Heliyon 6 (2020) e04304

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Avenanthramides as lipoxygenase inhibitors

Rikard Landberg [a,c], Kerstin Sunnerheim [b], Lena H. Dimberg [a,d,*]

[a] Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Department of Chemical Engineering, Mid Sweden University, Sundsvall, Sweden
[c] Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[d] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

ARTICLE INFO

Keywords:
Food science
Food analysis
Pharmaceutical chemistry
Pharmaceutical science
Phenol
Oats
Cinnamic acid
Structure-activity relationship
Antioxidant
Anti-inflammatory
Anthranilic acid

ABSTRACT

Avenanthramides (AVAs) present in oats are amides of anthranilic and cinnamic acids. AVAs are potent antioxidants and have anti-inflammatory properties. There are various potential mechanisms for their anti-inflammatory effects, including inhibition of lipoxygenases (LOX), which catalyse oxygenation of polyunsaturated fatty acids into potent signal molecules involved in inflammatory processes. In this study, AVAs were screened for LOX inhibition *in vitro* and structure-activity relationships were examined. Twelve different AVAs at 0.6 mM were tested as LOX inhibitors. The corresponding free cinnamic acids, the AVA analogue Tranilast® and the known LOX inhibitor *trans*-resveratrol were included for comparison. It was found that AVAs comprising caffeic or sinapic acid exhibited significant lipoxygenase inhibition (60–90%) ($P < 0.05$), whereas low or no inhibition was observed with AVAs containing *p*-coumaric or ferulic acid. No difference in inhibition was seen on comparing AVAs with their free corresponding cinnamic acids, which implies that the anthranilic acid part of the avenanthramide molecule does not affect inhibition. *Trans*-resveratrol showed inhibition, whereas no inhibition was seen for Tranilast® at the concentrations used in this study. This study suggests that aventahtramides comprising caffeic acid or sinapic acid partly exert their antioxidant and anti-inflammatory effects via lipoxygenase inhibition.

1. Introduction

Lipid oxidation is an important process in many different biological systems. In a food context, lipid oxidation is one of the major causes of food deterioration, as it may lead to off-flavours, changes in colour and texture and also decreased nutritional value of food products (Croguennec; 2016). Lipid oxidation can proceed in either a non-enzymatic or an enzymatic fashion. Lipoxygenases (LOX) constitute a family of non-haem iron-containing enzymes that catalyst oxidation of fatty acids and are widely distributed in plants and animals (Hildebrand, 1989; Kuhn et al., 2015; Horn et al., 2015). In plants, the substrate is mainly linoleic acid (LA) (C18:2, n-6) and α-linolenic acid (ALA) (C18:3, n-3), whereas in mammals it is arachidonic acid (AA) (C20:4, n-6). In mammals vegetable LA can be transformed to AA though enzyme mediated elongation and desaturation reactions. The same reactions also transform ALA to eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3). Lipid oxidation can be a problem in living systems, by altering the function of membranes and lipoprotein particles (Kuhn et al., 2015). In mammalian cells, LOX catalyse the initial step in the conversion of AA to the hydroperoxide, 5-hydroperoxy-6,8,11, 14-eicostatetraenoic acid, and are thereby key enzymes in the biosynthesis of a variety of bio-regulatory compounds such as leukotrienes (Pergola and Werz, 2010; Häggström and Funk, 2011; Rådmark et al., 2015). Leukotrienes are considered to be potent mediators of pro-inflammatory reactions. LOX enzymes are also associated to biosynthesis of compounds which may promote termination of acute inflammation reactions, and thereby function as anti-inflammatory agents. Such compounds are for example lipoxins, which are biosynthesised via oxygenation of AA, and resolvins, biosynthesised though oxygenation of the ω-3 fatty acids EPA and DHA (Pirault and Bäck, 2018; Recchiuti et al., 2019; Kuhn et al., 2015; Rådmark et al., 2015). Acute inflammation is a protective process. However, overbalance of pro-inflammatory vs anti-inflammatory agents over time may result in a variety of chronic inflammatory diseases, such as arthritis, bronchial asthma, and cardiovascular diseases (Yoon and Baek, 2005; Pergola and Werz, 2010; Chen, 2011; Häggström and Funk, 2011; Kuhn et al., 2015; Bruno et al., 2018). Therefore, inhibition of the pro-inflammatory LOX pathway is suggested to be interesting for prevention of these disesases. Many of the LOX inhibitors are found among the class of phenolic compounds, which have radical scavenging and/or iron chelating properties,

* Corresponding author.
E-mail address: Lena.Dimberg@slu.se (L.H. Dimberg).

https://doi.org/10.1016/j.heliyon.2020.e04304
Received 6 June 2019; Received in revised form 21 January 2020; Accepted 22 June 2020

and among fatty acid analogues (Yoon and Baek, 2005; Schneider and Bucar, 2005a, 2005b; Pergola and Werz, 2010; Chen, 2011).

Avenanthramides (AVAs) belong to a group of phenols comprising anthranilic and cinnamic acid derivatives (Figure 1a; Table 1). Among cereals, AVAs are found only in oats and so far 15 compounds have been identified (Dimberg et al., 1993; Matsukawa et al., 2000; Bratt et al., 2003; Collins, 2011; Ishihara et al., 2014; Readelli et al., 2015; Pridal et al., 2018; de Bruijn et al., 2019). The three most abundant AVAs in oat groats are **2p**, **2f** and **2c,** the **2** indicating 5-hydroxyanthranilic acid (as opposed to **1** for anthranilic acid, **3** for 5-hydroxy-4-methoxyanthranilic acid, **4** for 4-hydroxy anthranilic acids and **5** for 4,5-dihydroxyanthranilic acid), and the **p, f** and **c** indicating the cinnamic acids *p*-coumaric, ferulic and caffeic acid, respectively (Bratt et al., 2003; Fagerlund et al., 2009; Dimberg and Jastrebova, 2009; Ishihara et al., 2014; Readelli et al., 2015; de Bruijn et al., 2019). Other AVAs with cinnamic acids replaced by the corresponding avenalumic acids (5-phenyl-penta-2,4 dienoic) are also present in oats (Dimberg and Jastrebova, 2009; Collins, 2011; Ishihara et al., 2014; Pridal et al., 2018; de Bruijn et al., 2019). Sinapic acid (**s**) is found in oats, but no AVAs derived from this acid have been found in oat plant (Bratt et al., 2003; Collins, 2011).

