

# Identification and Quantification of Avenanthramides and Free and Bound Phenolic Acids in Eight Cultivars of Husked Oat (*Avena sativa* L) from Finland

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## Supporting Information

**ABSTRACT:** Finland is the second largest oat producer in Europe. Despite the existing knowledge of phenolics in oat, there is little information on the phenolic composition of oats from Finland. The aim of the study was to investigate the concentrations of free and bound phenolic acids, as well as avenanthramides in eight Finnish cultivars of husked oat (*Avena sativa* L.). Seven phenolic acids and one phenolic aldehyde were identified, including, in decreasing order of abundance: *p*-coumaric, ferulic, cinnamic, syringic, vanillic, 2,4-dihydroxybenzoic, and *o*-coumaric acids and syringaldehyde. Phenolic acids were mostly found as bound compounds. Significant varietal differences ( $p < 0.05$ ) were observed in the cumulative content of phenolic acids, with the lowest level found in cv. 'Viviana' ( $1202 \pm 52.9 \text{ mg kg}^{-1}$ ) and the highest in cv. 'Akseli' ( $1687 \pm 80.2 \text{ mg kg}^{-1}$ ). Avenanthramides (AVNs) 2a, 2p, and 2f were the most abundant. Total AVNs levels ranged from  $26.7 \pm 1.44$  to  $185 \pm 12.5 \text{ mg kg}^{-1}$  in cv. 'Avetron' and 'Viviana', respectively.

**KEYWORDS:** *Avena sativa* L., avenanthramides, dietary fiber, Finnish oats, phenolic compounds

## INTRODUCTION

Oat is a grain crop belonging to the family of *Poaceae* (or *Gramineae*).<sup>1</sup> Two main species of oat are produced in the world: *Avena sativa* L. and *Avena nuda* L.; the former, known as common oat, is the most cultivated.<sup>2</sup> *Avena sativa* L. is extensively grown in the cool and moist regions of Northern Europe and North America.<sup>3</sup> Finland and Poland are the biggest producers of oat within the European Union.<sup>4</sup> Oat-derived foods, e.g. ready to eat breakfast products, granola bars, oat-based cookies, and beverages, are commercially available across the world, although oat is mainly consumed as whole grain cereal due to the health benefits it provides as such.<sup>5</sup> The increasing interest of consumers toward whole grain oat is mainly driven by its advantageous macronutrient composition: lipids with a high degree of unsaturation, e.g. oleic and linoleic acids account for approximately 40 and 36% of total fatty acids, respectively;<sup>1</sup> proteins with a favorable composition of essential amino acids;<sup>6</sup> dietary fiber with a high content of  $\beta$ -glucan (2–8.5% w/w of oat seed), a soluble type of fiber that is able to lower plasma cholesterol by increasing the fecal excretion of bile acids.<sup>7</sup> An accruing body of evidence suggests that the protective effects of whole grain cereals against the development of noncommunicable diseases (NCD), including cardiovascular diseases and diabetes, are partly due to the presence of bioactive compounds, e.g. phenolic compounds, which are bound to and work synergistically with the dietary fibers.<sup>8,9</sup> In an 8-week placebo controlled study of 80 healthy overweight/obese volunteers, Vitaglione et al. (2015)<sup>10</sup> showed that subjects consuming whole grain wheat had greater excretion of ferulic acid and dihydroferulic acid in urine and lower plasma concentrations of markers of inflammation, e.g. PAI-1 (plasminogen activator inhibitor 1).

Phenolic compounds are secondary products of the plant metabolism and are structurally characterized by the presence of at least one aromatic ring bearing one or more hydroxyl groups.<sup>11</sup> They vary greatly in the molecular size, from simple monomers, to complex polymers, and can be divided into several subclasses, e.g. phenolic acids, flavonoids, stilbenes, coumarins, and tannins.<sup>12</sup> The level of phenolic compounds in whole grain cereals is influenced by the grain type, variety, and part of the grain analyzed.<sup>13</sup> Researchers have demonstrated that phenolic acids are among the most abundant phytochemicals in oat.<sup>11,14</sup> Phenolic compounds, particularly phenolic acids, are found in cereals either free or bound to cell wall components.<sup>15</sup> The free phenolics are mainly located in the vacuoles of the pericarp and, being soluble, can be extracted with organic solvents.<sup>16</sup> In cereals, including oat, only a small fraction of phenolic compounds exists in the free form.<sup>17</sup> Bound phenolic compounds form ether linkages with lignin and ester linkages with structural macromolecules such as polysaccharides and proteins.<sup>11</sup> Bound phenolics can be extracted following processes of alkaline and/or acid hydrolysis.<sup>18</sup> In addition to simple phenolic compounds, oat provides avenanthramides (AVNs), which are a group of hydroxycinnamoylanthranilate alkaloids unique to oat among the cultivated cereals.<sup>19</sup> It is estimated that more than 25 AVNs are found in oat, although the most common forms are esters of 5-hydroxyanthranilic acid with caffeic (2c aka C), *p*-coumaric (2p aka A), and ferulic (2f aka B) acids.<sup>20</sup> Avenanthramides

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were found to have anti-inflammatory effects and ameliorate skin disorders.<sup>21</sup> Several factors, e.g. genotype, abiotic, and biotic stressors, can influence the concentrations of AVNs in oat to a large extent. As a result, it is important to identify varieties of oat with high levels of AVNs, so as to provide consumers with raw materials of enhanced nutritional value.

