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Research Article

Nutritional properties of various oat and naked oat cultivars

Ernährungsphysiologischen Eigenschaften verschiedener Sorten von Hafer und Nackthafer

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Summary

Two oat (*Avena sativa* L.) and ten naked oat (*Avena nuda* L.) cultivars grown on an experimental farm in two consecutive years were analyzed for their content of β-glucan, ash, fat, protein and Osborne protein fractions. Concentration of the antioxidant substances avenanthramides, tocopherols, tocotrienols and total phenolic compounds was analyzed. The antioxidant capacity of the oat cultivars was studied by $ABTS^{\bullet+}$-scavenging assay and FRAP assay. Cultivar Vazec and conservation cultivar Klimt showed high contents of fat, avenanthramides and total phenolic compounds, as well as a high antioxidant capacity. Klimt also had a high total protein content, and the highest proportion of avenanthramide 2c, which has the highest antioxidant activity of the analyzed avenanthramides. On the other hand, Klimt was also relatively low in β-glucan and tocopherols and tocotrienols. The two cultivars of husked oat, Effektiv and Max, have high yields and low prolamin contents, but showed poor results in all analyzed antioxidant parameters. Comparison of the two cultivation periods showed large differences in many of the analyzed parameters. The hot, dry climate of 2015 had negative influences on the nutritional quality of the oat, especially concerning the antioxidant properties. No cultivar could be identified that was robust against these climatic influences.

Keywords: Oat, antioxidant capacity, avenanthramides, tocopherol, prolamins, β-glucan

Zusammenfassung

Zwei bespelzte Hafersorten (*Avena sativa* L.) und zehn Nackthafersorten (*Avena nuda* L.) welche auf Versuchsfeldern in zwei aufeinanderfolgenden Jahren geerntet wurden, wurden auf ihren Gehalt an β-Glucan, Asche, Fett, Protein und Osborne-Fraktionen getestet. Die Konzentration an den Antioxidantien Avenanthramiden, Tocopherolen, Tocotrienolen sowie der gesamten phenolischen Substanzen wurde ermittelt. Mittels $ABTS^{\bullet+}$-Scavenging-Assay and FRAP-Assay wurde zudem die antioxidative Kapazität der Haferproben untersucht. Die Sorten Klimt und Vazec wiesen hohe Konzentrationen an Fett, Avenanthramiden und gesamten phenolischen Substanzen auf. Klimt zeigte zudem einen hohen Proteingehalt sowie den höchsten Anteil an Avenanthramid 2c, welches das am stärksten antioxidativ wirksame der untersuchten Avenanthramide ist. Sowohl β-Glucangehalt als auch der Gehalt an Tocopherolen und Tocotrienolen waren jedoch bei Sorte Klimt relativ niedrig. Die zwei bespelzten Hafersorten Effektiv und Max waren ertragsstark und prolaminarm, wiesen jedoch bei sämtlichen antioxidativen Parametern deutlich niedrigere Werte auf. Im Vergleich der beiden Anbaujahre zeigten sich große Unterschiede bei fast allen untersuchten Parametern. Das trockene, heiße Klima im Jahr 2015 hatte durchwegs eine negative Auswirkung auf die ernährungsphysiologische Qualität des Hafers und besonders auf dessen antioxidative Eigenschaften. Es konnte keine Sorte identifiziert werden, welche sich gegenüber diesen klimatischen Einflüssen als robust erwiesen hätte.

Schlagworte: Hafer, Antioxidative Kapazität, Avenanthramide, Tocopherol, Prolamine, β-Glucan

1. Introduction

The nutritional composition of oat (*Avena sativa* L.) makes these cereal highly beneficial for the human diet. Oat is rich in the soluble dietary fiber β-glucan, with contents typically ranging between 3 and 5%, with only barley showing higher contents of up to 7% (Arendt and Zannini, 2013). Oat β-glucan forms viscous gels, causing the stomach content to expand in volume, prolonging the feeling of satiety (Kale et al., 2014). It also increases the viscosity of the digesta in the small intestine, effectively slowing nutrient uptake and therefore decreasing post-prandial blood glucose and insulin response (Granfeldt et al., 2008). The same mechanism is thought to be responsible for the LDL-cholesterol lowering properties of β-glucan by impeded dietary cholesterol uptake and bile acid reabsorption in the small intestine (Lazaridou and Biliaderis, 2007).

Oat is also high in protein, with groats containing between 12 and 20% protein (Arendt and Zannini, 2013). Oat protein is low in prolamins and high in albumins and globulins. Compared to albumins and globulins, prolamins have a lower content of lysine, which is the limiting amino acid in most cereal proteins (Klose and Arendt, 2012). As a result, oat protein has a higher biological value than other, more prolamin-rich cereals, with a Limiting Amino Acid Score (LAA) of 49.8 for wheat flour and 66.9 for oat flour (Suarez Lopez et al., 2006). Whether oat is suitable for consumption by patients suffering from celiac disease is still a subject of debate. While several studies showed no harmful effects of including oat in the diet of celiac patients (Janatuinen et al., 2002; Tapsas et al., 2014; Lionetti et al., 2017), others reported a varying tolerance of oat between patients (Lundin et al., 2003; Sjöberg et al., 2014). Comino et al. (2011) reported immunogenicity to be also dependent on oat cultivars.

Oat is the cereals highest in lipids, with groats typically containing around 7% lipids. Because of their high content of unsaturated fatty acids, oat is a valuable source of dietary fats but are also susceptible to lipid oxidation and the development of rancid off-flavors. High antioxidant concentration and antioxidant capacity are therefore not only nutritionally advantageous, but also crucial for storage stability (Zhou et al., 1999).