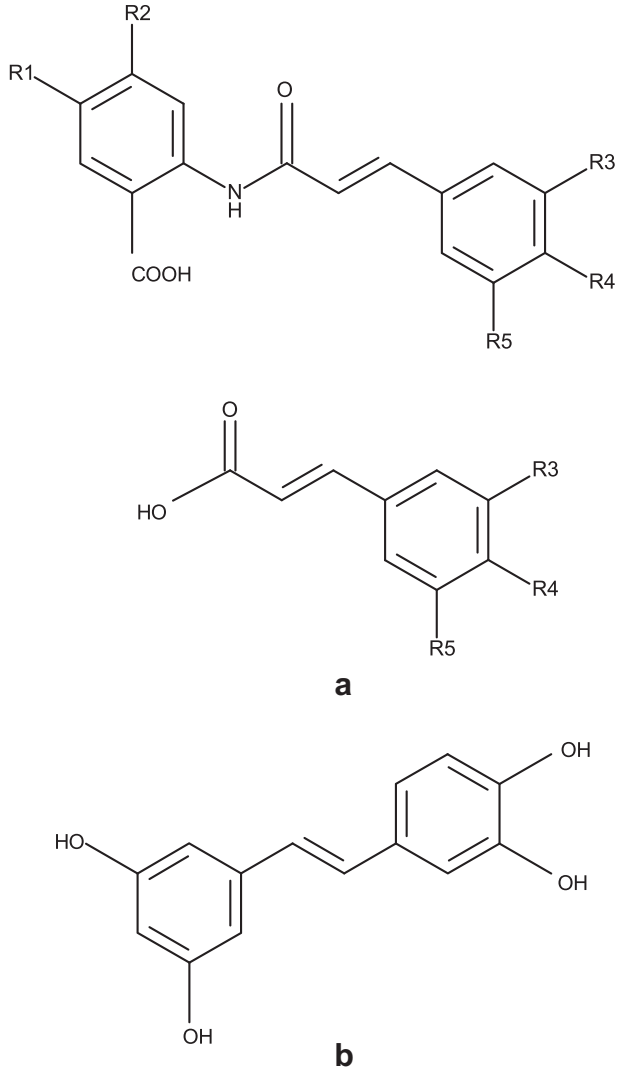

Figure 1. Structures of compounds used in the experiments: a) avenanthramides (**1s**, **2s** and **3s** are not found in oats), Tranilast®, and cinnamic acids (see Table 1 for the substitution pattern of the molecular skeletons) and b) *trans*-resveratrol.

Consumption of oats has been linked to a decreased risk of several chronic diseases and it has been suggested that AVAs contribute to the protective effects (Meydani, 2009; Tripathi et al., 2018). In *in vitro* studies, AVAs have been characterised as antioxidants with a clear structure-activity relationship (Dimberg et al., 1993; Bratt et al., 2003; Fagerlund et al., 2009; Lee-Manion et al., 2009; Ishihara et al., 2014). They have also been shown to be bioavailable (Zhang et al., 2017) and to exert antioxidant activity *in vivo* (Ji et al., 2003; Chen et al., 2004, 2007; Koenig et al., 2014). Furthermore, they have been found to exhibit anti-inflammatory, anti-proliferative and anti-itch activities *in vitro* and *in vivo* (Nie et al., 2006a, 2006b; Sur et al., 2008; Koenig et al., 2014; Reynertson et al., 2015; Hastings and Kenealey, 2017; Scarpa et al., 2018). In a food context, AVAs have been shown to be correlated to the fresh oat taste of oat products (Molteberg et al., 1996).

In this study it is hypothesized that AVAs partly protect oat foods from rancidity development through inhibition of LOX activity. Furthermore, a hypothesis is that AVAs may inhibit LOX and/or cyclooxygenase (COX-2) enzymes in mammals and thereby decrease the production of pro-inflammatory compounds, such as leukotrienes and prostaglandins. An AVA-enriched oat extract has been shown to inhibit cyclooxygenase (COX-2) activity and pro-inflammatory prostaglandin production from AA in mouse macrophages (Guo et al., 2010). Furthermore, a butanolic fraction of oat groats has been found to have anti-platelet activities mediated though inhibition of the COX and LOX pathways (Ahmed et al., 2013). The authors of the latter study did not speculate about the possible compounds involved, but a high amount of AVAs is probably present in a butanol extract, since methanol and ethanol are good extracting solvents for AVAs (Dimberg and Jastrebova, 2009).

The aim of the present work was to investigate whether pure AVAs and their corresponding free cinnamic acids (Figure 1a; Table 1), have LOX inhibitor activity *in vitro*. A further aim was to establish a structure-inhibition activity relationship. *Trans*-resveratrol (Fig. 1b), a phenolic stilbene known to possess LOX inhibitor activity (Fan and Mattheis, 2001; Chatterjee et al., 2011), was used as a positive control. Tranilast® (Figure 1a; Table 1), a known non-phenolic drug with anti-inflammatory properties (Pae et al., 2008; Darakhshan and Pour, 2015) and with remarkable chemical similarities to the AVAs was used as a negative control (Komatsu et al., 1988).