Finland is a major world producer of oat, with yields exceeding one million tonnes in 2016.<sup>22</sup> Nevertheless, data on the composition of Finnish oat cultivars are very limited. Rainakari et al. (2016)<sup>23</sup> measured the dietary fiber content of oat products from Finland. The investigation did not include phenolic compounds and took no notice of varietal differences, as based on commercial products. The European project "Avena Genetic Resources for Quality in Human Consumption"<sup>24</sup> investigated a wide range of European cultivars of *Avena*, yet cultivars from Finland were not included. Murariu et al. (2013)<sup>25</sup> performed an extensive investigation of 117 cultivars of *Avena* from several European countries, including the cultivar (cv) 'Ivory' from Sweden and Estonia. The study concluded that the Nordic agricultural conditions are ideal to produce oat; however, cultivars from Finland were not characterized. As Finland is a large producer and exporter of oat, compositional information on Finnish oat cultivars will provide data that are important both scientifically and commercially. The goal of the present research was to investigate the differences in the macro- and non-nutrient (phytochemical) composition of husked oat (*Avena sativa* L.) from eight Finnish cultivars: 'Akseli', 'Avetron', 'Peppi', 'Ivory', 'Marika', 'Riina', 'Rocky', and 'Viviana'. The study aimed to measure the fat and dietary fiber compositions of the selected cultivars, as well as to characterize the phenolic profile of the samples and to differentiate between free and bound phenolic acids, based on the extraction procedures. In addition, the study evaluated how the selected genotypes influenced the concentrations of AVNs.

## MATERIALS AND METHODS

**Chemicals.** General laboratory reagents were purchased from VWR International Oy (Helsinki, Finland). Methyl *tert*-butyl ether (MTBE), ethyl acetate, acetic acid (glacial), and sodium sulfate (anhydrous, granular,  $\geq 99\%$ ) were of analytical grade. Methanol and acetonitrile were of LC-MS grade. Hydrochloric acid (reagent grade, 37%) and sodium hydroxide (pellets pure) were purchased from Sigma-Aldrich, Inc. (Gillingham, England). Water was purified *in loco* with a Milli-Q water purification system (Millipore Co.). Analytical standards of 2,4-dihydroxy benzoic acid, vanillic acid, syringic acid, syringaldehyde, cinnamic acid, ferulic acid, *o*-coumaric acid, and *p*-coumaric acid were purchased from Sigma-Aldrich (Gillingham, England). Analytical standards of avenanthramides 2c, 2p, and 2f were purchased from ReseaChem GmbH (Burgdorf, Switzerland).

**Plant Materials and Sample Preparation.** A set of eight cultivars of husked oat (*Avena sativa* L.) was provided by The Natural Resources Institute Finland (Luke). The selected cultivars are named: 'Akseli', 'Avetron', 'Peppi', 'Ivory', 'Marika', 'Riina', 'Rocky', and 'Viviana'. The samples were collected from the farmers' network in the Sastamala area (61°20'25" N, 022°54'35" E), Southwestern Finland. A representative silo sample of 1 kg was taken from the harvest and delivered to the laboratory. Oats were grown and harvested in the year 2016. Husked oats (oat kernels) were milled with a Retsch ZM-100 ultra-centrifugal mill with a 0.5 mm screen insert (Düsseldorf, Germany), placed in sealed plastic bags, and stored at room temperature ( $20.3 \pm 2$  °C) under vacuum until analysis.

**Macronutrient Analysis.** Routine proximate analytical procedures were employed to determine the macronutrient composition of the oat samples. Three independent samples of each cultivar were analyzed. The dry matter (DM) was determined following the official AOAC method (925.10).<sup>26</sup> Dietary fiber was measured following the official

AOAC method (991.43).<sup>27</sup> Total fat was determined by adaptation of the Folch procedure.<sup>28</sup> Samples (0.5 g) were suspended in 5 mL of extraction mixture solvent (MTBE/methanol, 3/1, (v/v)), and agitated on an orbital shaker at RT for 20 min. The supernatants were separated by centrifugation (5 min; 700g; 18 °C). The remaining pellets were suspended in 2 mL of extraction mixture solvent (MTBE/methanol/water, 4/1.2/1, (v/v)) and stirred on vortex for 1 min. The supernatants were separated by centrifugation (5 min; 700g; 18 °C) and combined with the other supernatants. After adding 1.25 mL of water to the pooled supernatants and stirring for 1 min on vortex at RT, the samples were centrifuged (5 min; 700g; 18 °C) to induce phase separation. The organic phases containing lipids were collected and weighed after evaporation of the solvent under a stream of nitrogen.

**Extraction of Phenolic Acids.** Phenolic acids were extracted as described by Multari et al. (2016)<sup>29</sup> and Neacsu et al. (2015).<sup>30</sup> Four independent samples of each cultivar were analyzed. The term total phenolic acids indicates the sum of the cumulative free and bound phenolic acids. As syringaldehyde was the only phenolic aldehyde detected, it was considered as a phenolic acid in the data analysis for simplicity reasons.

**Extraction of Free Phenolic Acids.** Samples of milled husked oats (approximately 0.1 g) were suspended in HCl (3 mL; 0.2 M) and extracted into EtOAc (6 mL), and the layers were separated by centrifugation (5 min; 1800g; 18 °C). The extraction was repeated twice, and the EtOAc extracts were combined and left to stand over sodium sulfate (anhydrous) and then filtered. The solvent was removed under reduced pressure at a temperature not exceeding 40 °C. Then, the extracts were dissolved in methanol (1 mL) for UPLC-MS analysis. The remaining aqueous fraction, obtained after EtOAc extraction, was neutralized with NaOH (approximately 0.2 mL; 4 M) and freeze-dried.