Oat is also the only food containing avenanthramides, a group of over 20 highly anti-oxidative amides of hydroxycinnamic acids with anthranilic acid or its derivates. Avenanthramides were first identified in oat extracts in 1989 (Collins, 1989). The most common avenanthramides in oat is avenanthramide 2p (N-coumaroyl-5-hydroxyanthranilic acid), 2f (N-feruloyl-5-hydroxyanthranilic acid) and 2c (N-caffeeoyl-5-hydroxyanthranilic acid), also called avenanthramides A, B and C, respectively (Hitayezu et al., 2015). Avenanthramides are highly bioavailable antioxidants (Chen et al., 2007), and *in-vivo* studies have shown that avenanthramides inhibit the formation of reactive oxygen species after physical exercise and lower LDL-oxidation, both of which are mechanisms protecting against cardiovascular disease (Ji et al., 2003). Avenanthramides also have anti-inflammatory properties, inhibiting the production of pro-inflammatory cytokines by macrophages (Liu et al., 2004).

In this study, a comprehensive analysis of the nutritional profile of 12 cultivars of oat from two consecutive cultivation periods was conducted. The objective of this study was to identify cultivars with a highly beneficial nutritional profile and to investigate any climatic influences on the chemical composition of the oat. Special focus was put on the antioxidant properties of the cultivars, and the proportions of the albumins, globulins and prolamins in the oat protein. As cultivation conditions were identical in all cultivars and both years, any differences between samples within one year can be attributed to differences between cultivars, while differences between cultivation periods are due to climatic differences in the two years.

2. Material and methods

2.1 Plant material

Two husked oat (*Avena sativa* L.) cultivars (Effektiv and Max) and ten naked oat (full botanical name *Avena sativa* L. *ssp. nuda* Gillet & Magne) cultivars (Klimt, Tatran, Hronec, Vazec, Samuel, Oliver, Otakar, Kamil, Tibor and Saul), were grown on the experimental fields in Austria by Saatzucht Edelhof (48°36'29.5"N 15°13'29.2"E) in the years 2014 and 2015 on loamy sand soil (pH 5.8-6.3). Crops were cultivated organically, neither fertilizer nor pesticides were used. Sowing density was 400 seeds/m² for husked and 500 seeds/m² for naked oat. In 2014, the preceding crop was winter triticale, sowing date was 28th of March, harvest date was 22nd of August. Precipitation from March to August 2014 was 434 mm and the average temperature was 13.2°C. In 2015, the preceding crop was winter rye, sowing date was 23rd of March and harvest date 4th of August. Precipitation from March to August 2015 was 276 mm and the average temperature was 14.0°C.

All cultivars are registered in the EU (see Supplemental Table 1 for maintainers). The de-hulled grains of cultivars Effektiv and Max and the hull-less grains of the naked oat cultivars were ground with the ultra-centrifugal mill Retsch ZM 200 (Retsch, Haan, Germany). The oat flour was stored at -4°C in sealed plastic containers. Moisture content of the oat flour was determined by measuring the weight loss after drying for 3 h at 105°C (ICC standard method no. 110/1). All analytical results were expressed per dry matter.

2.2 Chemical Analysis

Fat content was determined according to the ICC standard method no. 136 (Soxhlet method), without acid hydrolysis prior to the extraction. Crude protein was determined from the nitrogen content according to the standard method no. 105-2 (Kjeldahl method). A factor of 5.34 was used for the conversion from nitrogen to protein (Mariotti et al., 2008). Ash content was determined according to the ICC standard method no. 104-1. The content of β-glucan was measured using the ICC standard method no. 166.

For the extraction of the Osborne fractions, 4 ml of distilled water was added to 250 mg of the ground oat samples, mixed on a vortex mixer, extracted for 10 min on an overhead shaker and then centrifuged for 20 min at 3220 g. The supernatant containing the albumin fraction was then collected and the pellet was once again extracted with water. The supernatant of both extractions was then combined, and water was added to a total volume of 10 ml. The pellet was then further extracted in the same way with 1 M aqueous sodium chloride solution to obtain the globulin fraction, and then with 60% ethanol for the prolamin fraction.

Albumin and globulin concentrations were determined photometrically via the Bradford assay using Roti-Quant solution, as per the manufacturer's instructions (Carl Roth, Germany). Prolamin concentration was measured via RP-HPLC on a Shimadzu HPLC system and a diode array detector (Shimadzu Cooperation, Kyoto, Japan). Water and acetonitrile with 0.05% trifluoroacetic acid were used as mobile phase, applying a gradient of 25 to 90% acetonitrile for 60 min. LabSolutions Software (Shimadzu Cooperation, Kyoto, Japan) was used to quantify the peaks relative to those obtained with the PWG gliadin standard.

2.3 Antioxidant analysis

Tocopherols and tocotrienols were extracted from the oat samples using the method of Panfili et al. (2003). The concentration was determined by NP-HPLC on a Shimadzu HPLC system with a fluorescence detector. The mobile phase was a mixture of 70% n-hexane und 30% ethyl ac-

Table 1. Contents of protein (determined by nitrogen content, conversion factor: 5.34), ash, fat and β-glucan in % per dry matter
Tabelle 1. Gehalte an Protein (berechnet über den Stickstoffgehalt, Umrechnungsfaktor: 5,34), Asche, Fett und β-Glucan in % pro Trockensubstanz