2. Materials and methods

2.1. Chemicals

Lipoxygenase, LOX 1 from soybean, (EC 1.13.11.12), was purchased from SIGMA Chemicals Co. (St. Louis, MO, USA), linoleic acid from Calbiochem® (U.S and Canada), sinapic acid from Fluka Chemie AG (Buchs SG, Switzerland) and ferulic-, caffeic- and *p*-coumaric acid from SIGMA Chemicals Co. (St. Louis, MO, USA). All AVAs were synthesised according to Bratt et al. (2003) (**1**- and **2**-series) or Fagerlund et al. (2009) (**3**-series). All other chemicals used were *pro analysi* and were used without further purification.

2.2. HPLC analysis

Reverse-phase high performance liquid chromatography (RP-HPLC) analysis was performed with an HP series 1100 instrument (Hewlett Packard, Waldbronn, Germany) equipped with diode array detector (DAD) and a HP ODS Hypersil column (5 μm, 125 mm × 4 mm). The isocratic mobile phase was acetonitrile:0.01M phosphate buffer (pH 2.8) containing 5% acetonitrile (70:30). Sample size injected was 10 μL and the flow rate was 1 mL/min. Products of LOX activity were detected at 234 nm (Villafuerte Romero and Barrett, 1997). Peaks were identified from retention times (Figure 2) and UV-spectra, and were manually integrated using the software HP ChemStation Version 05.01.

Table 1. Structures of compounds used in the experiments (refers to Figure 1a).

	R1	R2	R3	R4	R5
Avenanthramide[1]					
1p	H	H	H	OH	H
2p	OH	H	H	OH	H
3p	OH	OCH_3	H	OH	H
1f	H	H	OCH_3	OH	H
2f	OH	H	OCH_3	OH	H
3f	OH	OCH_3	OCH_3	OH	H
1c	H	H	OH	OH	H
2c	OH	H	OH	OH	H
3c	OH	OCH_3	OH	OH	H
1s	H	H	OCH_3	OH	OCH_3
2s	OH	H	OCH_3	OH	OCH_3
3s	OH	OCH_3	OCH_3	OH	OCH_3
Tranilast®	H	H	OCH_3	OCH_3	H
Cinnamic acid					
p			H	OH	H
f			OCH_3	OH	H
c			OH	OH	H
s			OCH_3	OH	OCH_3

[1] **1:** anthranilic acid; **2:** 5-hydroxyanthranilic acid; **3:** 5-hydroxy-4-methoxyanthranilic acid; **p**: *p*-coumaric acid; **f**: ferulic acid; **c**: caffeic acid; **s**: sinapic acid.

2.3. Preparation of solutions

Enzyme solution was prepared by thoroughly mixing 4 μL enzyme with 20 mL 0.1 M borate buffer ($Na_3(BO)_3$/HCl), pH 9. Substrate solution was prepared by dispersing 15 μL linoleic acid in 60 mL 0.1 M borate buffer (pH 9) containing 15 μL Tween 20, shaking thoroughly and then sonicating for 1 min. AVAs, Tranilast®, *trans*-resveratrol and the cinnamic acid derivatives were prepared as 9 mM stock-solutions in ethanol/borate buffer (15/85). All solutions were used promptly to avoid autoxidation and degradation of enzyme.

2.4. LOX assay

Enzyme solution (100 μL) was mixed with 200 μL of a stock-solution of the test compound and the mixture was incubated for 10 min. Then 2.7 mL of linoleic acid solution were added and the mixture was vortexed and incubated in a 30 °C water bath for 30 min. The enzymatic reaction was stopped by adding 100 μL of concentrated hydrochloric acid. The reaction mixture was extracted with 3.0 mL of ethyl acetate. A 2 mL sample of the organic phase was collected and evaporated to dryness in a centrifuge evaporator. The solid residue was dissolved in 200 μL of methanol and analysed by HPLC. As a control sample, ethanol/buffer solution without added test compound was used. In addition, a control in which hydrochloric acid was added to the enzyme solution prior to linoleic acid, in order to inactivate the enzyme, was included (Figure 2).

In order to compare inhibition of the different compounds, the same molar concentration (0.6 mM) in the final test solution was used in all tests. In a pre-test it was found that 0.6 mM was appropriate for ca 50% inhibition of the enzyme activity when using caffeic acid as the inhibitor.

For practical reasons, the controls and four test compounds were analysed in each experiment. Seven experiments (A-G) were performed to study the structure-LOX-inhibition activity relationships for AVAs/cinnamic acids. In the first four experiments (A-D), the importance of the substitution pattern in the antranilic acid part of the AVAs was elucidated, i.e. the cinnamic acid part was the same within each experiment. The following three experiments (E-G) studied the influence of the substitution pattern in the cinnamic acid part of the compounds, i.e. the anthranilic acid part was the same within each experiment (due to lack of material, the experiment with the **3**-series was omitted). The inhibition capacity of Tranilast® and *trans*-resveratrol was tested in one additional experiment (H).

Since the enzyme activity in the buffer solution decreased with time, and to avoid autoxidation of the substrate each experiment was performed with freshly prepared enzyme and substrate solutions. Enzyme activity was measured as the amount of conjugated dienes formed from linoleic acid, expressed as area units. The inhibition was calculated as the enzyme activity difference between reaction with and without added test compound. Since the enzyme activity slightly differed between the experiments, inhibition was expressed as percentage of uninhibited sample within each experiment.

2.5. Statistics

Analysis of variance with Tukey's pairwise comparison ($\alpha = 0.05$) was conducted using the software Minitab release 11.12 (Minitab Inc., State College, PA). Each experiment (A-H) was run separately with three replicates of each compound (except for **2s** in experiment G, which was run with two replicates). All comparisons were made within each experiment (A-H) since the conditions between experiments varied somewhat.

3. Results

The lipoxygenase activity expressed as the relative amount of conjugated diene formed from linoleic acid in the presence of the test compounds is shown in Figure 3, where each letter (A-H) represents one experiment.

