# *In vitro* bioaccessibility of avenanthramides in cookies made with malted oat flours

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Summary This study evaluated the effects of malting and *in vitro* digestion on the bioaccessibility of six avenanthramides (AVNs), soluble phenols (SPs) and antioxidant capacity (ORAC) in cookies (3B, 4B, 5B) prepared with oat flour malted for three (M3), four (M4), five (M5) days, respectively. Control cookies (CTRL) were made with non-malted oat flour (M0). Five-days malting increased the AVN content of native kernels up to 10-folds, whereas SPs and ORAC tripled their values. After the *in vitro* digestion, AVNs showed their bioaccessibility in the following order: 5B > 4B > 3B, with a recovery of 1703 μg AVNs per 50 g portion of 5B cookies, compared to 135 μg of CTRL. Results showed that the inclusion of 27% malted oat flour was effective to formulate functional cookies with satisfactory technological properties, high AVN content and bioaccessibility.

Keywords: Antioxidant effect, avenanthramides, functional cookies, *in vitro* digestion, oat malting.

## Introduction

Advances in food and nutrition sciences have shed light on the health benefits of many bioactive compounds in foods. In the case of cereals, a great deal of attention has been focused on the health benefits of polyphenols, which are found in high concentrations and show considerable antioxidant capacity (Angelino *et al.*, 2017).

Among cereals, oats have attracted nutrition professionals and commercial attention (Pridal *et al.*, 2018). The beneficial effects of oats can be traced in part to β-glucan (Daou & Zhang, 2012), the active compound in soluble fibre, mainly present in the caryopsis endosperm (Panato *et al.*, 2017). Moreover, polyphenols, the active antioxidant ingredients of the aleurone layer of oats (Antonini *et al.*, 2017; Panato *et al.*, 2017), confer additional health benefits (Peterson, 2001). Avenanthramides (AVNs) are a group of several amino phenolic molecules specifically found in oats (Collins, 1989). Three main AVNs are present in oats, chemically constituted by 5-hydroxyanthranilic acid conjugated with *p*-coumaric, caffeic or ferulic acid and labelled as 2p, 2c and 2f, respectively (Bratt *et al.*, 2003).

Other minor AVN forms have been found, where the avenalumic acid is present instead of the cinnamic acid (Collins *et al.*, 1991) and are labelled as $2p_d$ and $2f_d$ (Pridal *et al.*, 2018). Moreover, it has been found by Bratt *et al.* (2003) an AVN derived from sinapic acid, reported as 2s, whose concentration is not easily detectable in raw extract of oats. AVNs show a broad spectrum of health promoting activities including: antioxidant, anti-inflammatory, anti-itching, anti-irritant, anti-atherogenic, anti-proliferative properties (Perrelli *et al.*, 2018).

Avenanthramides are adsorbed and metabolised in the human body (Chen *et al.*, 2007; Zhang *et al.*, 2017; Schär *et al.*, 2018). It is worth to note that only a small part of intact AVNs was found in the plasma and urine, whereas a large amount was metabolised particularly to hydroxycinnamic acids during their passage through the gastrointestinal tract and into the circulation (Schär *et al.*, 2018). Hydroxycinnamic acids, in turn, underwent reduction, methylation, sulphation or glucuronidation, and were also metabolised to smaller hydroxybenzoic acids (Schär *et al.*, 2018).

Various processing techniques are applied to oats and other plant seeds in order to stimulate *de novo* synthesis of phytochemicals, thus impacting their potential health protective effects (Xiang *et al.*, 2017). A common physiological method to increase AVN and polyphenol contents in oats involves manipulating germination, which takes place from the embryo, when favoured by water, $O_2$ and temperature (Skoglund *et al.*, 2008). The seeds germinate either in the light or in the dark. In the dark, the photosynthetic activity of

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the sprouts is avoided but the secondary metabolites are synthesised and the phenol concentration increases (Xiang *et al.*, 2017). A controlled germination method called "malting", usually used for brewing, consists of steeping and germination in the dark (Gupta *et al.*, 2010). After malting, the caryopses are kilned at temperatures ranging from 50 to 220 °C in order to dry the grains, block or retard any biochemical reactions and enhance flavour compounds and colour (Gupta *et al.*, 2010).