**Extraction of Bound Phenolic Acids.** The freeze-dried pellets were suspended in NaOH (3 mL; 1 M) and stirred at room temperature for 4 h under nitrogen. The pH was reduced to 2 with HCl (approximately 0.4 mL; 10 M), and the samples were extracted into EtOAc (6 mL). This was repeated twice. The EtOAc extracts were combined, and the solvent was removed under reduced pressure at a temperature not exceeding 40 °C. The extracts were dissolved in methanol (1 mL) for UPLC-MS analysis. The pH of the remaining aqueous fractions was brought to 7 with NaOH (approximately 1.9 mL; 4 M), and the aqueous fractions were freeze-dried. Then, the dried aqueous fractions were suspended in HCl (3 mL; 2 M) and incubated at 95 °C for 30 min with intermittent mixing. The samples were cooled and extracted with EtOAc (6 mL). This was repeated twice. The EtOAc extracts were combined, and the solvent was removed under reduced pressure at a temperature not exceeding 40 °C. Extracts were dissolved in methanol (1 mL) for UPLC-MS analysis.

**Extraction of Avenanthramides.** Avenanthramides were extracted by adapting the method from Bryngelsson et al. (2002).<sup>31</sup> Briefly, milled oat samples (5.0 g) were extracted twice with 80% methanol (35 mL) for 30 min at RT using a magnetic stirrer. Then, samples were centrifuged (10 min; 600g; 18 °C), and the supernatants were dried under reduced pressure at a temperature not exceeding 40 °C. Extracts were dissolved in methanol (2 mL), filtered through PTFE membrane filters (Pall Corporation, Port Washington, NY, USA), and analyzed by HPLC. Three independent samples of each cultivar were analyzed. Avenanthramides that contain the avenalumoyl structure instead of the hydroxycinnamoyl structure, i.e. type II avenanthramides, are referred to as avenaluminins (ALs) in the text. Exemplary avenanthramide structures are found as [Supporting Information](#) (SI 2).

**UPLC-MS Analysis of Phenolic Acids.** The liquid chromatography separation of the phenolic acids was obtained adapting the method from Multari et al. (2016)<sup>29</sup> and Neacsu et al. (2015).<sup>30</sup> It was performed on an UPLC-PDA-ESI-MS system consisting of a Waters Acquity UPLC in combination with a Waters 2996 PDA detector and a Waters Quattro Premier mass spectrometer (Waters Corp., Milford, MA). The column used was a Kinetex C18 column (100 × 4.6 mm; 2.6  $\mu$ m i.d.; 100 Å) from Phenomenex (Torrance, USA). The mobile-phase solvents were water containing 0.1% acetic acid (A) and acetonitrile containing 0.1% acetic acid (B). The gradient used to separate the different phenolic

Table 1. Macronutrient Composition of the Oat Samples<sup>a</sup>

cultivar	moisture	dry matter	fat	dietary fiber
Akseli	95.7 ± 1.75 c,b	904 ± 1.75 a,b	46.1 ± 1.35 e	350 ± 6.50 a
Avetron	109 ± 1.74 a,b	891 ± 1.74 c,b	58.8 ± 1.75 a	284 ± 7.77 e,f
Ivory	111 ± 0.67 a,b	889 ± 0.67 c,b	43.7 ± 0.57 f,e	289 ± 2.10 d,e,f
Marika	89.8 ± 3.33 c,b	910 ± 3.33 a,b	38.7 ± 0.22 g	319 ± 2.32 b,c
Peppi	118 ± 1.37 a	882 ± 1.37 c	50.1 ± 0.69 d	326 ± 2.22 b
Riina	100 ± 1.35 b	900 ± 1.35 b	52.1 ± 0.13 d,c	314 ± 5.03 b,c
Rocky	92.9 ± 2.40 c,b	907 ± 2.40 a,b	54.0 ± 0.38 b,c	284 ± 3.74 f
Viviana	84.5 ± 14.8 c	915 ± 14.8 a	56.2 ± 0.77 b	312 ± 1.36 c

<sup>a</sup>Data (g kg<sup>-1</sup> DM) is presented as mean ± SD and represents mean of minimum three independent measurements. Values with unlike letters (a-g) within the same column differ significantly ( $p < 0.05$ )

compounds was as follows: 10% B (0–1.5 min), 55% B (1.5–16.5 min), 80% B (16.5–30.0 min), 10% B (30.0–32.0 min). The flow rate was 840  $\mu\text{L min}^{-1}$ , the injection volume was 10  $\mu\text{L}$ , and the PDA was set at 210–600 nm. After splitting, the LC eluent (400  $\mu\text{L min}^{-1}$ ) was directed into the mass spectrometer equipped with an electrospray interface. The mass spectrometer was run in both negative and positive ion modes with the following source settings: capillarity voltage, 3.0 kV (ES<sup>+</sup>) and 5.0 kV (ES<sup>-</sup>); cone voltage, 15.0 V (ES<sup>+</sup>) and 22.0 V (ES<sup>-</sup>); extractor voltage 3.0 V (ES<sup>+</sup>) and 4.0 V (ES<sup>-</sup>); source temperature, 120 °C; desolvation temperature, 300 °C; desolvation gas flow, 700 L/h; cone gas flow, 100 L/h. Ions were scanned across the range of  $m/z$  120–450. All the phenolic compounds were identified using the UV spectra and the parent ions ( $m/z$  obtained from both positive ion scan  $[\text{M} + \text{H}]^+$  and negative ion scan  $[\text{M} - \text{H}]^-$ ). The quantification of the phenolic compounds by PDA was performed using external standards. The list of the individual phenolic compounds identified by UPLC-PDA-MS is included as Supporting Information (SI 1).