3.1. Structure-activity relationship of LOX inhibition

3.1.1. Influence of the anthranilic acid moiety

No significant difference in inhibition activity was found between the free cinnamic acids *p*-coumaric acid (**p**), ferulic acid (**f**), caffeic acid (**c**) and sinapic acid (**s**) and their corresponding AVAs (Figure 3, experiments A-D), except that **s** showed significantly higher inhibition than **3s**. Moreover, no differences were found within the **1**-, **2**- or **3**- series of AVAs (experiments A-D) except for **2f**, which exerted slightly higher inhibition than **3f,** and for **3s**, which showed lower inhibition than **1s** and **2s**. Compared to controls, the **p**-serie inhibited the LOX enzyme 0–30% (experiment A), the **f**-serie 0–20% (experiment B), the **c**-serie 50–70%

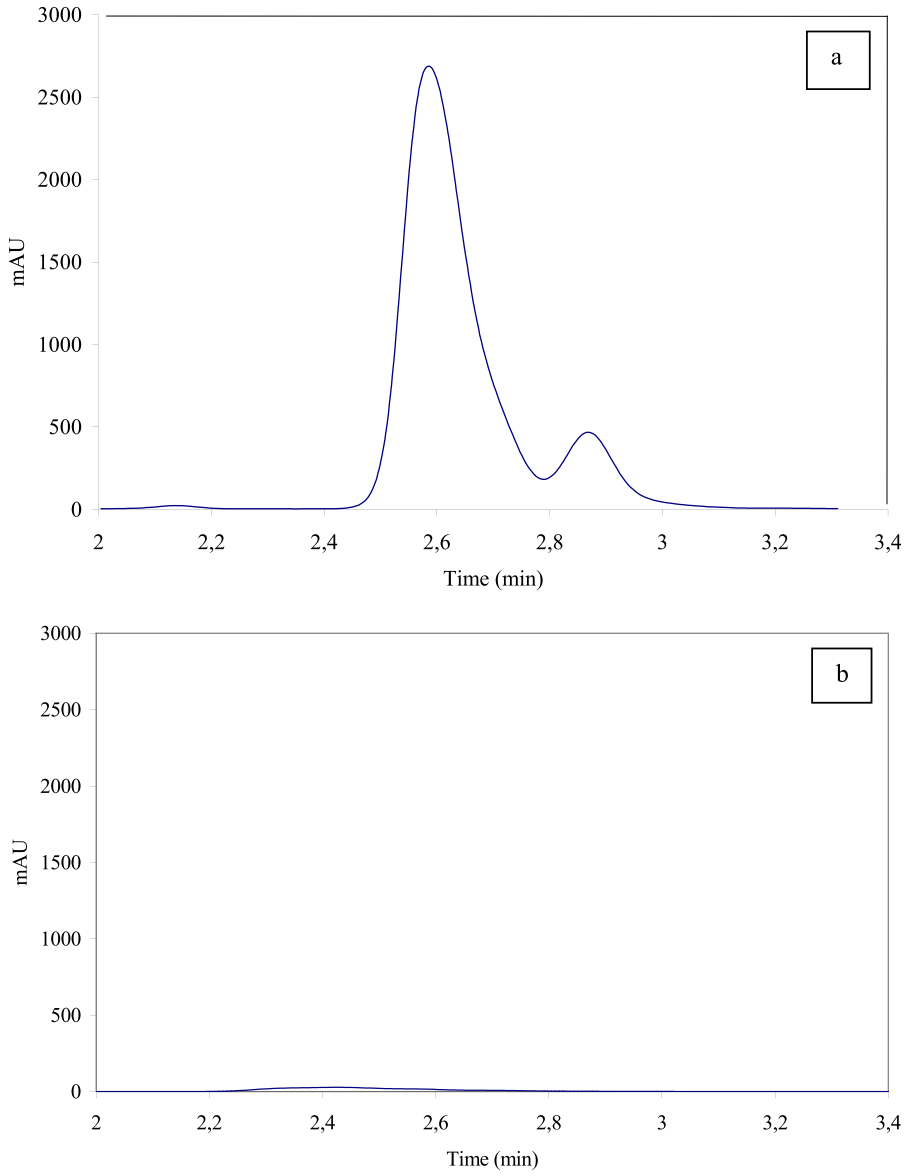

Figure 2. Reverse-phase chromatogram of lipoxygenase products detected at 234 nm of a) an active lipoxygenase enzyme and b) an inactivated lipoxygenase enzyme.

(experiment C) and the **s**-serie 60–90% (experiment D). These results indicate that the anthranilic acid part of the avenanthramides was of minor importance for the inhibition.

3.1.2. *Influence of the cinnamic acid moiety*

Among the free cinnamic acids, caffeic (**c**) and sinapic (**s**) acids showed significant inhibition of LOX activity (60–90%) compared to the control, whereas *p*-coumaric (**p**) and ferulic (**f**) acids showed low or no inhibition (Figure 3, experiments A-D, E). The difference in inhibition between **c** and **s** was not significant (experiment E). A similar pattern was observed when the **1**-series of AVAs was studied (experiment F), i.e. **1c** and **1s** significantly inhibited LOX, whereas **1p** and **1f** did not. Again, there was no significant difference between the inhibition of **1c** and **1s**. The experiment with the **2**-series of avenanthramides showed that **2c** and **2s**, but also **2f**, had significant inhibitory effects and that all were significantly different in their activity, following the order **2s** > **2c** > **2f** (experiment G). The LOX activity was approximately 1.2 times higher in the sample with AVA **2p** compared with the control (experiment G). However, this was not observed in experiment A. The **3**-series could not be evaluated in this context, due to lack of material. However, from experiments A-D it was evident that **3c** and **3s** inhibited the LOX activity by 50–60% and **3p** inhibited it by around 30%, whereas no inhibitory activity was found for **3f**. This confirms that the cinnamic acid part of the AVAs, especially those containing caffeic and sinapic acids, was important for LOX inhibition.