The AVN content increases notably with oat malting due to the increase in hydroxycinnamoyl-CoA: hydroxyanthranilate N-hydroxycinnamoyl transferase (HHT), the enzyme responsible for AVN biosynthesis (Bryngelsson *et al.*, 2003). After kilning, malted oats, polished from the radicles, undergo the milling process, with the production of whole grain flour, rich in polyphenols and AVNs. This flour may then be used for bakery products, which could be functionalised with AVNs (Skoglund *et al.*, 2008).

The bioaccessibility of a food is the nutrient fraction released from the food matrix by the digestive juices, and it provides information on how many nutrients become available for absorption. Several *in vitro* simulated digestion methods provide useful alternatives to animal models for the screening of nutrient bioaccessibility (Hur *et al.*, 2011). Bioaccessibility may be used to predict bioavailability, which is the fraction of nutrients that passes into systemic circulation (Angelino *et al.*, 2017). Both static and dynamic methods are used to evaluate bioaccessibility (Angelino *et al.*, 2017). Static methods consist of multiple phases (oral, gastric, intestinal) set up at different incubation times, under agitation but without the removal of the digestion products. Dynamic methods simulate the conditions of the four main digestive compartments by remote computer control, and they are combined with a dialysis system that removes the digested products during their production (Angelino *et al.*, 2017).

Several authors have highlighted the relationship between cereal processing and bioaccessibility of nutrients (Li *et al.*, 2016; Huang *et al.*, 2017). Among the technological processes, only the traditional milling was considered, whereas it was omitted the malting effect, the transformation of malted oat grains into wholegrain flours and the mixing with other ingredients to obtain bakery products. In this regard, Li *et al.* (2016) showed that conventional ingredients, such as flour, eggs, sugar, oil and leavening agents, influence the bioaccessibility of the polyphenols.

The aim of the present study was to develop a malting process able to increase AVNs, soluble phenols (SPs) and antioxidant capacity in oat grains, which once milled to obtain wholegrain flours could be used to produce cookies with high polyphenol and AVN content, satisfactory sensorial properties and bioaccessibility. Moreover, we wanted to find out the AVN forms released from the food matrix by the action of digestive enzymes after the *in vitro* digestion process of cookies.

## Material and methods

### Chemicals

Ethanol (analytical grade) was purchased from VWR International (Radnor, USA). Water (LC–MS grade), acetonitrile (LC–MS grade), formic acid (LC–MS grade), glacial acetic acid, 2,2$^0$-Azobis (2-amidinopropane)dihydrocloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman 2-carboxylic acid (Trolox), fluorescein, Folin-Ciocalteu's reagent, caffeic acid, mucin, a-amylase, uric acid, BSA, pepsin, pancreatin, lipase, bile, urea, glucose, glucuronic acid, glucosamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaCl, KCl, KSCN, $Na_2SO_4$, $KH_2PO_4$, $NaHCO_3$, NaOH, $CaCl_2$, $MgCl_2$, $NH_4Cl$, HCl were purchased from CARLO ERBA Reagents S.r.l. (Milano, IT).

### Malting procedure

Naked oats (cultivar Luna) were provided by COBI, Consorzio Italiano di Produttori dell'Orzo e della Birra (Ancona, Italy). The industrial malting plant (Sfoggiatech Srl, Treviso, Italy), ordinarily used by COBI for malting barley, was adapted to produce oats with high levels of AVNs, by regulating times and temperatures of steeping water and subsequent heating treatment. The malting procedure was performed for 5 days, from M0 (non-malted oat grains) to M5 (oat grains malted for 5 days), using the following procedure: steeping at 20 °C for 24 h, germination in the dark at 15 °C and kilning in an air oven at 100 °C for 12 h. The heat treatment was sufficient to control microbial growth. Cleaning was performed by air dehulling to remove the radicles. Milling was carried out by "Il mulino di Nino", Azienda Agricola Roncarati (Ancona, Italy) using a roll mill at a 90% sifting rate and equipped with a ≤ 0.5 mm ring sieve.