Cultivar	Protein (%)		Ash (%)		Fat (%)		β-glucan (%)	
	2014	2015	2014	2015	2014	2015	2014	2015
Effektiv	12.67 ^{bc}	11.10 ^{D}	2.16 ^{a}	2.05 ^{A}	4.94 ^{a}	5.73 ^{B}	2.99 ^{e}	2.84 ^{CD}
Max	11.39 ^{a}	10.14 ^{A}	2.26 ^{c}	2.23 ^{AB}	5.31 ^{ab}	5.62 ^{B}	3.68 ^{g}	3.03 ^{DE}
Klimt	15.98 ^{i}	12.74 ^{J}	2.42 ^{f}	2.51 ^{D}	7.19 ^{ef}	7.70 ^{H}	2.98 ^{e}	2.53 ^{BC}
Tatran	13.74 ^{g}	12.43 ^{I}	1.91 ^{a}	2.25 ^{BC}	7.60 ^{fg}	7.58 ^{H}	2.94 ^{de}	3.41 ^{F}
Hronec	13.06 ^{d}	10.88 ^{C}	1.89 ^{a}	2.01 ^{A}	6.07 ^{cd}	6.08 ^{CDE}	2.60 ^{c}	2.78 ^{CD}
Vazec	13.32 ^{ef}	12.15 ^{G}	2.24 ^{def}	2.24 ^{BC}	7.52 ^{g}	7.35 ^{G}	2.64 ^{cd}	3.50 ^{DE}
Samuel	14.59 ^{h}	11.83 ^{F}	2.08 ^{bc}	2.12 ^{ABC}	6.21 ^{cde}	6.54 ^{D}	3.23 ^{f}	2.28 ^{BC}
Oliver	12.69 ^{bc}	10.90 ^{C}	2.02 ^{ab}	2.04 ^{A}	5.72 ^{bc}	6.02 ^{C}	2.03 ^{a}	1.93 ^{A}
Otakar	13.47 ^{f}	11.30 ^{E}	2.18 ^{cde}	2.10 ^{ABC}	7.18 ^{fg}	7.04 ^{F}	2.46 ^{bc}	2.18 ^{AB}
Kamil	13.16 ^{de}	12.26 ^{H}	2.24 ^{def}	2.12 ^{ABC}	7.31 ^{fg}	7.36 ^{G}	2.76 ^{cd}	3.86 ^{G}
Tibor	12.87 ^{b}	10.60 ^{B}	2.15 ^{bcde}	1.98 ^{A}	6.72 ^{def}	6.85 ^{E}	3.76 ^{g}	3.36 ^{F}
Saul	13.12 ^{c}	12.03 ^{G}	2.25 ^{def}	2.09 ^{AB}	5.67 ^{bc}	5.41 ^{A}	3.62 ^{f}	3.08 ^{EF}

Differences between samples of the same cultivation year at a significance level of $p < 0.05$ are marked with different superscript letters. Differences between samples from 2014 are marked with lower case letters, differences between the 2015 samples with capital letters.

etate. Samples were quantified relative to the tocopherol and tocotrienol standards (Supelco/Sigma-Aldrich, USA). For the extraction of phenolic compounds, a protocol optimized for maximum avenanthramide extraction was used (Maliarova et al., 2015). Extracts were stored at -30°C. Avenanthramide concentration was measured via RP-HPLC on a Shimadzu HPLC system equipped with a diode array detector (Shimadzu Cooperation, Kyoto, Japan). Water and acetonitrile with 0.25% trifluoroacetic acid were used as mobile phase with a gradient of 12 to 95% acetonitrile for 50 min. LabSolutions Software (Shimadzu Cooperation, Kyoto, Japan) was used to quantify the peaks relative to the avenanthramide standards A, B and C (Sigma-Aldrich, USA). Total phenolic content (TPC) was determined using the Folin-Ciocalteu method by using a modified protocol given by Singleton et al. (1999). Radical scavenging capacity was determined with $ABTS^{\bullet+}$-radical assay (Re et al., 1999), expressed as µmol Trolox-equivalents (TE)/g sample. For further analysis of antioxidant capacity, a ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) according to (Benzie and Strain, 1999) was performed.

2.4 Statistical evaluation

All results are given as mean values of triplicates, unless otherwise specified. All data was tested for outliers (Grubb's test). One-way ANOVA (analysis of variance) was used for finding differences between cultivars and years at a significance level of $p < 0.05$. The post hoc test Fisher's least significant difference test (LSD) was used to visualize the differences (shown by different superscript letters in results and discussion). Multi-factor ANOVA was used to study the effects of genotype and year on the parameters, and to investigate the genotype-year interactions. To identify correlations between two factors, the Pearson product-moment correlation coefficient was determined. All statistical analyses were conducted using Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA, USA.

3. Results and discussion

3.1 Contents of dry matter, ash, fat, protein and β-glucan

Dry matter contents ranged between 91.5 and 96.5%, with higher values in samples from 2015. As dry matter content is dependent on storage conditions, these differences in dry matter content are within the expected range. Results of the analysis of ash, fat, protein and β-glucan are summarized in Table 1. No significant difference between the values of ash, fat and β-glucan content in the two different harvest seasons were found. Fat contents ranged between 5.3 and 7.4%, which was in accordance with the literature values (Zhou et al., 1999). With Soxhlet method, polar lipids like phospholipids, which can make up to 20% of oat lipids, as well as lipids bound to proteins and carbohydrates, are not fully extracted; therefore, the actual fat content might be higher (Zhou et al., 1999). Effektiv, Max and Saul cultivars were lowest in fat, Klimt, Tatran and Vazec were the cultivars highest in fat. High fat contents in oat indicate a higher risk of a development of rancid off-flavors, and risk of clumping during milling. As the fatty acid composition in oat is highly beneficial for human consumption, a high fat content is nevertheless seen as a sign of quality in oat (Decker et al., 2014).

Ash contents varied in a relatively narrow range between 1.9% (Hronec) and 2.5% (Klimt). Oat is high in potassium, calcium and magnesium; so, oat cultivars with high ash content and therefore high mineral content can pose a beneficial addition to human diet (Arendt and Zannini, 2013).

None of the cultivars showed high β-glucan contents, with values ranging between 2 and 3.5%. In a two-year study with US oat cultivars, β-glucan contents between 3.9 and 6.4% were found (Lim et al., 1992) and according to Arendt and Zannini (2013), contents of up to 8% are possible. Oliver and Otakar were especially low in β-glucan, while Max, Kamil, Tibor and Saul showed the highest β-glucan values of the analyzed cultivars.

Protein contents in 2014 were significantly higher than in 2015 (Table 1). Values between 11.4 and 16.0% (2014) and 10.1 and 12.7% (2015) were observed. Klimt, Tatran and Samuel exhibited high protein contents, while cultivars Effektiv, Max, Hronec, Oliver and Tibor showed low protein contents in both years. These values are consistent with protein contents found in previous studies, which reported protein contents between 12 and 20% of dry matter (Arendt and Zannini, 2013).