**HPLC-DAD Analysis of Avenanthramides.** Avenanthramides were identified and quantified as described by Mattila et al. (2005).<sup>32</sup> The system employed was an Agilent 1100 high-performance liquid chromatograph equipped with a diode array detector (HPLC-DAD) (Agilent, Santa Clara, CA, USA). The HPLC pumps, autosampler, column oven, and diode array system were operated by the ChemStation computer program. The analytical column was Phenomenex Kinetex C18 (100 × 3.0 mm; 5  $\mu\text{m}$  i.d.; 100 Å); the column oven was set at 35 °C. The mobile phase consisted of 0.05 M phosphate buffer (A) at pH 2.4 and methanol (B) with the following gradient: 5–60% B in 50 min; 60–90% B in 6 min. The flow was set at 0.6 mL min<sup>-1</sup>. Avenanthramides were quantitated at the wavelength of 350 nm. In addition, to AVNs 2c, 2p, and 2f, two unknown AVNs (AVNa and AVNb) and five avenaluminins, i.e. type II AVNs, were tentatively identified according to their UV spectra (SI 2) and quantitated using AVN 2p calibration curve, due to the lack of commercial standards.

**Statistical Analysis.** Data reported are mean of minimum triplicate observations, and values were expressed as mean ± SD. The statistical analysis was carried out using SPSS 23.0 for Windows (IBM, Armonk, NY, USA). The Shapiro–Wilk test was applied to verify the normal distribution of the variables. When the statistical distribution was not normal, a logarithmic transformation of the variables was performed. The Levene's test was applied to detect possible nonhomogeneity of the variances. The data were analyzed using One-Way-Analysis of Variance (ANOVA) to compare the groups, and the Tukey's test was performed to allow for multiple comparisons. Differences among groups were considered significant at  $p < 0.05$ . Not detected (n/d) values were not included in the statistical analysis.

## RESULTS AND DISCUSSION

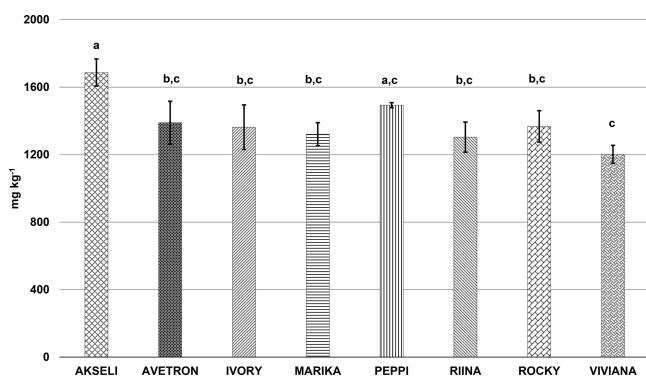
**Macronutrient Composition.** The macronutrient composition of the oat samples is shown in Table 1. The dry matter (DM) accumulation exhibited significant differences among the oat cultivars ( $p \leq 0.001$ ), ranging from 882 ± 1.37 g kg<sup>-1</sup> in cv. 'Peppi' to 915 ± 14.8 g kg<sup>-1</sup> in cv. 'Viviana'. The average DM content in these oat samples was 89.98%, which is in line with

previous investigations.<sup>14</sup> The fat content varied significantly ( $p \leq 0.001$ ) among the oats, with the highest value observed for cv. 'Avetron' (58.8 ± 1.75 g kg<sup>-1</sup> DM). All the oats had a fat content above 40 g kg<sup>-1</sup> DM, apart from cv. 'Marika' that presented a fat content of 38.7 ± 0.22 g kg<sup>-1</sup> DM. These results agree with earlier studies, in which an average fat content of about 50 g kg<sup>-1</sup> DM was reported.<sup>33</sup> Noteworthy is the investigation carried out by Hu et al. (2014),<sup>34</sup> in which oat from Sweden, a country sharing with Finland common botanical and agronomic characteristics, had a fat content of 51.3 g kg<sup>-1</sup> DM. Oat has been traditionally considered as a good source of vegetable fats, which are mainly located in the endosperm.<sup>5</sup> Apart from maize, oat provides more fats than other cereals,<sup>1</sup> being an excellent source of energy and unsaturated fatty acids. The quality and quantity of oil in oat can be influenced by several factors, e.g. extraction method, cultivar, and storage conditions. However, studies performed on oat oil reported that husked oats contain more than 70% of unsaturated fatty acids,<sup>35</sup> and that palmitic, oleic, and linoleic acids are the main fatty acids regardless of the genetic and environmental factors.<sup>1,36</sup>

Dietary fiber (DF) has long been considered critical for the physiological effects resulting from the consumption of whole grain oat.<sup>37</sup> The content of total dietary fiber from the oat samples is reported in Table 1. Among the samples, cv. 'Akseli' had the highest content of dietary fiber 350 ± 6.50 g kg<sup>-1</sup> DM, which statistically differed from all the other oats ( $p < 0.001$ ). The average dietary fiber level of the oats analyzed in this study was 31%, which is higher than most of the oat samples analyzed in analogous investigations.<sup>1,38</sup> This difference can be ascribed to the presence, in the selected oats, of the husk, the outer covering of the seed that is rich in dietary fiber.<sup>39</sup> The husks can account for up to 30% of the total kernel weight and are rich in hemicellulose, lignin, and cellulose.<sup>40</sup> Conversely, naked oats (free from the external husk) are richer in lipids, proteins, and starch.<sup>41</sup>

**Identification and Quantification of Phenolic Acids.** The total content of phenolic acids from the selected Finnish oats statistically differed among the samples ( $p < 0.001$ ) (Figure 1) and ranged from 1202 ± 52.9 to 1687 ± 80.2 mg kg<sup>-1</sup>. The concentrations of phenolic acids in the cultivars studied were, in the following order: 'Akseli' > 'Peppi' > 'Rocky' > 'Avetron' > 'Ivory' > 'Marika' > 'Riina' > 'Viviana'. Bound phenolic acids markedly outweighed the free in the cumulative content. Bound phenolic acids accounted for more than 99% of the total phenolic acids in all the cultivars (Table 2). Nevertheless, statistically significant differences were identified ( $p < 0.01$ ), with cv. 'Peppi' showing the highest share of bound phenolic acids (99.5 ± 0.03%). It is widely recognized that phenolic acids mostly occur as bound compounds in cereals, including oats.<sup>11</sup>





**Figure 1.** Total phenolic acid content of the oat cultivars. Results are expressed as sum of the cumulative free and bound phenolic acids and represent mean of four independent measurements. Values with unlike letters (a-c) differ significantly ( $p < 0.05$ ).