Aliquots (50 g) of each flour obtained from malted oats (M0–M5) were retained daily for quantitative analysis of AVNs, SPs and ORAC.

### Preparation of cookies

Figure 1 summarises the steps for the realisation of cookies, which were labelled as follows: 3B, cookies obtained with 27% flour from oat malted for 3 days; 4B, cookies obtained with 27% flour from oat malted for 4 days; 5B, cookies obtained with 27% flour from oat malted for 5 days. The other ingredients used in

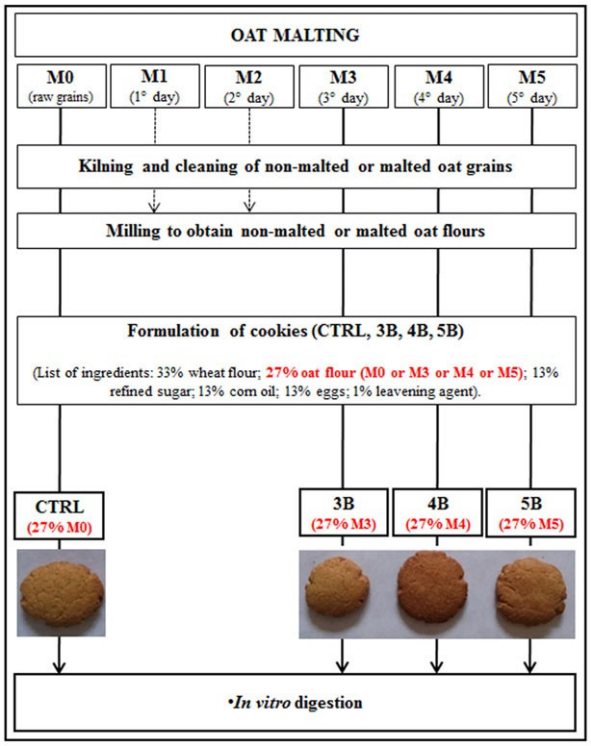


Figure 1 Flow chart of cookie formulations. Flours obtained from non-malted (M0) or malted (M3 or M4 or M5) oat grains were mixed, at 27%, with other ingredients to make control (CTRL) and experimental (3B, 4B, 5B) cookies. The level of avenanthramides (AVNs), soluble phenols (SPs) and antioxidant capacity (ORAC) was followed along the production chain, from raw grains to cookies. The *in vitro* digestion was performed on cookies only. [Colour figure can be viewed at wileyonlinelibrary.com]

the dough are listed in Fig. 1. Control cookies (CTRL) were made with 27% non-malted oat flour (M0) and the same percentages of the other ingredients used for the 3B, 4B, 5B cookies (Fig. 1). Cookies were baked for 30 min in a ventilated oven at 180 °C and cooled at room temperature. Cookies were milled in a ZM 200 ultracentrifugal mill with a 0.5 mm ring sieve (Retsch, Haan, Germany) and analysed for the content of SPs, AVNs, ORAC, before and after the *in vitro* digestion process.

### Extraction of SPs and AVNs

Flours derived from malted oats (M0–M5 samples) and non-digested cookies (CTRL, 3B, 4B, 5B) underwent the extraction of SPs, including AVNs, as reported (Antonini *et al.*, 2016). One gram of each sample was treated with 3 9 10 mL of 80% acidified EtOH (in 0.1% glacial acetic acid), pH 2.8, in a water bath at 50 °C, under shaking. After each extraction, the samples were centrifuged at 1000 g for 10 min. The three supernatants were collected and stored at —20 °C until analysis. The extractions were performed in triplicate for each sample.

### In vitro digestion of malted oat-based cookies

The *in vitro* digestion was performed in a single bioreactor, as described by Oomen *et al.* (2003). A constant mixing was obtained by placing the bottles in a rotator at a speed of 55 r.p.m. The bottles were rotated head-over-heels. The digestion was performed at 37 °C and consisted in three successive steps, i.e., oral, gastric and intestinal phases, each one composed by a specific mixture of digestive juices, as described in Table 1.