3.2 Antioxidant properties

The results of the analyses of tocopherol and tocotrienol content are provided in Table 2. Tocopherol and tocotrienol analyses of the cultivars showed that only the α-forms of

both substances were present. In 2015, the tocopherol and tocotrienol content was significantly higher than in 2014. The lowest value of total tocopherols and tocotrienols in 2014 was 2.3 mg/100 g sample (cultivar Tatran), highest was 3.2 mg/100 g (Hronec). In 2015, 2.8 mg/100 g was the lowest content of total tocopherols and tocotrienols (Tatran), and 6.5 mg/100 g the highest (Otakar). Cultivars Effektiv, Klimt, Tatran, Vazec and Samuel showed lower tocopherol and tocotrienol contents while Oliver, Otakar and Saul contained very large amounts. The α-tocotrienol content was higher than the α-tocopherol content in both years and all cultivars except for Vazec. While α-tocotrienol has a higher antioxidant capacity than α-tocopherol (Serbinova et al., 1991) and is therefore of higher benefit for protection of oat lipids against oxidation, α-tocopherol has a higher bioavailability and, in addition to its antioxidant properties, also positive effects on cell division and thrombocyte aggregation (Sen et al., 2006). Other studies have reported oat vitamin E contents to be around 1.6 mg/100 mg (Arendt and Zannini, 2013) or even lower (Berga and Zute, 2012); so the values found in the present study were significantly higher. Tocol levels in cereals are highest in lipid rich milling fractions like germ and bran, so a correlation between total fat content and tocopherol and tocotrienol content was to be expected (Ko et al., 2003). But interestingly, no statistically significant correlation could be found between fat content and the concentration of tocopherol and tocotrienol.

Antioxidant capacity (measured in $ABTS^{\bullet+}$-radical assay and FRAP assay) and total phenolic content in all cultivars were higher in 2014 than 2015 (Table 3). Cultivars Effektiv, Max and Hronec exhibited low antioxidant capacity and total phenolic content, while Klimt, Tatran, Samuel and Oliver showed high antioxidant capacity. Klimt, Vazec and Kamil exhibited a high total phenolic content. Few studies have been conducted on the factors that influence oat antioxidant activities. One Swedish study (Dimberg et al., 2005) investigated the concentration of antioxidative substances in three organically and conventionally grown oat cultivars from three consecutive harvests. There, the factor year was also found to have the greatest influence on antioxidant concentration, with concentrations in samples from a warm and dry year about 25% lower than from years with average precipitation.

As expected, strong correlations between antioxidant capacity measured in $ABTS^{\bullet+}$-radical assay and FRAP assay ($r = 0.96$, $p < 0.001$) could be observed. Antioxidant capacity and total phenolic content also correlated strongly, with results from $ABTS^{\bullet+}$-radical assay and FRAP assay correlating equally strongly with total phenolic content (r in both correlations = 0.83, $p < 0.001$). High phenolic content and antioxidant capacity are desirable traits in oat, as antioxi-

Table 2. Contents of α-tocopherol and α-tocotrienol, and the sum of α-tocopherol and α-tocotrienol (all values expressed as mg/100 g dry matter)
Tabelle 2. Gehalte an α-Tocopherol und α-Tocotrienol und die Summe aus beiden Werten (alle Werte als mg/100 g Trockenmasse angegeben)

Cultivar	α-tocopherol (mg/100 g)		α-tocotrienol (mg/100 g)		Total (mg/100 g)	
	2014	2015	2014	2015	2014	2015
Effektiv	0.92 ^{bc}	1.00 ^{B}	1.73 ^{cd}	2.54 ^{B}	2.65 ^{abc}	3.54 ^{BC}
Max	1.52 ^{g}	0.91 ^{AB}	2.30 ^{g}	2.57 ^{B}	2.98 ^{cd}	3.48 ^{B}
Klimt	0.89 ^{b}	0.86 ^{A}	1.59 ^{bcd}	2.42 ^{B}	2.48 ^{ab}	3.28 ^{B}
Tatran	0.95 ^{bcd}	0.98 ^{AB}	1.37 ^{a}	1.85 ^{A}	2.31 ^{a}	2.83 ^{A}
Hronec	1.24 ^{f}	1.34 ^{C}	1.97 ^{ef}	2.94 ^{C}	3.21 ^{d}	4.28 ^{E}
Vazec	0.94 ^{bcd}	2.02 ^{E}	1.57 ^{bc}	1.82 ^{A}	2.50 ^{ab}	3.84 ^{D}
Samuel	0.97 ^{cd}	1.76 ^{D}	1.75 ^{d}	2.03 ^{A}	2.72 ^{bc}	3.79 ^{CD}
Oliver	0.90 ^{bc}	2.64 ^{G}	1.45 ^{ab}	3.06 ^{C}	2.35 ^{a}	5.70 ^{G}
Otakar	1.06 ^{e}	2.69 ^{G}	2.12 ^{fg}	3.76 ^{D}	3.15 ^{d}	6.45 ^{H}
Kamil	0.93 ^{bcd}	2.26 ^{F}	1.95 ^{e}	2.88 ^{C}	2.88 ^{cd}	5.14 ^{F}
Tibor	0.72 ^{a}	2.32 ^{F}	1.64 ^{cd}	3.05 ^{C}	2.64 ^{abc}	5.38 ^{F}
Saul	1.00 ^{de}	2.68 ^{G}	2.13 ^{fg}	3.54 ^{D}	3.12 ^{d}	6.22 ^{H}

Differences between samples of the same cultivation year at a significance level of $p < 0.05$ are marked with different superscript letters. Differences between samples from 2014 are marked with lower case letters, differences between the 2015 samples with capital letters.

dative oat extracts have been shown to reduce intracellular inflammatory activities and lessen the symptoms of colits in mice (Hasnat et al., 2015), and prevent ROS-induced stress in the livers and kidneys of diabetic mice (Marmouzi et al., 2017).

