Supplementary 2).

Effect of processing on antioxidant levels

Changes in individual AVNs, free and bound phenols and their respective ORAC values were evaluated along the processing chain, from grain to flour (Table 1).

Dehulling markedly decreased the three AVN forms (2c, 2p, 2f) in all samples. Cv. Donata showed the highest average AVN losses (75%), while average AVN losses in cv. Flavia were lower (30% for F1; 45% for F2). Milling did not change AVN concentrations in the three forms in either of the cvs. (Table 1). Dehulling reduced FPs by 40% in both Donata dehulled groats, whereas FPs remained stable in both Flavia dehulled groats; BPs in all samples were dramatically reduced by 67%. ORAC values showed the same pattern of reduction as the corresponding phenol fractions (Table 1).

Milling did not affect FPs and their antioxidant capacities in any of the samples. However, it further reduced BPs, with reductions ranging from 25 to 40% in both cvs. with a consequent drop in the ORAC values of BPs to one half of the values found in the dehulled groats (Table 1).

Figure 2 shows the total phenols and total AVNs of the D1 (Fig. 2a) and F1 (Fig. 2b) grains dehulled by hand compared to those that were dehulled using industrial processes. After the industrial dehulling, total phenols showed 32 (D1) and 37% (F1) decay compared to manual dehulling. Moreover, total AVNs showed a 60 and 29% reduction in D1 and F1, respectively when the two kind of dehulling were compared.

Figure 2 also shows images of hulls (Fig. 2c), oat groat after manual dehulling (Fig. 2d) and industrial dehulling (Fig. 2e). It can be observed that after manual dehulling, the groat was still enveloped by the natural fluff, whereas after industrial dehulling, the surface appeared worn and brighter.

The total AVNs in hand hulled fractions were: 130 ± 10 and $90 \pm 8 \mu\text{g g}^{-1} \text{ d.w.}$ for D1 and F1, respectively. The total phenols were: 11.6 ± 1.0 and $9.25 \pm 0.87 \text{ mg g}^{-1} \text{ d.w.}$ for D1 and F1, respectively.