The suspension (≈ 200 mL), derived from the last digestion phase (intestinal phase), was centrifuged at 16000 g at 4 °C for 30 min and an aliquot (50 mL) of the clear supernatant was extracted with 300 mL ethyl acetate. The extract was dried under nitrogen, dissolved in 1.5 mL of 80% ethanol and analysed for SP, ORAC and AVN concentration. A blank sample was prepared with identical chemicals, but without the food matrix, and underwent the same conditions as the samples. The digestions were carried out in triplicate for each sample.

Relative bioaccessibility (%) was calculated as follows:

$$\text{Phenols of digested fraction} / \text{Phenols of cookies (d.w.)} \times 100$$

Absolute bioaccessibility (1g per serving) was calculated as follows:

$$\text{Phenols of cookies (d.w.)} \times (\text{Relative bioaccessibility}, \%) \times \text{serving size}(50\ \text{g})$$

The relative and absolute bioaccessibility values of individual and total AVNs, as well as the ORAC, were calculated as reported above for phenols.

### Folin–Ciocalteu assay of SPs

The SP content of all sample (M0–M5 flours; CTRL, 3B, 4B, 5B digested and non-digested cookies) was measured using the Folin-Ciocalteu method (Singleton *et al.*, 1999). The ethanolic extract (100 1L), was mixed with 50 1L of Folin–Ciocalteu reagent. After mixing thoroughly, 300 1L of saturated sodium carbonate solution was added and the mixture was shaken for 0.5 min. Finally the solution was brought up to 1 mL with distilled water. After 30 min of

Table 1 Three-stage *in vitro* digestion procedure (A) and composition of digestive juices (B)

(A) Digestion phases

| | Sample | Extraction mixture | Time (min) | Temperatures (°C) |
|---|---|---|---|---|
| Oral | Cookies (2 g) | 30 mL of salivary solution[a] | 5 | 37 |
| Gastric | Suspension after the oral phase | 45 mL of gastric solution[b] | 120 | 37 |
| Intestinal | Suspension after the gastric phase | 90 mL of duodenal solution 320 mL of biliar solution[d] | 120 | 37 |

(B) Constituents and composition of 1 L of salivary, gastric, duodenal and biliar juices

| Solution and concentration (g $L^{-1}$) | Salivary[a] (mL) | Gastric[b] (mL) | Duodenal[c] (mL) | Biliar[d] (mL) |
|---|---|---|---|---|
| NaCl (175.3) | 1.7 | 15.7 | 40.0 | 30.0 |
| KCl (89.6) | 10.0 | 9.2 | 6.3 | 4.2 |
| KSCN (20.0) | 10.0 | | | |
| $NaH_2PO_4$ (88.8) | 10.0 | 3.0 | | |
| $Na_2SO_4$ (57.0) | 10.0 | | | |
| $KH_2PO_4$ (8.0) | | | 10.0 | |
| $NaHCO_3$ (84.7) | | | 40.0 | 68.3 |
| NaOH (40.0) | 1.8 | | | |
| $CaCl_2$ (22.2) | | 18 .0 | 9.0 | 10.0 |
| $MgCl_2$ (5.0) | | | 10.0 | |
| $NH_4Cl$ (30.6) | | 10.0 | | |
| HCl (440.3) | | 8.3 | 0.5 | 0.5 |
| Urea (25.0) | 8.0 | 3.4 | 4.0 | 10.0 |
| Glucose (65.0) | | 10.0 | | |
| Glucuronic acid (2.0) | | 10.0 | | |
| Glucosamine hydrochloride (33.0) | | 10.0 | | |
| **Solid** | **mg** | **mg** | **mg** | **mg** |
| Mucin | 50 | 3000 | | |
| a-Amylase (13 U $mg^{-1}$) | 145 | | | |
| Uric acid | 15 | | | |
| BSA | | 1000 | 1000 | 1000 |
| Pepsin (738 U $mg^{-1}$) | | 1000 | | |
| Pancreatin (4 9 USP) | | | 3000 | |
| Lipase (90 U $mg^{-1}$) | | | 500 | |
| Bile | | | | 6000 |

reaction at 25 °C in the dark, the absorbance was measured at 765 nm in a UV–vis spectrophotometer (Perkin-Elmer). The amount of total SPs was calculated using caffeic acid (from 50 to 400 1g $mL^{-1}$) as a standard.