Avenanthramide analysis showed that contents were much higher in 2014 than in 2015 (Table 4). In 2014, total content of avenanthramides 2p, 2f and 2c ranged between 12.1 and 36.9 mg/100 g; while in 2015, contents between 1.7 and 6.2 mg/100 g were found. Other studies reported avenanthramide concentrations between 0.2 und 80 mg/100 g (Dimberg et al., 2005; Tong et al., 2014; Maliarova et al., 2015). Effektiv, Max and Hronec had the lowest, Tatran and Otakar the highest avenanthramide contents. Of the three analyzed avenanthramides, avenanthramide 2f was the most abundant, and avenanthramide 2c the least abundant in all cultivars (Table 4). *In-vitro* studies have shown avenanthramide 2c to have the highest, 2p the lowest antioxidant activity, a high proportion of avenanthramide 2c is therefore beneficial (Fagerlund et al., 2009). Cultivars Effektiv, Max and Hronec have low overall avenanthramide concentrations and an especially low content of avenanthramide 2c. Tatran and Otakar had the highest avenanthramide concentrations of the analyzed cultivars, and also a more beneficial avenanthramide profile. Avenanthramide concentration correlated strongly with antioxidant capacity measured with $ABTS^{\bullet+}$-radical assay and with FRAP assay ($r = 0.95$ and 0.94, respectively, $p < 0.001$). As was expected, avenanthramide content also correlated strongly with total phenolic content ($r = 0.84$, $p < 0.001$).

Aside from the three quantified peaks, six more peaks could be observed in the chromatogram (Figure 1). These are most probably other, less common avenanthramides for which no commercial HPLC standards are currently available.

3.3 Protein fractions

While albumin concentrations were higher in 2015 than in 2014, the opposite trend could be observed in globulins. No difference in prolamin concentrations could be observed between the harvest years (Table 5). Albumin contents ranged between 0.95 and 1.9% of dry matter, or between 7.8 und 17.1% of total protein content. Globulins made up between 3.6 and 6.8% of dry mass or 34.7 and 42.7% of total protein. Prolamins constituted between 0.5 and 1.4 of dry matter or 3.7 and 10.9% of total protein, with cultivars Effektiv, Max, Samuel, Oliver, Kamil and Tibor showing relatively low levels of prolamins. Other studies have found albumins to make up 1–20%, globulins 70–80% and prolamins 4–14% of total protein (Klose et al., 2009; Runyon et al., 2013; Rasane et al., 2015), which indicates that the globulin fraction in this study was not fully extracted. Higher volumes of solvent and longer extraction times seem to be needed. As discussed by Sunilkumar and Tareke (2017), numerous different extraction methods for the Osborne fractionization in oat have been used, so results vary widely. Further research to identify the optimal extraction parameters for a standardized Osborne fractionization of oat protein is needed.

The RP-HPLC spectra of the prolamin fraction showed major differences in chromatogram patterns between the cultivars (Figure 2). While all cultivars showed clusters of peaks between minutes 21 and 23 and minutes 26 and 29, the number, shape, and distribution of peaks were characteristic for each cultivar. Chromatograms could therefore be used for oat cultivar identification, as is already a standard method for wheat cultivars (Belitz et al., 2008).

4. Conclusion

In the multi-factorial ANOVA, significant effects ($p < 0.001$) of cultivar, year and cultivar × year interaction could be observed in all parameters except for prolamin and ash content, on which the year had no significant effect (Supplemental Table 2). On the parameters ash, β-glucan, fat and prolamin content, the factor cultivar had the biggest influence. On the parameters of protein, albumin, globulin, tocol, avenanthramide and total phenolic content and antioxidant capacity, the factor year had the biggest effect. In all parameters, the cultivar × year interaction had by far the smallest effect.

The two husked cultivars Effektiv and Max showed very similar results in all analyzed parameters, with lower contents of fat, protein, avenanthramides, tocopherols, tocotrienols, total phenolic compounds and antioxidant capacity than the naked oat cultivars. Naked oat cultivar Hronec had a similar chemical profile to Effektiv and Max, showing low fat and protein contents and very low values in all antioxidant parameters. These three cultivars also had particularly low concentrations of avenanthramides. The nutritional profile of the conservation cultivar Klimt was highly beneficial, with high fat and protein contents, high avenanthramide and total phenolic content, and high antioxidant capacity. On the other hand, cultivar Klimt

Table 3. Antioxidant capacity, measured in ABTS$^{\bullet+}$-assay (µmol TE/g) and FRAP-assay (µmol Fe^{2+}-ions/g), and total phenolic content (TPC) in mg/g FAE. Samples were analyzed in duplicates that were measured three times, results are means of these six values.
Tabelle 3. Antioxidative Kapazität, gemessen mit ABTS$^{\bullet+}$-Assay (µmol TE/g Trockensubstanz) und FRAP-assay (µmol Fe^{2+}-Ionen/g Trockensubstanz) und Gesamtgehalt phenolischer Substanzen (TPC) in mg/g FAE. Die Analyse erfolgte in Duplikaten die jeweils dreifach gemessen wurden, die Ergebnisse hier sind die Mittelwerte dieser sechs Werte.