They are generally linked by ester and ether bonds to cell wall polysaccharides.<sup>13</sup> This finds further corroboration in the present investigation. The cultivars 'Akseli' and 'Peppi' showed the highest content of both dietary fiber ( $350 \pm 6.5$  and  $326 \pm 2.22 \text{ kg}^{-1} \text{ DM}$ , respectively) and cumulative bound phenolic acids ( $1684 \pm 80.2$  and  $1493 \pm 15.1 \text{ mg kg}^{-1}$ , respectively). Rolled oats are oat groats produced industrially from dehusked kernels, by cutting, steaming, and flaking.<sup>23,42</sup> Since the present investigation showed that most of the phenolic acids were bound to fiber, rolling oat into flakes can substantially decrease the phenolic content of oat. Conversely, low processed oat can serve as a natural and cost-effective source of functional ingredients. The corroborated association between dietary fiber and bioactive phenolic compounds could enhance the interest of the scientific community and consumers toward the selected oat cultivars. Across the samples, ferulic acid and *p*-coumaric acid contributed to the majority of bound acids. Ferulic acid also provided the greatest share of free phenolic acids. Nevertheless, regardless the contribution of ferulic acid, the absolute values of free phenolic acids were low, averaging  $2.90 \pm 0.69 \text{ mg kg}^{-1}$ , i.e.  $0.21 \pm 0.02\%$  of the cumulative phenolic acid content. Previous investigations carried out on oat confirmed that ferulic acid and *p*-coumaric acid prevailed in the bound form.<sup>43</sup>

Table 2 shows the content of individual phenolic acids identified in the oat samples (exemplary UPLC chromatograms are included as Supporting Information, SI 1). Eight different compounds were found. The selected oats differed in their qualitative profiles, as only four phenolic acids were ubiquitously identified across the samples: ferulic acid, *o*-coumaric acid, *p*-coumaric acid, and syringic acid. Ferulic and *p*-coumaric acids were the major compounds. Both of them were mainly identified in the bound form. Ferulic acid and *p*-coumaric acid accounted each for about 45% of the total phenolic acid content (Figure 2). Syringic acid was also identified in all the samples yet with marked differences between free and bound forms: cv. 'Rocky' contained syringic acid only as free ( $2.18 \pm 0.13 \text{ mg kg}^{-1}$ ), while cvs 'Marika' and 'Viviana' had only the bound form ( $6.17 \pm 0.54$  and  $6.45 \pm 0.53 \text{ mg kg}^{-1}$ , respectively). Syringic acid represented about  $0.54 \pm 0.2\%$  of the total phenolic content. Vanillic acid (on average  $0.42 \pm 0.14\%$  of the total phenolic content) was identified in all the oat samples in the bound form, apart from cv. 'Rocky' where it was identified as both free and bound compound ( $0.3 \pm 0.1$  and  $5.77 \pm 0.23 \text{ mg kg}^{-1}$ , respectively) and cv. 'Riina' where it was absent. Cinnamic acid ( $0.72 \pm 0.2\%$  of the total phenolic acid content)

was found across the samples in the bound form, but it was not detected in cv. 'Peppi'. Syringaldehyde was identified as free compounds in cvs 'Riina', 'Rocky', and 'Marika', while 2,4-dihydroxybenzoic acid was identified in cvs 'Peppi', 'Ivory', and 'Avetron' as bound compound.