Table 1 Effect of processing on antioxidant levels

Parameters	Processing	D1	D2	F1	F2
AVNs ($\mu\text{g g}^{-1}$ d.w.)					
2c	Raw grain	282.6 $\pm$ 21.0 ^{Aa}	246.4 $\pm$ 11.1 ^{Ba}	68.5 $\pm$ 6.3 ^{Ca}	100.6 $\pm$ 5.6 ^{Da}
	Dehulled groat	55.3 $\pm$ 6.8 ^b	37.8 $\pm$ 5.9 ^b	45.7 $\pm$ 5.2 ^b	44.7 $\pm$ 6.5 ^b
	Flour	63.0 $\pm$ 5.6 ^b	40.4 $\pm$ 5.0 ^b	58.6 $\pm$ 7.0 ^b	50.8 $\pm$ 6.7 ^b
2p	Raw grain	403.7 $\pm$ 36.1 ^{Aa}	333.3 $\pm$ 14.4 ^{Ba}	123.4 $\pm$ 14.2 ^{Ca}	171.7 $\pm$ 11.7 ^{Da}
	Dehulled groat	106.1 $\pm$ 11.3 ^b	92.9 $\pm$ 10.5 ^b	84.8 $\pm$ 4.8 ^b	95.7 $\pm$ 8.2 ^b
	Flour	114.4 $\pm$ 10.8 ^b	112.3 $\pm$ 10.1 ^b	85.7 $\pm$ 7.0 ^b	113.6 $\pm$ 11.0 ^b
2f	Raw grain	417.4 $\pm$ 46.9 ^{Aa}	340.1 $\pm$ 7.7 ^{Ba}	113.7 $\pm$ 16.2 ^{Ca}	168.3 $\pm$ 17.2 ^{Da}
	Dehulled groat	101.4 $\pm$ 6.5 ^b	82.0 $\pm$ 7.2 ^b	83.6 $\pm$ 1.7 ^b	97.6 $\pm$ 8.6 ^b
	Flour	113.1 $\pm$ 11.5 ^b	95.9 $\pm$ 10.5 ^b	93.7 $\pm$ 11.7 ^b	116.1 $\pm$ 11.7 ^b
Polyphenols (mg g^{-1} d.w.)					
FPs	Raw grain	1.89 $\pm$ 0.10 ^{Aa}	1.99 $\pm$ 0.11 ^{Aa}	1.27 $\pm$ 0.10 ^{Ba}	1.49 $\pm$ 0.13 ^{Ba}
	Dehulled groat	1.05 $\pm$ 0.08 ^b	1.29 $\pm$ 0.10 ^b	1.24 $\pm$ 0.09 ^a	1.27 $\pm$ 0.10 ^a
	Flour	1.11 $\pm$ 0.06 ^b	1.30 $\pm$ 0.03 ^b	1.23 $\pm$ 0.03 ^a	1.28 $\pm$ 0.05 ^a
BPs	Raw grain	2.02 $\pm$ 0.15 ^{Aa}	2.24 $\pm$ 0.28 ^{Aa}	2.64 $\pm$ 0.18 ^{Ba}	2.71 $\pm$ 0.32 ^{Ba}
	Dehulled groat	0.73 $\pm$ 0.06 ^b	0.77 $\pm$ 0.05 ^b	0.71 $\pm$ 0.06 ^b	0.76 $\pm$ 0.05 ^b
	Flour	0.55 $\pm$ 0.03 ^c	0.48 $\pm$ 0.07 ^c	0.43 $\pm$ 0.09 ^c	0.56 $\pm$ 0.07 ^c
Antioxidant capacity ($\mu\text{mol TE g}^{-1}$ d.w.)					
ORAC of FPs	Raw grain	195.3 $\pm$ 13.0 ^{Aa}	186.2 $\pm$ 13.6 ^{Aa}	122.7 $\pm$ 9.3 ^{Ba}	100.0 $\pm$ 9.4 ^{Ca}
	Dehulled groat	122.9 $\pm$ 8.8 ^b	128.5 $\pm$ 11.7 ^b	114.8 $\pm$ 10.6 ^a	111.2 $\pm$ 3.0 ^a
	Flour	135.2 $\pm$ 5.4 ^b	129.7 $\pm$ 7.5 ^b	129.3 $\pm$ 8.7 ^a	120.7 $\pm$ 8.4 ^a
ORAC of BPs	Raw grain	60.8 $\pm$ 2.6 ^{Aa}	46.5 $\pm$ 4.8 ^{Ba}	46.3 $\pm$ 1.7 ^{Ba}	66.2 $\pm$ 2.8 ^{Ca}
	Dehulled groat	31.9 $\pm$ 7.9 ^b	35.3 $\pm$ 3.1 ^b	38.1 $\pm$ 2.8 ^b	36.6 $\pm$ 3.0 ^b
	Flour	15.4 $\pm$ 1.1 ^c	17.8 $\pm$ 1.0 ^c	13.7 $\pm$ 1.5 ^c	12.0 $\pm$ 1.1 ^c

Each value is shown as mean $\pm$ standard deviation and is expressed on dry weight (d.w.)

AVNs avenanthramides, D1 cv. Donata in loamy soil; D2 cv. Donata in medium texture soil, F1 cv. Flavia in loamy soil, F2 cv. Flavia in medium texture soil, FPs free phenols, BPs bound phenols, ORAC Oxygen Radical Absorbance Capacity

^{A–D} Values with different capital letters in the same row indicate significant differences ($p \leq 0.05$) with respect to genotype (D1 vs. F1; D2 vs. F2) or soil type (D1 vs. D2; F1 vs. F2)

^{a–c} Values with different lower case letters in the same column, for each parameter, indicate significant differences ($p \leq 0.05$) along the processing chain, from raw grain to flour

The effects of genotypes, soils and processing on β -glucan and macronutrients

Table 2 shows the effect of genotypes and soils on β -glucan, protein, starch and total dietary fiber in raw grains, dehulled groats and flours. β -glucan showed higher values in cv. Flavia (+20%) than in cv. Donata in both soil types. The analysis of variance showed that the genotype effect was highly significant ($p \leq 0.001$) for the accumulation of β -glucan in the raw grains (Supplementary 2).

The β -glucan concentration markedly increased from raw grain to flour in all samples.

The protein content did not vary with soils and cultivars in raw grains, and the dehulling process did not affect

protein concentration, which decreased significantly during milling ($p < 0.05$).

Regarding starch content, no statistically significant difference was observed between soils and cultivars in raw grains. The starch concentration was threefold higher in flour than it was in raw grain.

The total fiber varied according to genotype, with $F1 > D1$, or soil type, with $D2 > D1$. The analysis of variance showed that the accumulation of the total fiber in the raw grains was influenced, to a greater extent, by the soil and combination, soil $\times$ genotype ($p \leq 0.001$), and to a minor extent by the genotype ($p \leq 0.05$) (Supplementary 2).

The fiber content was halved with dehulling and further reduced with milling.