3.1.3. *Controls*

Trans-resveratrol was an effective lipoxygenase inhibitor when present in the assay at a concentration of 0.60 mM, but Tranilast® was not (Figure 3, experiment H).

4. Discussion

Although it has been shown that different phenols have LOX-inhibiting activity, the mechanisms involved have not been fully identified. In the active form of LOX, there is a ferric ion (Fe^{3+}) at the active site. When linoleic acid is oxidised, the electron from the hydrogen atom is transferred to Fe^{3+} and its proton to a proton acceptor. The Fe^{3+} ion is thus reduced to a ferrous ion (Fe^{2+}), rendering the enzyme inactive. The linoleic acid radical formed is re-arranged to a conjugated diene radical, which reacts with oxygen to form a peroxy radical. The peroxy radical reacts further with a proton and the electron from Fe^{2+} to form a

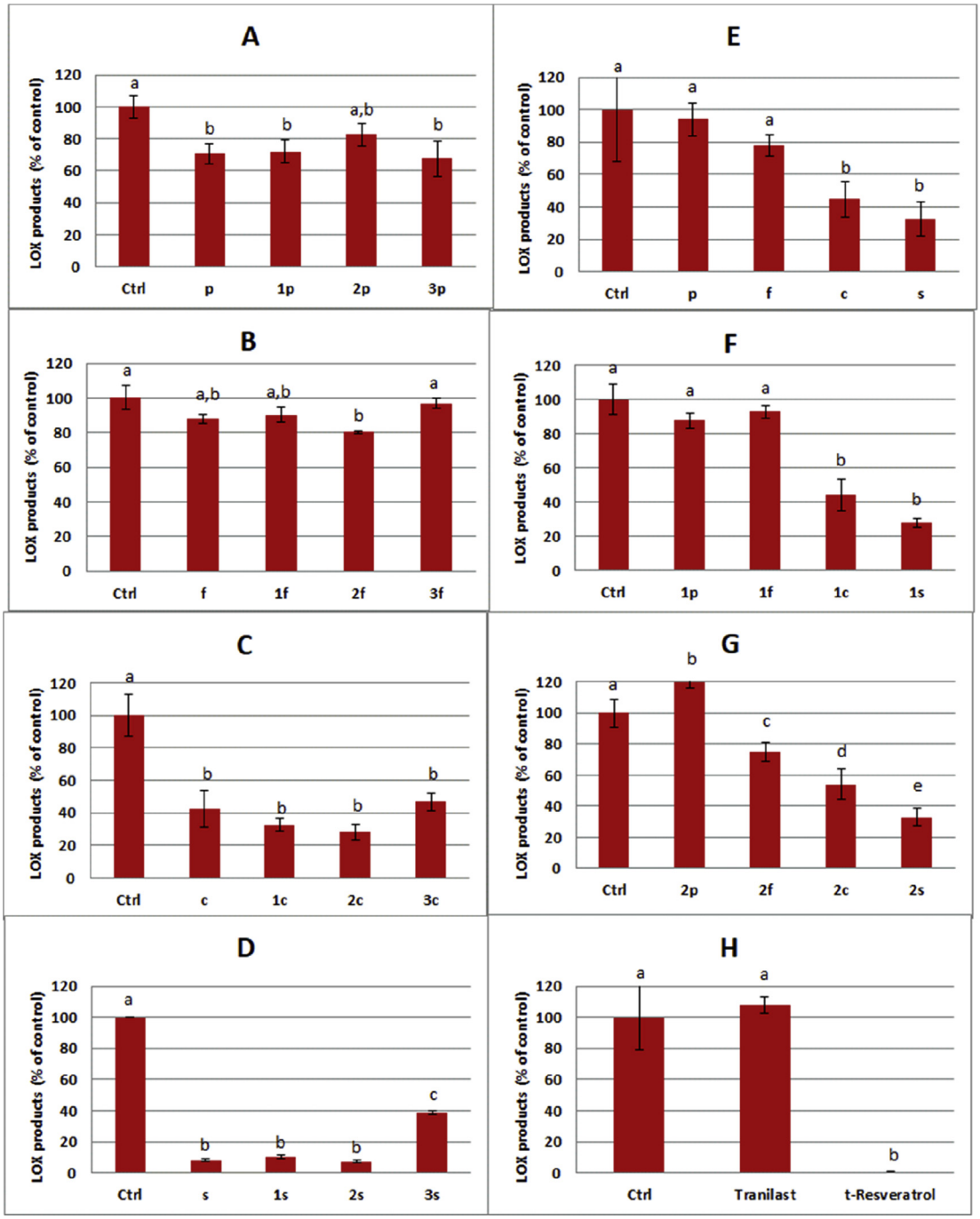

Figure 3. Products formed after lipoxygenase (LOX) activity on linoleic acid in the presence of avenanthramides, their corresponding cinnamic acids, Tranilast® or *trans*-resveratrol at 0.6 mM, expressed as percentage of controls (uninhibited samples). Each capital letter (A–H) represents one experiment. Error bars are given as coefficient of variation (CV) (except for **2s** in experiment G, which is given as the difference between the two replicates). Different superscript letters within each experiment A-H indicate significant differences ($p < 0.05$) between the compounds tested.

hydroperoxide, Fe^{2+} is oxidised to Fe^{3+}, the enzyme is thereby re-activated and the reaction can re-start with another linoleic acid (Hildebrand, 1989; Häggström and Funk, 2011). The mechanism of LOX inhibition can be explained in several ways, e.g. the inhibitor may scavenge the linoleic acid radicals formed, it may chelate or reduce the Fe^{3+} ion in the active site of the enzyme, or it may interact with the enzyme molecule.