Cultivar	ABTS (µmol TE/g)		FRAP (µmol Fe^{2+}/g)		TPC (mg FAE/g)	
	2014	2015	2014	2015	2014	2015
Effektiv	2.35 ^{b}	1.79 ^{B}	11.33 ^{b}	6.32 ^{D}	1.57 ^{b}	1.50 ^{E}
Max	2.39 ^{b}	1.75 ^{AB}	12.53 ^{c}	6.74 ^{F}	1.43 ^{a}	1.32 ^{CD}
Klimt	3.00 ^{f}	1.91 ^{C}	16.27 ^{h}	6.20 ^{C}	2.11 ^{f}	1.52 ^{EF}
Tatran	3.11 ^{g}	2.06 ^{G}	15.52 ^{g}	7.01 ^{H}	1.91 ^{d}	1.33 ^{D}
Hronec	1.96 ^{a}	1.72 ^{A}	9.31 ^{a}	6.07 ^{B}	1.54 ^{b}	1.24 ^{B}
Vazec	2.85 ^{e}	1.97 ^{DE}	14.86 ^{e}	7.31 ^{J}	2.18 ^{g}	1.73 ^{H}
Samuel	2.96 ^{f}	1.94 ^{CD}	15.15 ^{f}	6.86 ^{G}	1.96 ^{e}	1.35 ^{D}
Oliver	2.85 ^{e}	2.02 ^{FG}	14.80 ^{e}	6.95 ^{GH}	1.98 ^{e}	1.27 ^{BC}
Otakar	2.84 ^{e}	1.99 ^{EF}	14.68 ^{e}	6.55 ^{E}	1.98 ^{e}	1.52 ^{EF}
Kamil	2.67 ^{c}	1.94 ^{CD}	13.79 ^{d}	7.16 ^{I}	1.97 ^{e}	1.62 ^{G}
Tibor	2.76 ^{d}	2.03 ^{FG}	12.71 ^{c}	6.69 ^{F}	1.87 ^{d}	1.13 ^{A}
Saul	2.65 ^{c}	1.93 ^{CD}	11.41 ^{b}	5.87 ^{A}	1.75 ^{c}	1.58 ^{FG}

Differences between samples of the same cultivation year at a significance level of $p < 0.05$ are marked with different superscript letters. Differences between samples from 2014 are marked with lower case letters, differences between the 2015 samples with capital letters.

Table 4. Contents of avenanthramides 2p, 2f and 2c, and the total amount of these avenanthramides in mg/100 g dry matter. Analysis was performed in duplicates.
Tabelle 4. Gehalte an den Avenanthramiden 2p, 2f und 2c und der Gesamtgehalt an diesen Avenanthramiden in mg/100 g Trockensubstanz. Die Analyse erfolgte in Duplikaten.

Cultivar	2p (mg/100 g)		2f (mg/100 g)		2c (mg/100 g)		total (mg/100 g)	
	2014	2015	2014	2015	2014	2015	2014	2015
Effektiv	3.79 ^{a}	0.59 ^{A}	5.60 ^{a}	0.71 ^{A}	2.69 ^{a}	0.45 ^{A}	12.08 ^{a}	1.75 A
Max	3.47 ^{a}	0.58 ^{A}	5.83 ^{a}	0.88 ^{B}	3.36 ^{b}	0.66 ^{B}	12.66 ^{a}	2.11 C
Klimt	9.32 ^{de}	1.03 ^{B}	12.05 ^{c}	1.08 ^{C}	9.57 ^{f}	0.95 ^{C}	30.94 ^{d}	3.06 D
Tatran	8.86 ^{cd}	1.78 ^{D}	13.93 ^{e}	2.57 ^{J}	7.14 ^{e}	1.89 ^{H}	29.93 ^{d}	6.24 J
Hronec	3.83 ^{a}	0.60 ^{A}	7.38 ^{b}	0.77 ^{A}	2.80 ^{a}	0.49 ^{A}	14.01 ^{b}	1.85 B
Vazec	8.47 ^{c}	1.43 ^{C}	14.21 ^{e}	1.55 ^{E}	7.22 ^{e}	1.36 ^{E}	29.91 ^{d}	4.34 F
Samuel	7.28 ^{b}	1.43 ^{C}	13.00 ^{d}	1.84 ^{F}	6.86 ^{de}	1.52 ^{F}	27.14 ^{c}	4.79 H
Oliver	10.37 ^{f}	1.33 ^{C}	15.54 ^{f}	1.95 ^{H}	6.87 ^{de}	1.18 ^{D}	32.78 ^{e}	4.46 G
Otakar	11.76 ^{g}	1.80 ^{D}	17.92 ^{g}	2.11 ^{I}	7.25 ^{e}	1.54 ^{F}	36.94 ^{f}	5.45 I
Kamil	8.77 ^{cd}	1.83 ^{D}	12.52 ^{cd}	1.90 ^{G}	6.46 ^{cd}	1.71 ^{G}	27.75 ^{c}	5.43 I
Tibor	8.93 ^{cd}	1.36 ^{C}	12.53 ^{cd}	1.58 ^{E}	6.05 ^{c}	1.22 ^{D}	27.51 ^{c}	4.17 E
Saul	9.69 ^{ef}	1.07 ^{B}	14.56 ^{e}	1.16 ^{D}	6.13 ^{c}	0.84 ^{C}	30.39 ^{d}	3.07 D

Differences between samples of the same cultivation year at a significance level of $p < 0.05$ are marked with different superscript letters. Differences between samples from 2014 are marked with lower case letters, differences between the 2015 samples with capital letters.