### Oxygen radical absorbance capacity

The ethanolic extract was used to assess the antioxidant capacity of all samples (M0–M5 flours; CTRL, 3B, 4B, 5B digested and non-digested cookies) through the Oxygen Radical Absorbance Capacity (ORAC) assay. The ORAC assay was performed using a Fluostar Optima plate reader fluorimeter (BMG Labtech, Offenburgh, Germany), equipped with a temperature-controlled incubation chamber and automatic injection pump, as previously reported (Antonini *et al.*, 2016). Fluorescence was read at 485 nm ex. and 520 nm em. until complete extinction. A calibration curve was constructed each time with six concentrations of the standard Trolox (from 25 to 500 1M).

### HPLC-PDA-MS analysis of AVNs

The filtered ethanolic extract of all samples (M0–M5 flours; CTRL, 3B, 4B, 5B digested and non-digested cookies) was directly analysed in a Waters instrument, equipped with Alliance HT 2795 High Performance Liquid Chromatography (HPLC), 2996 Photo Diode Array (PDA). A C18 column, LiChroCART® (250 9 4 mm), with a particle size of 5 1m, was used. The mobile phase used consisted of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B). The gradient was changed as follows: 0–5 min 2.4% A (isocratic), 5–20 min to 24% A, 20–38 min to 40% A, 38–50 min to 75% A. The total running time was 45 min. The injected sample volume was 50 1L and the flow rate was 0.8 mL $min^{-1}$. UV spectra were recorded from 220 to 420 nm, whereas the chromatograms were registered at 330 nm. Electrospray ionisation (ESI) was operated in positive and negative ion mode in a range of 150–370 amu. Capillary voltage was set at 3 kV, source temperature at 100 °C and desolvation temperature at 300 °C. The cone and desolvation nitrogen gas flows were 50 and 500 L $h^{-1}$, respectively. Data were processed using MassLynx 4.1 (Waters, Milford, MA, USA). To identify and quantify the three main AVNs (2p, 2f, 2c), retention time, UV spectra, MS-ESI(+) and ESI(—) spectra were compared with those of chemically synthesised AVNs (Scarpa *et al.*, 2018). Minor AVNs ($2p_d$ and $2f_d$), not commercially available and not yet synthesised by us, were identified and quantified using the main AVN standards: 2p for $2p_d$; 2f for $2f_d$ and 2s (Pridal *et al.*, 2018).

### Statistical analysis

Soluble phenols and AVN analysis were performed in triplicate, and the results were reported as the mean value standard deviations (SD). The ORAC assay was based on eight independent determinations for each sample; each value was the mean SD.

Statistical differences were calculated using the Student's *t*-test and one-way ANOVA with the SPSS® 17.0 software (SPSS Inc., IBM, Chicago, IL, USA).

## Results and discussion

### Effect of malting on oat nutrients

We first searched for a malting procedure that could yield the highest possible AVN concentration while, at the same time, preserving the integrity of the caryopsis. The malting process, adapted to our specific oat cultivar *Luna*, resulted in a low germination rate in the dark. The average breakage of the caryopses was 12 3%.

Table 2 shows the daily increase in individual and total AVNs, as well as SPs and antioxidant capacity (ORAC), in oat grains during malting. Interestingly, both SP and ORAC values tripled from M0 to M5.

Total AVNs showed an approximate 10-fold increase at day M5 compared to M0. Among the individual AVNs, the 2c was the most representative at M0, thus confirming our data on different naked oat grains (Antonini *et al.*, 2016), and becoming more evident at M5 (Table 2). Regarding the concentration of minor AVNs, $2p_d$, $2f_d$ and 2s were very low at M0, but showed remarkable values at M5. It is worthy to note that the minor AVN forms showed a higher percentage increase than the main forms during 5-days malting, reaching the 48-fold increase in the case of AVN 2s.

The subsequent steps of kilning and cleaning were found to have no effect on AVN levels, which is consistent with other reports (Dimberg *et al.*, 2001).