Caffeic and sinapic acids and their corresponding AVAs were the most efficient inhibitors in the present study. These compounds are also strong antioxidants when tested in diphenylpicrylhydrazyl (DPPH) and non-enzymatic linoleic acid diene formation assays (Bratt et al., 2003; Fagerlund et al., 2009; Lee-Manion et al., 2009; Ishihara et al., 2014), both of which methods rely on a radical scavenging mechanism. The stronger antioxidation activity of **c** and **s** derivatives found was expected, as they are able to stabilise the phenolic radical formed better than **p** and **f** derivatives. However, if the enzymatic inhibition shown in the present study had been caused solely by radical scavenging, the **3**-series of AVAs would have been expected to be more active than the **2**-series, which in turn would have been more active than the **1**-series. This was not the case. Instead, it was found that there was no significant difference between the free cinnamic acids and their corresponding avenanthramides from the **1**-, **2**- and **3**-series (Figure 3, experiments A-D). We therefore

suggest that chelation of the Fe^{3+} iron within the active site of the enzyme is important. However, it seems that a phenolic structure is also important, since the non-phenolic molecule Tranilast®, with the same basic skeleton as the AVAs (Figure 1 a; Table 1), did not have any inhibitory properties under the test conditions. Tranilast® is an anti-inflammatory substance (Komatsu et al., 1988; Pae et al., 2008; Darakhshan and Pour, 2015), but the anti-inflammatory activity appears to be dependent on other mechanisms rather than through LOX inhibition. Other studies have also demonstrated that cinnamic acid analogues lacking the redox-active phenolic hydroxyl groups lose all LOX inhibitory activity and that radical scavenging is not sufficient to ensure efficient LOX inhibition (Doiron et al., 2017; Touaibia et al., 2018).

We could not determine whether the inhibition observed in the present study was caused by an interaction between the phenolic compounds and the enzyme, since the initial velocity of product formation was not monitored. However, it is unlikely that the active compounds acted as competitive inhibitors, since their structures are very different from that of the substrate, i.e. linoleic acid. Nevertheless, other interactions between inhibitors and the enzyme may occur, as other studies have shown that caffeic acid derivatives exert both non-competitive (Koshihara et al., 1984) and uncompetitive (Sud'ina et al., 1993) inhibition. These compounds have also been reported to inhibit the enzyme by chelating iron ions, thereby preventing catalysis (Young, 1999).

We recognize that high concentrations of AVAs have been used in our *in vitro* experiments. AVAs are bioavailable (Chen et al., 2004, 2007; Zhang et al., 2017), but whether dietary AVAs at intake levels normally achieved through consumption of oat products exert LOX inhibitory activity *in vivo*, and thereby inhibit production of pro-inflammatory compounds, remains to be elucidated. In this context it may also be of importance to emphasize that the lipid content in groats of various oat cultivars is comparably high (49–135 g kg^{-1}) and that LA and ALA comprise about 40% and 1%, respectively, of the fatty acids present (Bryngelsson et al., 2002; Sterna et al., 2016). Theoretically, this may imply that oat derived LA, as a precursor to AA, could adversely affect the inflammatory processes and thereby counteract the possible protective effect of the avenanthramides. However, studies performed on healthy humans consuming a Western diet, have failed to show that additional dietary intake of LA is positively associated with pro-inflammation markers, such as e.g. interleukin-6 (Il-6), interleukin-1β (IL-1β), tumour necrosis factor (TNF-α) and c-reactive protein (CRP) in the plasma (Innes and Calder, 2018; Fritsche, 2015).

In conclusion, AVAs have been shown to possess anti-inflammatory properties and the present study provides *in vitro* findings suggesting that this could be due to inhibition of LOX activity. These findings, together with findings from previous studies on antioxidant activity (Dimberg et al., 1993; Bratt et al., 2003; Fagerlund et al., 2009; Lee--Manion et al., 2009; Ishihara et al., 2014), may indicate that oat AVAs (especially the **c**-derivatives, as **s**-derivatives are not found in oats) can protect oat foods from rancidity development and contribute to anti-inflammatory effects in mammals. Further studies on foods and *in vivo* studies in mammals are needed to confirm this.

Declarations

Author contribution statement

Rikard Landberg: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kerstin Sunnerheim: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lena H. Dimberg: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Dr Katharina Bratt and Dr Amelie Fagerlund are acknowledged for synthesis of the avenanthramides used in this study.

References

Ahmed, S., Gul, S., Gul, H., Bangash, M.H., 2013. Anti-inflammatory and anti-platelet activities of *Avena sativa* are mediated through the inhibition of cyclooxygenase and lipoxygenase enzymes. Int. J. Endorsing Health Sci. Res. 1, 62–65.

Bratt, K., Sunnerheim, K., Bryngelsson, S., Fagerlund, A., Engman, L., Andersson, R.E., Dimberg, L.H., 2003. Avenanthramides in oats (*Avena sativa L.*) and structure-antioxidant activity relationships. J. Agric. Food Chem. 51, 594–600.

de Bruijn, W.J.C., van Dinteren, S., Gruppen, H., Vincken, J.-P., 2019. Mass spectrometric characterisation of avenanthramides and enhancing their production by germination of oat (*Avena sativa*). Food Chem. 277, 682–690.

Bruno, F., Spaziano, G., Liparulo, A., Roviezzo, F., Nabavi, S.M., Sureda, A., Filosa, R., D'Agostino, B., 2018. Recent advances in the search for novel 5-lipoxygenase inhibitors for the treatment of asthma. Eur. J. Med. Chem. 153, 65–72.

Bryngelsson, S., Mannerstedt-Fogelfors, B., Kamal-Eldin, A., Andersson, R., Dimberg, L.H., 2002. Lipids and antioxidants in groats and hulls of Swedish oats (*Avena sativa* L). J. Sci. Food Agric. 82, 606–614.