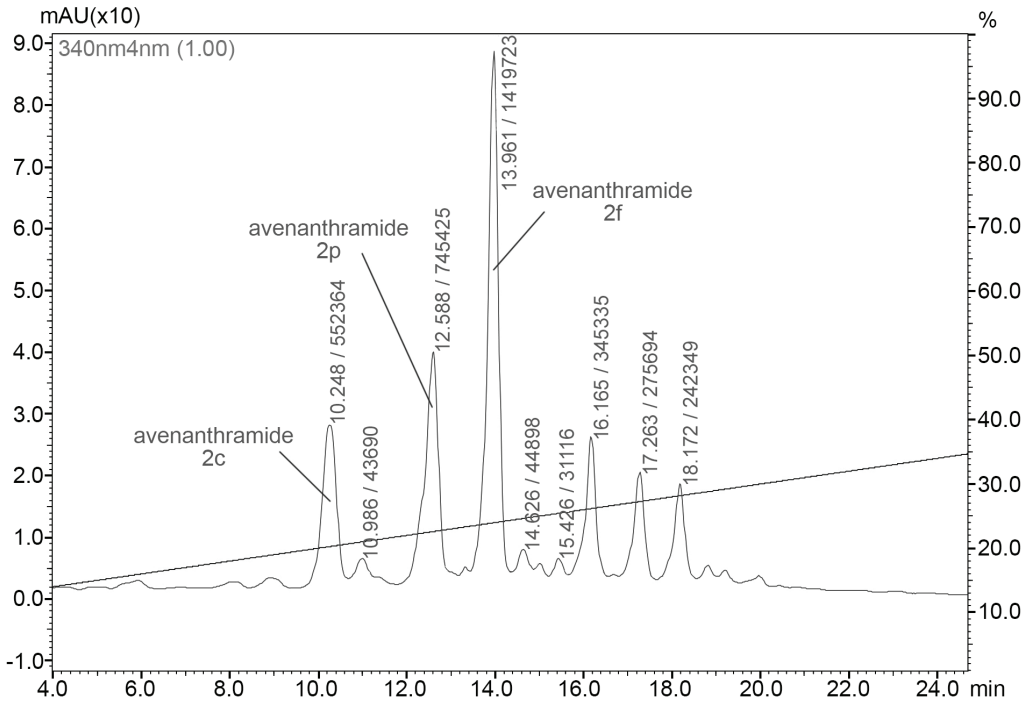

Figure 1. RP-HPLC chromatogram of avenanthramides
Abbildung 1. RP-HPLC Spektrum der Avenanthramide

Table 5. Concentration of albumins, globulins and prolamins in % per dry matter. Results of albumins and prolamins were analyzed in triplicates, prolamins were analyzed in duplicates.
Tabelle 5. Konzentrationen an Albuminen, Globulinen und Prolaminen in % der Trockensubstanz. Albumine und Globuline wurden in Dreifachbestimmung gemessen, Prolamine wurden in Duplikaten analysiert.

Cultivar	Albumins (%)		Globulins (%)		Prolamins (%)	
	2014	2015	2014	2015	2014	2015
Effektiv	1.02 ab	1.53 BC	4.61 a	4.08 BCD	0.57 ab	0.68 C
Max	0.95 a	1.51 B	4.40 a	3.93 BC	0.59 abc	0.59 A
Klimt	1.25 efg	1.56 BCD	6.83 d	5.03 EF	1.06 d	0.83 F
Tatran	1.35 fg	1.58 BCD	4.77 ab	4.58 DEF	0.78 bc	0.76 D
Hronec	1.35 efg	1.65 CDE	4.66 ab	3.57 AB	0.80 bcd	0.60 A
Vazec	1.18 bcd	1.52 BCD	4.84 ab	4.48 AB	1.45 e	0.79 DEF
Samuel	1.21 cde	1.48 B	5.24 bc	4.03 BCD	0.49 a	0.65 C
Oliver	1.58 h	1.76 E	4.46 a	3.18 A	0.49 a	0.60 AB
Otakar	1.08 abc	1.93 F	5.25 bc	4.80 EF	0.74 abc	0.77 DE
Kamil	1.37 g	1.46 AB	5.52 c	3.96 BC	0.49 a	0.82 EF
Tibor	1.04 ab	1.31 A	4.50 a	3.81 B	0.64 abc	0.62 ABC
Saul	1.30 efg	1.70 DE	4.71 ab	5.11 F	0.85 cd	0.68 C

Differences between samples of the same cultivation year at a significance level of $p < 0.05$ are marked with different superscript letters. Differences between samples from 2014 are marked with lower case letters, differences between the 2015 samples with capital letters.

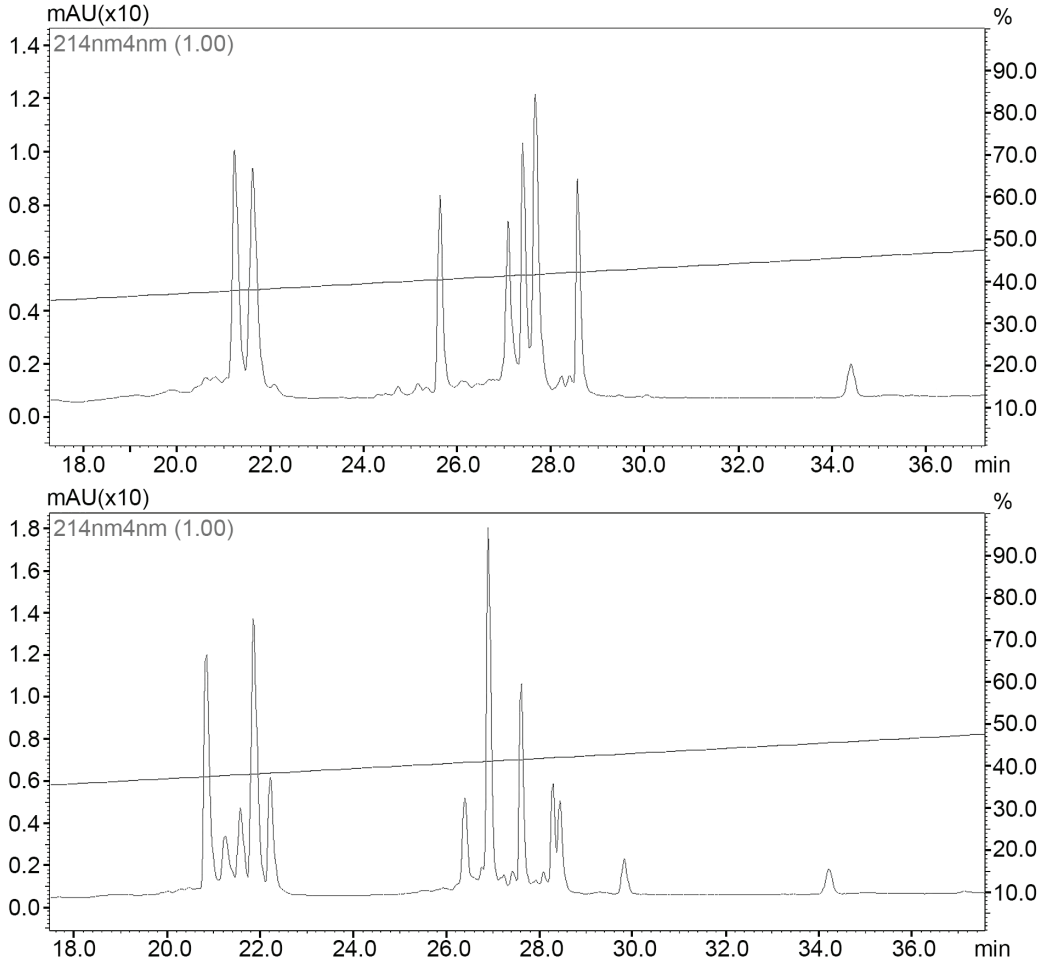

Figure 2. RP-HPLC chromatogram of prolamins, cultivars Effektiv (above) and Tatran
Abbildung 2. RP-HPLC Spektren der Prolaminfraktion, hier von den Sorten Effektiv (oben) und Tatran