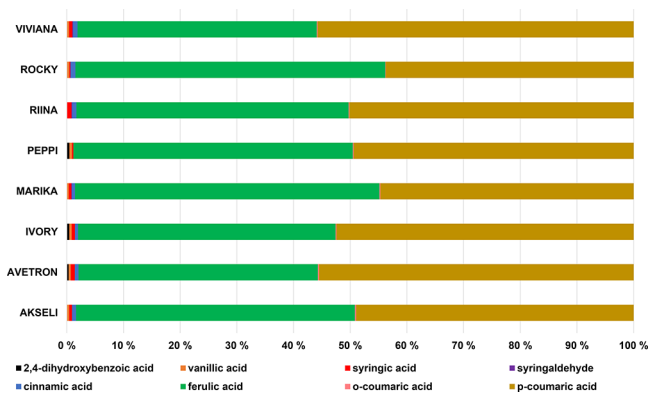
The selected Finnish oats were markedly different in the quantitative profiles, as the concentrations of the individual phenolic acids varied significantly across the cultivars, not including vanillic acid. For example, bound cinnamic acid in cv. 'Rocky' ( $11.7 \pm 2.5 \text{ mg kg}^{-1}$ ) was about 35% higher than in cv. 'Ivory' ( $7.65 \pm 0.82 \text{ mg kg}^{-1}$ ) ( $p < 0.05$ ); bound ferulic acid in cv. 'Akseli' ( $829 \pm 73.8 \text{ mg kg}^{-1}$ ) was about 35% higher than in cv. 'Viviana' ( $532 \pm 27.3 \text{ mg kg}^{-1}$ ) ( $p < 0.001$ ); bound *p*-coumaric acid in cv. 'Akseli' ( $826 \pm 53.0 \text{ mg kg}^{-1}$ ) was about 29% higher than in cv. 'Marika' ( $590 \pm 35.4 \text{ mg kg}^{-1}$ ) ( $p < 0.001$ ). The attentive observation of the data suggests that oats were rich in hydroxycinnamates, e.g. ferulic acid, *o*-coumaric acid, and *p*-coumaric acid. This is in agreement with previous investigations that attested the ubiquity of hydroxycinnamates in whole grain cereals.<sup>44</sup> In addition, ferulic acid and *p*-coumaric acid were described as the most abundant phenolic compounds in oat by analogous studies.<sup>45</sup> Noteworthy is the investigation carried out by Cai et al. (2012),<sup>46</sup> in which the concentrations of ferulic and *p*-coumaric acids increased several folds after fungal fermentation of oat. During fermentation, fungi produce different types of enzymes that can soften the kernel structure and release the phenolic compounds bound to the cell wall.<sup>11</sup> Cai et al. (2012)<sup>46</sup> showed that ferulic and *p*-coumaric acids are mainly present as bound phenolics in oats, as corroborated by the present study. In addition, enzymes might degrade polymeric phenolic compounds during the fermentation process.<sup>47</sup> To some extent, an analogous outcome might be achieved by performing processes of hydrolysis at high temperatures to enhance the extractability of bound phenolic compounds, as carried out herein. Flavonoids are important antioxidants in foods but traditionally have been detected in low quantities in cereals.<sup>11</sup> In the current study, several flavonoids were investigated, e.g. quercetin, kaempferol, isorhamnetin, and rhamnetin; however, they were not detected in any of the samples (data not shown).

**Identification and Quantification of Avenanthramides (AVNs).** In addition to simple phenolic compounds, ten AVNs were detected in the selected oats (Table 3). An explanatory chromatogram of oat AVNs and the corresponding UV spectra is provided as Supporting Information (SI 2). Total levels of AVN ranged from  $26.7 \pm 1.44$  to  $185 \pm 12.5 \text{ mg kg}^{-1}$  (Figure 3). Cv. 'Viviana' showed the highest total AVN content, with levels of AVN 2c, 2p, and 2f at  $39.2 \pm 5.14$ ,  $29.6 \pm 5.13$ , and  $21.9 \pm 0.35 \text{ mg kg}^{-1}$ , respectively. On the contrary, cv. 'Avetron' presented the lowest total AVN content, with the concentrations of AVNs 2c, 2p, and 2f being  $3.50 \pm 0.45$ ,  $6.0 \pm 0.29$ , and  $4.82 \pm 0.31 \text{ mg kg}^{-1}$ , respectively. The total content of AVNs differed markedly across the samples ( $p < 0.05$ ), with up to 7-fold difference among the selected cultivars, implying that the variety has a great effect on the concentration of AVNs in oat. This observation is consistent with the investigation of Chen et al. (2018),<sup>2</sup> which detected very different levels of AVNs among nine varieties of oat from China. Several studies showed that AVNs 2c, 2p, and 2f are the main kind of AVNs found in oat.<sup>48</sup> This is in agreement with the present investigation. Nevertheless, the literature provides inconsistent data on the concentration of AVNs in oats. Xie et al. (2017)<sup>49</sup> reported a total AVN content (as sum of 2c, 2p, and 2f) for Canadian oats averaging  $36 \text{ mg kg}^{-1}$ .

Table 2. Content of Individual Phenolic Acids of the Oat Cultivars<sup>a</sup>

	2,4-dihydroxy benzoic acid		vanillic acid		syringic acid		syringaldehyde		cinnamic acid		ferulic acid		o-coumaric acid		p-coumaric acid		cumulative free phenolics	cumulative bound phenolics
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound		
Akseli	n/d	7.1 ± 0.96 a	2.11 ± 0.11 a	6.3 ± 0.1 c	n/d	n/d	0.6 ± 0.4 b	829 ± 73.8 a	n/d	12.1 ± 1.59 a	0.6 ± 0.4 b	829 ± 73.8 a	n/d	3.78 ± 0.65 a,b	n/d	826 ± 53.0 a	2.71 ± 0.35 b,c,d	1684 ± 80.2 a
Avetron	n/d	4.86 ± 0.56 b	2.04 ± 0.12 a	8.0 ± 0.62 a,b	n/d	n/d	n/d	586 ± 68.0 b,c,d	n/d	9.2 ± 1.2 a,b,c	n/d	618 ± 33.1 b,c,d	n/d	2.84 ± 0.24 a,b	n/d	771 ± 69.4 a,b	2.04 ± 0.12 d	1387 ± 127 b,c
Ivory	n/d	5.71 ± 0.64 a,b	n/d	7.61 ± 0.71 b	n/d	n/d	2.55 ± 0.26 a	618 ± 33.1 b,c,d	n/d	7.65 ± 0.82 b,c	2.55 ± 0.26 a	618 ± 33.1 b,c,d	n/d	2.30 ± 0.59 b,c	n/d	713 ± 65.6 a,b,c	2.55 ± 0.26 c,d	1360 ± 132 b,c
Marika	n/d	4.59 ± 0.58 a	n/d	6.17 ± 0.54 c	1.13 ± 0.08 a	n/d	0.8 ± 0.2 b	709 ± 38.2 a,c	n/d	7.17 ± 0.56 c	0.8 ± 0.2 b	709 ± 38.2 a,c	n/d	2.39 ± 0.92 b,c	n/d	590 ± 35.4 c	1.93 ± 0.16 d	1319 ± 68.4 b,c
Peppi	n/d	6.65 ± 0.35 a	n/d	3.89 ± 0.16 d	n/d	n/d	5.0 ± 0.2 a	731 ± 8.41 a,c	n/d	n/d	5.0 ± 0.2 a	731 ± 8.41 a,c	n/d	2.54 ± 0.46 b,c	n/d	737 ± 13.9 a,b,c	5.0 ± 0.2 a,b	1488 ± 15.1 a,b
Riina	n/d	n/d	1.92 ± 0.13 a	8.9 ± 0.5 a	1.69 ± 0.15 a	n/d	n/d	626 ± 59.2 c,d	n/d	9.57 ± 2.21 a,b,c	n/d	626 ± 59.2 c,d	n/d	1.68 ± 0.92 c,b	n/d	654 ± 66.1 b	3.61 ± 0.19 b,c,d	1300 ± 89.1 b,c
Rocky	n/d	0.3 ± 0.1	2.18 ± 0.13 a	n/d	1.11 ± 0.25 a	n/d	0.7 ± 0.2 b	747 ± 68.0 a,c	n/d	11.7 ± 2.5 a,b	0.7 ± 0.2 b	747 ± 68.0 a,c	n/d	1.11 ± 0.05 c	n/d	598 ± 55.6 b,c	4.29 ± 0.28 b,c	1363 ± 93.1 b,c
Viviana	n/d	4.76 ± 0.7 a	n/d	6.45 ± 0.53 c,b	n/d	n/d	1.03 ± 0.3 b	532 ± 27.3 d	n/d	9.2 ± 1.11 a,b,c	1.03 ± 0.3 b	532 ± 27.3 d	n/d	1.69 ± 0.58 c,b	n/d	646 ± 43.7 b,c	1.03 ± 0.3 e	1201 ± 52.9 c