Chatterjee, M., Das, S., Janarthan, M., Ramachandran, H.K., Chatterjee, M., 2011. Role of 5-lipoxygenase in resveratrol mediated suppression of 7,12-dimethylbenz(α) anthracene-induced mammary carcinogenesis in rats. Eur. J. Pharmacol. Mol. Cell. Pharmacol. 668, 99–106.

Chen, C.Y., Milbury, P.E., Collins, F.W., Blumberg, J.B., 2007. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. J. Nutr. 137, 1375–1382.

Chen, C.Y., Milbury, P.E., Kwak, H.K., Collins, F.W., Samuel, P., Blumberg, J.B., 2004. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. J. Nutr. 134, 1459–1466.

Chen, S., 2011. Natural products triggering biological targets – a review of the anti-inflammatory phytochemicals targeting the arachidonic acid pathway in allergy asthma and rheumatoid arthritis. Curr. Drug Targets 12, 288–301.

Collins, F.W., 2011. Oat phenolics: biochemistry and biological functionality. In: Webster, F.H., Woods, P.J. (Eds.), Oats: Chemistry and Technology. AACC International, Inc., USA, pp. 157–217.

Croguennec, T., 2016. Lipid oxidation. In: Jeantet, R., Croguennec, T., Schuck, P., Brulé, G. (Eds.), Handbook of Food Science and Technology 1: Food Alteration and Food Quality. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 99–132.

Darakhshan, S., Pour, A.B., 2015. Tranilast: a review of its therapeutic applications. Pharmacol. Res. 91, 15–28.

Dimberg, L., Jastrebova, J., 2009. Quantitative analysis of oat avenanthramides. In: Shewry, P.R., Ward, J.L. (Eds.), Healthgrain Methods: Analysis of Bioactive Components in Small Grain Cereals. AACC International, Inc., USA, pp. 113–127.

Dimberg, L.H., Theander, O., Lingnert, H., 1993. Avenanthramides- A group of phenolic antioxidants in oats. Cereal Chem. 70, 637–641.

Doiron, J.A., Leblanc, L.M., Hébert, M.J.G., Levesque, N.A., Paré, A.F., Jean-François, J., Cormier, M., Surette, M.E., Touaibia, M., 2017. Structure–activity relationship of caffeic acid phenethyl ester analogs as new 5-lipoxygenase inhibitors. Chem. Biol. Drug Des. 89, 514–528.

Fagerlund, A., Sunnerheim, K., Dimberg, L.H., 2009. Radical-scavenging and antioxidant activity of avenanthramides. Food Chem. 113, 550–556.

Fan, X., Mattheis, J.P., 2001. Inhibition of oxidative and antioxidative enzymes by trans-resveratrol. Food Chem. Toxicol. 66, 200–203.

Fritsche, K.L., 2015. The science of fatty acids and inflammation. Adv. Nutr. 6, 293S–301S.

Guo, W., Nie, L., Wu, D., Wise, M.L., Collins, F.W., Meydani, S.N., Meydani, M., 2010. Avenanthramides inhibit proliferation of human colon cancer cell lines *in vitro*. Nutr. Canc. 62, 1007–1016.