showed the lowest concentration of tocopherols and tocotrienols of all analyzed cultivars. Cultivars Tatran, Vazec, Otakar, Kamil and Tibor had a similar profile to Klimt, but showed lower protein contents, and Otakar, Kamil and Tibor had substantially higher tocopherol and tocotrienol contents. Otakar notably had the highest concentration of avenanthramides in both analyzed years. Cultivars Samuel, Oliver and Saul were relatively low in fat and had high antioxidant capacity and total phenolic content. Protein content was high in cultivar Samuel but lower in Oliver and Saul. The β-glucan content was low in all analyzed cultivars, and the cultivars Oliver and Otakar showing especially poor results.

The hot, dry weather conditions of 2015 negatively affected protein and total phenolic content. Antioxidant capacity was also higher in 2014 than in 2015, with results obtained by FRAP-assay showing stronger differences between the two analyzed years than those obtained with $ABTS^{\bullet+}$-scavenging assay. However, α-tocopherol concentrations in cultivars Vazec, Samuel, Oliver, Otakar, Kamil, Tibor and Saul were higher in 2015 than in 2014; while in the other cultivars, α-tocopherol concentrations were relatively constant in both years. The concentrations of α-tocotrienol were higher in 2015 than in 2014 in all cultivars.

Cultivars Effektiv, Max, Samuel, Oliver, Kamil and Tibor showed the lowest levels of prolamins and might therefore be used for the selection of oat cultivars that are suitable for consumption by celiac patients. Further research to study the immunogenicity of the different cultivars is necessary to gain further insight as to which cultivars might be tolerated by celiac patients.

Historically, husked oat has been preferred over naked oat because of its suitability as horse feed, and their higher resistance to kernel damage. Naked oat has the advantage of being free-threshing, but show lower yields and are more susceptible to kernel damage during harvest, and mold (Kirkkari et al., 2001; Arendt and Zannini, 2013). Also, the fine trichomes covering naked oat kernels can act as irritants and have to be removed by polishing prior to further processing, but trichome numbers vary between cultivars (Kirkkari et al., 2009). This study showed naked oat has a more nutritionally beneficial profile, especially regarding fat and protein content and antioxidant properties. Especially for cultivation for human consumption, these nutritional advantages might outweigh the disadvantage of the more delicate kernels of naked oat.

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Supplemental Table 1. Maintainers of cultivars according to the EU Commission Plant Variety Database
Zusatztabelle 1. Inhaber der untersuchten Sorten laut Plant Variety Database der EU Kommission

Cultivar	Maintainer	Country
Effektiv	Landwirtschaftliche Fachschule Saatzucht Edelhof	Austria
Max	Saatzucht Bauer GmbH & Co KG	Germany
Klimt	Klimt Gerhard, Ing.	Austria
Tatran	Národné poľnohospodárske a potravinárske centrum, VÚRV Piešťany	Slovakia
Hronec	Národné poľnohospodárske a potravinárske centrum, VÚRV Piešťany	Slovakia
Vazec	Národné poľnohospodárske a potravinárske centrum, VÚRV Piešťany	Slovakia
Samuel	Groetzner Management GmbH, Germany & Nufarm GmbH	Germany
Oliver	Selgen, a.s.	Czechia
Otakar	Selgen, a.s.	Czechia
Kamil	Selgen, a.s.	Czechia
Tibor	Selgen, a.s.	Czechia
Saul	Selgen, a.s.	Czechia

Supplemental Table 2. Variance components for the analyzed parameters calculated from the mean squares of the multi-factor analysis of variance. Factor Cultivar: 11 degrees of freedom (DF), factor year: 1 DF
Zusatztabelle 2. Varianzkomponenten der analysierten Parameter aus den mittleren Abweichungsquadraten der multifaktoriellen Varianzanalyse. Faktor Sorte: 11 Freiheitsgrade, Faktor Jahr: 1 Freiheitsgrad

	Ash		β-glucan		Fat		Protein		Albumins		Globulins		Prolamins	
	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Cultivar	0.127	<0.001	2.485	<0.001	4.388	<0.001	5.118	<0.001	0.091	<0.001	0.999	<0.001	0.111	<0.001
Year	0.002	0.672ns	0.450	<0.001	0.429	<0.001	74.701	<0.001	1.801	<0.001	6.803	<0.001	0.032	0.472ns
C × Y	0.044	<0.001	1.171	<0.001	0.140	<0.001	0.851	<0.001	0.051	<0.001	0.362	<0.001	0.064	<0.001
Residual	0.009		0.029		0.008		0.007		0.006		0.078		0.007	

	α-tocopherol		α-tocotrienol		Total tocols		Total avenanthramides		ABTS		FRAP		TPC	
	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Cultivar	71.791	<0.001	100.588	<0.001	282.819	<0.001	7773.280	<0.001	398.537	<0.001	17.204	<0.001	0.416	<0.001
Year	1086.510	<0.001	1455.690	<0.001	5057.450	<0.001	520154.000	<0.001	15754.800	<0.001	1743.050	<0.001	7.408	<0.001
C × Y	98.675	<0.001	39.229	<0.001	221.882	<0.001	4401.620	<0.001	122.773	<0.001	10.693	<0.001	0.184	<0.001
Residual	0.399		1.715		2.320		15.060		1.244		0.028		0.003	

MS = mean squares, ns = not significant

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