<sup>a</sup>Data (mg kg<sup>-1</sup>) is presented as mean ± SD and represents mean of four independent measurements. n/d = not detected (i.e., below the detection level). Values with unlike letters (a-d) within the same column differ significantly ( $p < 0.05$ ).



**Figure 2.** Phenolic acids in oat cultivars reported as individual percentages of the total phenolic acid content.

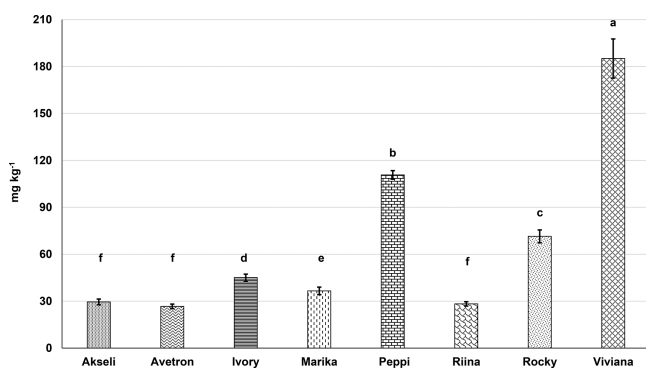
Chen et al. (2018)<sup>2</sup> showed the variety 'Longyan' from China to provide about 146 mg kg<sup>-1</sup> of total AVNs (sum of 2c, 2p, and 2f). It is clear that location, climate, variety, processing methods, and their interactions, are all factors playing a significant role in the process of AVN biosynthesis. Investigations on model systems suggest that AVNs might exert beneficial effects, e.g. anti-inflammatory activity, on human health.<sup>50,51</sup> Results from the present investigation indicate that to gain additional health benefits from the consumption of oats, the selection of genotype is an essential factor in the production of oats with high AVN content.

In conclusion, the selected Finnish oats showed favorable macro- and non-nutrient (phytochemical) profiles, due to their content in fats, which are likely to be unsaturated,<sup>1</sup> dietary fiber, and phytochemicals, i.e. phenolic acids and avenanthramides (AVNs). Accumulating evidence suggests that phenolic acids are mainly present as insoluble esters instead of aglycones (free forms), with hydroxycinnamates being esterified to hemicellulose arabinoxylans in the cereal cell wall.<sup>44</sup> In this study, seven phenolic acids, one phenolic aldehyde, and ten avenanthramides were identified and quantified from eight Finnish oat cultivars. Some of the phytochemicals were found at high concentrations, e.g. ferulic acid, *p*-coumaric acid, AVNs 2c and 2p. These compounds are known for their beneficial health effects, e.g. anti-inflammatory activity, hypoglycemic effects, cardiovascular protection.<sup>51,52</sup> Almost all the phenolic acids were identified in the bound form. The selected cultivars differed greatly in the phytochemical composition. The cv. 'Akseli' showed the highest content of phenolic acids, whereas cv. 'Viviana' had the highest content of avenanthramides. From a commercial standpoint, these two cultivars provide new opportunities for local food producers and manufacturers, as oat breeders could selectively grow them, due to their high levels of bioactive compounds. The cultivars 'Akseli' and 'Viviana' might be employed in the formulation of functional products, which could ameliorate the diet of targeted consumers. From a nutritional standpoint, their consumption could have a beneficial impact on colon health.<sup>53</sup> Human intervention studies showed that fiber from whole grain cereals can deliver phenolic compounds into the lower gut and that the release of phenolic acids by gut microbiota can increase the abundance of *Firmicutes*, *Bacteroidetes*, and *Lactobacilli*, by a two-way interaction mechanism.<sup>10</sup> It is acknowledged that phenolic metabolites lower the colonic pH value and modulate the composition of the gut microbiota.<sup>54</sup> While the release of bound phenolic compounds by the action of gut microbiota is being studied, actual knowledge on the bioavailability of