Fig. 2 Comparison of total phenol and total AVN contents in manual or industrial dehulling in cv. Donata (a) and Flavia (b). c Hulls derived from manual dehulling; d oat groat after manual dehulling;

e oat groat after industrial dehulling. Asterisk indicates a statistically significant difference ($p \leq 0.05$) between industrial and manual dehulling

Table 2 Effect of processing on β -glucan and macronutrient levels

Parameters	Processing	D1	D2	F1	F2
β -Glucan	Raw grain	1.4 $\pm$ 0.1 ^{Aa}	1.5 $\pm$ 0.1 ^{Aa}	1.8 $\pm$ 0.1 ^{Ba}	1.9 $\pm$ 0.2 ^{Ba}
	Dehulled groat	2.1 $\pm$ 0.3 ^b	1.8 $\pm$ 0.1 ^b	2.1 $\pm$ 0.4 ^a	2.3 $\pm$ 0.2 ^a
	Flour	2.8 $\pm$ 0.3 ^c	2.9 $\pm$ 0.4 ^c	3.2 $\pm$ 0.4 ^b	3.1 $\pm$ 0.5 ^b
Protein	Raw grain	11.8 $\pm$ 0.2 ^{Aa}	11.5 $\pm$ 0.1 ^{Aa}	11.8 $\pm$ 0.1 ^{Aa}	12.1 $\pm$ 0.2 ^{Aa}
	Dehulled groat	11.6 $\pm$ 0.2 ^a	11.4 $\pm$ 0.1 ^a	11.6 $\pm$ 0.3 ^a	12.0 $\pm$ 0.2 ^a
	Flour	11.0 $\pm$ 0.2 ^b	10.8 $\pm$ 0.2 ^b	10.8 $\pm$ 0.1 ^b	11.3 $\pm$ 0.3 ^b
Total dietary fiber	Raw grain	35.2 $\pm$ 0.3 ^{Aa}	38.2 $\pm$ 0.2 ^{Ba}	37.0 $\pm$ 0.6 ^{Ca}	37.8 $\pm$ 0.3 ^{BCa}
	Dehulled groat	15.5 $\pm$ 0.1 ^b	14.3 $\pm$ 0.1 ^b	14.6 $\pm$ 0.2 ^b	15.3 $\pm$ 0.1 ^b
	Flour	13.4 $\pm$ 0.1 ^c	11.9 $\pm$ 0.2 ^c	12.2 $\pm$ 0.2 ^c	13.1 $\pm$ 0.3 ^c
Starch	Raw grain	21.4 $\pm$ 1.8 ^{Aa}	21.1 $\pm$ 1.5 ^{Aa}	22.8 $\pm$ 1.7 ^{Aa}	23.1 $\pm$ 1.9 ^{Aa}
	Dehulled groat	40.3 $\pm$ 3.2 ^b	40.9 $\pm$ 2.9 ^b	42.7 $\pm$ 3.8 ^b	41.1 $\pm$ 3.0 ^b
	Flour	59.8 $\pm$ 4.1 ^c	60.1 $\pm$ 4.4 ^c	61.2 $\pm$ 4.0 ^c	59.3 $\pm$ 4.8 ^c

Each value is shown as mean $\pm$ standard deviation and is expressed as %, on dry weight

D1 cv. Donata in loamy soil, D2 cv. Donata in medium texture soil, F1 cv. Flavia in loamy soil, F2 cv. Flavia in medium texture soil

^{A-C} Values with different capital letters in the same row indicate significant differences ($p \leq 0.05$) with respect to genotype (D1 vs. F1; D2 vs. F2) or soil type (D1 vs. D2; F1 vs. F2)

^{a-c} Values with different lower case letters in the same column, for each parameter, indicate significant differences ($p \leq 0.05$) along the processing chain

Discussion

In the present study, changes in the nutrient content, including TPs and AVNs, were thoroughly investigated in two husked oat genotypes grown in loamy and medium texture soils.

The two soils had different total nitrogen contents, but we did not find any difference in the protein contents of the two crops. Three reasons may account for the similarity in the protein contents of the two genotypes: (a) rainfall, which could wash out the nitrogen content of the soil making its absorption by the roots difficult; (b) the C/N ratio in the two soils; (c) crop yields. Since the first two parameters, rainfall and C/N ratio, were nearly the same in the two sites, we concluded that the identical protein concentrations were due to the different crop yields. In fact, as reported by Redaelli et al. (2014), oat protein content is inversely correlated to yield.

Our investigation showed that the genotype was the main determinant of TP and AVN concentrations, but most of the differences between the two cultivars were nullified by processing.