Häggström, J.Z., Funk, C.D., 2011. Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. Chem. Rev. 111, 5866–5898.
Hastings, J., Kenealey, J., 2017. Avenanthramide-C reduces the viability of MDA-MB-231 breast cancer cells through an apoptotic mechanism. Canc. Cell Int. 17, 93–104.
Hildebrand, D.F., 1989. Lipoxygenases. Physiol. Plantarum 76, 249–253.
Horn, T., Adel, S., Schumann, R., Sur, S., Kakularam, K.R., Polamarasetty, A., Redanna, P., Kuhn, H., Heydeck, D., 2015. Evolutionary aspects of lipoxygenases and genetic diversity of human leukotriene signaling. Prog. Lipid Res. 57, 13–39.
Innes, J.K., Calder, P.C., 2018. Omega-6 Fatty Acids and inflammation. Prostaglandins Leukot. Essent. Fatty Acids 132, 41–48.
Ishihara, A., Kojima, K., Fujita, T., Yamamoto, Y., Nakajima, H., 2014. New series of avenanthramides in oat seed. Biosc. Biotech. Biochem. 78, 1975–1983.
Ji, L.L., Lay, D., Chung, E., Fu, Y., Peterson, D.M., 2003. Effects of avenanthramides on oxidant generation and antioxidant enzyme activity in exercised rats. Nutr. Res. 23, 1579–1590.
Koenig, R., Dickman, J.R., Kang, C., Zhang, T., Chu, Y.-F., Ji, L.L., 2014. Avenanthramide supplementation attenuates exercise-induced inflammation in postmenopausal women. Nutr. J. 13, 1–11.
Komatsu, H., Kojima, M., Tsutsumi, N., Hamano, S., Kusama, H., Ujiie, A., Ikeda, S., Nakazawa, M., 1988. Mechanism of inhibitory action of tranilast on the release of slow reacting substance of anaphylaxis (SRS-A) *in vitro*: effect of tranilast on the release of arachidonic acid and its metabolites. Jpn. J. Pharmacol. 46, 53–60.
Koshihara, Y., Neichi, T., Murota, S.-I., Lao, A.N., Fujimoto, Y., Tatsuno, T., 1984. Caffeic acid is a selective inhibitor for leukotriene biosynthesis. Biochim. Biophys. Acta 792, 92–97.
Kuhn, H., Banthiya, S., van Leyen, K., 2015. Mammalian lipoxygenases and their biological relevance. Biochim. Biophys. Acta 185, 308–330.
Lee Manion, A.M., Price, R.K., Strain, J.J., Dimberg, L.H., Sunnerheim, K., & Welch, R.W.. *In vitro* antioxidant activity and antigenotoxic effects of avenanthramides and related compounds. J. Agric. Food Chem. 57, 10619-10624
Matsukawa, T., Isobe, T., Ishihara, A., Iwamura, H., 2000. Occurrence of avenanthramides and hydroxycinnamoyl-CoA: hydroxyanthranilate N-hydroxycinnamoyltransferase activity in oat seeds. Z. Naturforsch. 55c, 30–36.
Meydani, M., 2009. Potential health benefits of avenanthramides of oats. Nutr. Rev. 67, 731–735.
Molteberg, E.L., Solheim, R., Dimberg, L.H., Frölich, W., 1996. Variation in oat groats due to vareity, storage and heat treatment. II: sensory quality. J. Cereal. Sci. 24, 273–282.
Nie, L., Wise, M.L., Peterson, D.M., Meydani, M., 2006. Avenanthramide, a polyphenol from oats, inhibits vascular smooth muscle cell proliferation and enhances nitric oxide production. Atherosclerosis 186, 260–266.
Nie, L., Wise, M., Peterson, D., Meydani, M., 2006. Mechanism by which avenanthramide-c, a polyphenol of oats, blocks cell cycle progression in vascular smooth muscle cells. Free Radic. Biol. Med. 41, 702–708.
Pae, H.O., Jeong, S.O., Koo, B.S., Ha, H.Y., Lee, K.M., Chung, H.T., 2008. Tranilast, an orally active anti-allergic drug, up-regulates the anti-inflammatory heme oxygenase-1 expression but down-regulates the pro-inflammatory cyclooxygenase-2 and inducible nitric oxide synthase expression in RAW264.7 macrophages. Biochem. Biophys. Res. Commun. 371, 361–365.
Pergola, C., Werz, O., 2010. 5-Lipoxygenase inhibitors: a review of recent developments and patents. Expert Opin. Ther. Pat. 20, 355–375.
Pridal, A.A., Böttiger, W., Ross, A.B., 2018. Analysis of avenanthramides in oat products and estimation of avenanthramide intake in humans. Food Chem. 253, 93–100.
Pirault, J., Bäck, M., 2018. Lipoxin and resolvin receptors transducing the resolution of inflammation in cardiovascular disease. Front. Pharmacol. 9, 1–12.
Rådmark, O., Werz, O., Steinhilber, D., Samuelsson, B., 2015. 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. Biochim. Biophys. Acta 185, 331–339.
Readelli, R., Dimberg, L., Germier, C.U., Berardo, N., Locatello, S., Guerrini, L., 2015. Variability of tocopherols, tocotrienols and avenanthramides content in European oat germplasm. Euphytica 207, 273–292.
Reynertson, K.A., Chon, S., Mahmood, K., 2015. Anti-inflammatory activities of colloidal oatmeal (*Avena sativa*) contribute to the effectiveness of oats in treatment of itch associated with dry, irritated skin. J. Drugs Dermatol. JDD 14, 43–48.
Recchiuti, A., Mattoscio, D., Isopi, E., 2019. Roles, actions, and therapeutic potential of specialized pro-resolving lipid mediators for the treatment of inflammation in cystic fibrosis. Front. Pharmacol. *1*0, 1–18.
Scarpa, E.S., Antonini, E., Palma, F., Mari, M., Ninfali, P., 2018. Antiproliferative activity of vitexin-2-*O*-xyloside and avenanthramides on CaCo-2 and HepG2 cancer cells occurs through apoptosis induction and reduction of pro-survival mechanisms. Eur. J. Nutr. 57, 1381–1395.
Schneider, I., Bucar, F., 2005. Lipogenase inhibitors from natural plants sources. Part 1: medicinal plants with inhibitory activity on arachidonate 5-lipoxygenase, 5-lipoxygenase/cyclooxygenase. Phytother Res. 19, 81–102.
Schneider, I., Bucar, F., 2005. Lipogenase inhibitors from natural plants sources. Part 2: medicinal plants with inhibitory activity on arachidonate 12-lipoxygenase and 15-lipoxygenase and leukotriene receptor antagonists. Phytother Res. 19, 263–272.
Sterna, V., Zute, S., Brunava, L., 2016. Oat grain composition and its nutrition benefice. Agric. Agric. Sci. Procedia 8, 252–256.
Sud'ina, G.F., Mirzoeva, O.K., Pushkareva, M.A., Korshunova, G.A., Sumbatyan, N.V., Varfolomeev, S.D., 1993. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett. 329, 21–24.
Sur, R., Nigam, A., Grote, D., Liebel, F., Southall, M.D., 2008. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. Arch. Dermatol. Res. 300, 569–574.
Touaibia, M., Hébert, M.J.G., Levesque, N.A., Doiron, J.A., Doucet, M.S., Jean-François, J., Cormier, M., Boudreau, L.H., Surette, M.E., 2018. Sinapic acid phenethyl ester as a potent selective 5-lipoxygenase inhibitor: synthesis and structure–activity relationship. Chem. Biol. Drug Des. 89, 514–528.
Tripathi, V., Singh, A., Ashraf, M.T., 2018. Avenanthramides in oats: medicinal importance and future perspectives. Phcog. Rev. 12, 66–71.
Villafuerte Romero, M., Barrett, D.M., 1997. Rapid methods for lipoxygenase assay in sweet corn. J. Food Sci. 62, 696–700.
Yoon, J.-H., Baek, S.J., 2005. Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med. J. 46, 585–596.
Young, R.N., 1999. Inhibitors of 5-lipoxygenase: a therapeutic potential yet to be fully realized? Eur. J. Med. Chem. 34, 771-685.
Zhang, T., Shao, J., Gao, Y., Chen, C., Yao, D., Chu, Y.F., Johnson, J., Kang, C., Yeo, D., Ji, L.L., 2017. Absorption and elimination of oat avenanthramides in humans after acute consumption of oat cookies. Oxidative Med. Cell. Longevity X, 1–7.