### Nutrient content of cookies

As highlighted in Fig. 1, the oat grains malted for 3, 4 and 5 days were milled and the respective flours were used to make cookies. The percentage of oat flour (27%), chosen in accordance with technological preliminary tests, was mixed with 33% wheat flour, previously analysed in our laboratory for polyphenol content. The preparation of the dough was carried out in an artisanal bakery that supplies baked products to the local town.

Figure 2 show the concentrations of total AVNs (Fig. 2a), SPs (Fig. 2b) and ORAC (Fig. 2c) in experimental cookies (3B, 4B, 5B), with respect to the CTRL.

Experimental cookies were characterised by higher levels of SPs, ORAC and total AVNs than CTRL, being the latter formulated with non-malted oat grains (Fig. 2). Moreover, all the analysed parameters increased along the malting days, thus confirming the data of wholegrain flours (Table 2).

Figure S1 shows the HPLC chromatograms of M5 flour and 5B cookies, showing the peaks of all the identified AVN forms.

### In vitro digestion of cookies

Table 3 shows the antioxidant recovery of experimental cookies, in terms of relative and absolute bioaccessibility of individual and total AVNs, SPs and ORAC, after the three-stage *in vitro* digestion process. The individual and total AVN bioaccessibility of the cookies was in the following order: 5B > 4B > 3B. The only exception was represented by the AVN 2c, which did not differ within the set and was characterised by the lowest relative and absolute bioaccessibility (Table 3). This finding is consistent with what has been reported in the literature (Li *et al.*, 2016). Figure S2 shows the HPLC chromatogram and the UV-DAD Mass Spectrometry analysis used to identify the AVN forms in the digested cookies.

The bioaccessibility of both SPs and ORAC was in the following order: 3B > 4B > 5B (Table 3). Hence, the cookie formulation with the lowest phenol concentration showed the highest bioaccessibility. This result

Table 2 Avenanthramides (AVNs), soluble phenols (SPs) and antioxidant capacity (ORAC) during 5-days malting

| | MO | M1 | M2 | M3 | M4 | M5 |
|---|---|---|---|---|---|---|
| AVNs (1g $g^{-1}$ d.w.) | | | | | | |
| 2c | 14 ± 0.7 | 45 ± 2.3 | 58 ± 3.4 | 92 ± 5.9 | 107 ± 5.3 | 120 ± 6.8 |
| 2p | 12 ± 0.5 | 22 ± 0.9 | 29 ± 1.1 | 54 ± 2.9 | 56 ± 3.1 | 56 ± 3.5 |
| 2f | 9 ± 0.8 | 17 ± 0.8 | 32 ± 1.6 | 67 ± 3.4 | 70 ± 2.9 | 98 ± 6.2 |
| 2s | 1 ± 0.01 | 2 ± 0.4 | 6 ± 0.3 | 21 ± 0.9 | 35 ± 2.1 | 48 ± 2.8 |
| $2p_d$ | 5 ± 0.3 | 22 ± 0.9 | 40 ± 2.7 | 66 ± 3.5 | 70 ± 3.8 | 81 ± 3.6 |
| $2f_d$ | 1 ± 0.01 | 5 ± 0.1 | 13 ± 0.7 | 24 ± 1.1 | 31 ± 1.9 | 37 ± 2.8 |
| Total | 42 ± 2.7 a | 113 ± 5.5 b | 178 ± 8.6 c | 324 ± 16.2 d | 369 ± 16.8 e | 440 ± 20.4 f |
| SPs (1g $g^{-1}$ d.w.) | 660 ± 50 a | 875 ± 55 b | 1029 ± 62 c | 1350 ± 35 d | 1492 ± 48 e | 1983 ± 120 f |
| ORAC (1molTE $g^{-1}$ d.w.) | 46 ± 3 a | 59 ± 2 b | 70 ± 5 c | 84 ± 4 d | 95 ± 3 e | 111 ± 6 f |

M0–M5 indicate the days of malting. d.w., dry weight. Values are the mean SD of three individual determinations for SPs and AVN individual concentrations. ORAC values are the mean SD of eight different analysis. [a,b]Different letters indicate statistically significant differences among samples (from M0 to M5), for each parameter ($P < 0.05$, one-way ANOVA).