Regarding phenols, it was found that dehulling and milling reduced FP and BP values, excluding the original differences stemming from genotype. In raw grains, the ratio between FPs and BPs was about 0.5 in cv. Flavia and 1.0 in cv. Donata. The FP/BP ratio became 1.5 ± 0.1 after dehulling and 2.5 ± 0.2 after milling in both cvs. Thus, free phenols (FPs) proved to be the dominant fraction in dehulled groats and flours. This result could have significant implications for nutrition. In fact, FPs are considered to be bio-available in humans, whereas BPs can become bio-available with help from intestinal microbiota (Andreasen et al. 2001). Interestingly, the ORAC values of FPs in raw grains were significantly higher than BPs, in both cultivars and soils. Very likely, FPs show a wide heterogeneous composition of phenolic molecules, including AVNs, which provide a higher number of hydroxyl groups able to better scavenge peroxy radicals than BPs, in the ORAC test (Antonini et al. 2016).

From a nutritional standpoint, ORAC values were shown to be extremely high in terms of daily dose. In fact, considering 100 g of dehulled oats per meal, as would be found for example in a soup, the antioxidant contribution of cv. Donata reaches 15,000 ORAC units, which is more than enough to cover the daily recommended antioxidant intake (Ou et al. 2013).

In the present study, we have shown that genotype is the most important factor determining AVN concentration. Indeed, AVN concentration was found to be higher in cv. Donata than it was in cv. Flavia. Nevertheless, the interaction, soil $\times$ genotype, also had an impact on AVN

content. In point of fact, in cv. Donata, the maximum AVN concentration was obtained in loamy soil, whereas in cv. Flavia, the medium texture soil yielded the highest AVN concentration. Regarding the major AVN fractions, the highest concentrations were found in the 2p and 2f forms, whereas the 2c form showed the lowest concentrations.

In literature, 2c has been reported to be the most abundant AVN form and to be the most active antioxidant in a number of oat cvs.; however, these data refer to different cvs. from those investigated herein (Peterson 2001). The discrepancy between our findings and data reported in literature simply suggests that the relative ratios of AVN among the three forms should be thoroughly investigated in several cultivars to shed light on possible variations (Ninfali et al. 2015).

AVNs are also present in oat bran and hulls, and the discarding of hulls notably reduces AVN contents (Yang et al. 2014). When comparing manual dehulling to industrial dehulling, it appears that the relative adhesion of hulls to groats, a characteristic linked to genotype, was an important factor in determining how much oats are impacted by processing. In fact, in cv. Donata, there were greater AVN losses than in cv. Flavia, both in industrial and manual dehulling.

Therefore, the most important determinants for nutrient maintenance in oats are genotype and the rotor speed of the industrial dehulling, which must be carefully calibrated to obtain maximum dehulling efficiency with minimum oat breakage. Indeed, Doehlert et al. (2009) analyzed 18 different genotypes grown in six different environments and identified the optimal rotor speed of dehulling in the range 1614–1821 rpm, taking into account the physical and chemical oat kernel characteristics of each cultivar.

Wholegrain oat flour can be used to make products that offer the antioxidant and anti-inflammatory properties of AVNs. Because AVNs can resist the heat associated with baking, our results, together with those of other researchers (Dimberg et al. 2001), can be useful in providing a scientific basis for the preparation of AVN fortified bakery products.

Regarding β -glucan, the loss of hulls, which do not contain this nutrient, increases β -glucan concentration in dehulled groats. The β -glucan value also increases in flour, due to the loss of bran. Since β -glucan is an important nutrient, debranned flour may be useful in reaching the 3 g diet threshold indicated by the EFSA for health claims (European Commission 2012).

There are different opinions regarding the effect of processing on β -glucan content in the literature. Some authors point to the stability of the nutrient (Li et al. 2014), while others find a decrease (Hu et al. 2009) or an increase in its concentration (Doehlert and Moore 1997). The present study showed that β -glucan increased with milling,

indicating that β -glucan, in our cultivars, was not influenced by processing.

From raw grain to flour, proteins showed only small changes, indicating that they are primarily concentrated in the endosperm of oat kernels (Wang et al. 2007); starch increased significantly, as it is the main constituent of the starchy endosperm (Li et al. 2014), which is not influenced by processing.

In conclusion, oat genotype and processing technology are of central importance for the preservation of AVNs and phenols, which are essential elements in healthy oat products. By giving due importance to these factors, highlighted in the present investigation, producers of oat-based foods can maximize the health benefits of their products.

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