**Table 3.** Content of Individual Avenanthramides of the Oat Cultivars<sup>a</sup>

	2c	2p	2f	AVNa	AVNb	AL 1	AL 2	AL 3	AL 4	AL 5	cumulat 2c+2p+2f	cumulat ALs
Akseli	5.22 ± 0.75 e	6.90 ± 0.26 ef	4.69 ± 0.06 f	n/d	n/d	n/d	3.84 ± 0.58 c	1.55 ± 0.15 c	3.31 ± 0.06 ef	3.99 ± 0.15 ef	16.8 ± 0.98 f	12.7 ± 0.89 d
Avetron	3.50 ± 0.48 f	6.00 ± 0.29 f	4.82 ± 0.31 f	n/d	1.20 ± 0.10 d	n/d	2.33 ± 0.23 c	1.83 ± 0.21 c	2.92 ± 0.46 f	4.09 ± 0.35 edf	14.3 ± 1.01 g	11.2 ± 0.5 d
Ivory	7.19 ± 0.64 d	8.40 ± 0.32 de	12.3 ± 0.42 d	1.62 ± 0.12 c	2.34 ± 0.06 c	n/d	2.27 ± 0.15 c	1.69 ± 1.00 c	4.33 ± 0.21 de	4.98 ± 0.17 d	27.8 ± 1.36 d	13.3 ± 0.7 d
Marika	5.97 ± 0.84 de	7.14 ± 0.31 ef	8.89 ± 0.36 e	n/d	2.07 ± 0.12 c	1.12 ± 0.15 d	1.89 ± 0.25 c	1.61 ± 0.15 c	3.18 ± 0.06 ef	4.69 ± 0.15 de	22.0 ± 1.53 e	12.5 ± 0.8 d
Peppi	19.0 ± 0.46 b	20.9 ± 1.14 b	18.5 ± 0.93 b	3.00 ± 0.22 b	2.98 ± 0.10 b	4.58 ± 0.15 b	11.4 ± 0.32 b	4.14 ± 0.15 b	12.5 ± 0.64 b	13.6 ± 0.53 b	58.5 ± 1.57 b	46.3 ± 0.9 b
Riina	3.57 ± 0.23 f	6.80 ± 0.40 f	4.47 ± 0.23 f	n/d	1.11 ± 0.10 d	1.21 ± 0.15 d	2.39 ± 0.12 c	1.82 ± 0.23 c	3.38 ± 0.45 ef	3.52 ± 0.56 f	14.8 ± 0.76 fg	12.3 ± 0.6 d
Rocky	10.2 ± 0.70 c	10.9 ± 0.51 c	16.9 ± 0.64 c	2.41 ± 0.31 b	3.12 ± 0.10 b	3.14 ± 0.38 c	4.07 ± 0.40 c	4.18 ± 0.46 b	8.65 ± 0.75 c	7.84 ± 0.56 c	38.0 ± 1.71 c	27.9 ± 2.0 c
Viviana	39.2 ± 5.14 a	29.6 ± 5.13 a	21.9 ± 0.35 a	5.68 ± 0.23 a	4.84 ± 0.35 a	15.3 ± 2.29 a	26.8 ± 3.57 a	5.74 ± 0.50 a	15.6 ± 2.00 a	20.5 ± 0.29 a	90.7 ± 5.71 a	83.9 ± 6.5 a

<sup>a</sup>Data (mg kg<sup>-1</sup>) is presented as mean ± SD and represents mean of three independent measurements. n/d = not detected (i.e., below the detection level). Values with unlike letters (a-g) within the same column differ significantly ( $p < 0.05$ ). AVNs 2c, 2f, and 2p are esters of 5-hydroxyanthranilic acid with caffeic (2c aka C), *p*-coumaric (2f aka B), and ferulic (2f aka A) acids. AVNa and AVNb are unknown avenanthramides.



**Figure 3.** Total avenanthramides content of the oat cultivars. Results are expressed as mean of three independent measurements. Values with unlike letters (a-f) differ significantly ( $p < 0.05$ ).

phenolics from whole grain oat is limited and inconsistent. The metabolic fate of phenolic compounds depends largely on their level of esterification to cell wall polysaccharides. In general, the selected Finnish oats were particularly rich in dietary fiber and fiber-bound phenolic acids. Further research is required to elucidate the bioaccessibility and bioavailability of phenolic compounds from whole grain oats and to advance the knowledge on their processing as raw materials of added-value for food products. As the levels of total and individual phenolic acids and AVNs varied considerably across the samples, the present study provides information to shape the future oat breeding programs within Finland and the Northern regions of Europe.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jafc.7b05726](https://doi.org/10.1021/acs.jafc.7b05726).

List of the individual phenolic compounds identified by UPLC-PDA-MS (SI 1) ([PDF](#))  
Avenanthramide (SI 2) ([PDF](#))

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### Author Contributions

S.M. designed and performed all the experiments reported in this study, apart from the fat analysis, carried out the UPLC-PDA-MS analysis of the phenolic compounds, analyzed data, performed the statistical analysis, and wrote the manuscript; J.-P.S. supervised the analytical work and revised the manuscript; P.O.-C. performed the fat analysis; J.-M.P. provided the oat samples, carried out the analysis of AVNs, and revised the manuscript; V.H. was responsible for the collection of the oat samples; B.Y. revised the manuscript. All the authors approved the final version of the manuscript for publication.

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The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AOAC, American Organization of Analytical Chemists; AVNs, avenanthramides; cvs., cultivars; cv., cultivar; DF, dietary fiber; DM, dry matter; MTBE, methyl *tert*-butyl ether; PAI-1, plasminogen activator inhibitor; RT, retention time; SD, standard deviation

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