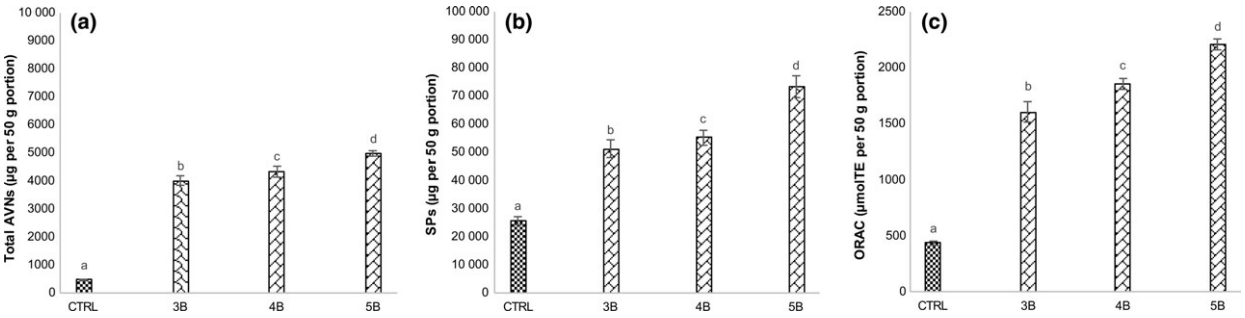


Figure 2 Total avenanthramides (AVNs) (a), soluble phenols (SPs) (b), antioxidant capacity (ORAC) (c), detected in control (CTRL) and experimental (3B, 4B, 5B) cookies. Values are the mean SD of three determinations for SPs and AVNs. ORAC values are the mean SD of eight different analysis. [a,b]Different letters indicate statistically significant differences among samples ($P < 0.05$, one-way ANOVA).

was surprising, as we expected an increase in SP bioaccessibility, in parallel with the increasing SP concentration of the cookies (Fig. 2) and in line with the malting process (Table 2).

The discrepancy between SP and AVN bioaccessibility may be linked to several aspects:

- *Instability of some phenolic compounds.* Oat phenols, representing about 6% of the oat caryopsis, are a complex mixture, including, beyond the AVNs, phenolic acids such as *p*-hydroxybenzoic, vanillic, syringic, ferulic, caffeic, *p*-cumaric and synapic acids, as well as flavonoids such as apigenin, glycosyl vitexin, isovitexin (Peterson, 2001; Skoglund *et al.*, 2008). The discrepancy may be associated to the loss of some phenolic compounds in the digested product, due to their instability under the neutral or alkaline conditions of the *in vitro* digestion system (Friedman & Jürgens, 2000). In this regard, Sun *et al.* (2015) showed that total anthocyanins were degraded during intestinal digestion, being instead retained during the gastric phase. If these conditions occur, it may be that the 5B cookies, with a greater pool of unstable phenols, was subjected to more consistent losses than 4B and 3B cookies.
- Changes in the pool of the total phenols during malting. Xu *et al.* (2009) studied the variability in oat phenolic compounds, showing a different pattern between free and bound phenols during steeping and germination due to the softening of the caryopsis and enzymatic activities. In this regard, the discrepancy may be linked to the increase in the released AVNs which were bound to cell structures (Xu *et al.*, 2009). If these conditions occur, the observed increase in AVN bioavailability in the 5B digested cookies is of great importance, considering that AVNs are exclusively found in oats (Collins, 1989).

Table 3 Relative and absolute bioaccessibility of avenanthramides (AVNs), soluble phenols (SPs) and antioxidant capacity (ORAC) detected on set B cookies

| | Malted oat-based cookies | Relative bioaccessibility (%) | Absolute bioaccessibility (1g or 1molTE per serving) |
|---|---|---|---|
| AVN 2c | 3B | 11 ± 1 a | 124 ± 6 a |
| | 4B | 11 ± 1 a | 131 ± 9 a |
| | 5B | 12 ± 1 a | 134 ± 7 a |
| AVN 2p | 3B | 45 ± 2 a | 260 ± 13 a |
| | 4B | 43 ± 2 a | 282 ± 14 a |
| | 5B | 52 ± 3 b | 308 ± 15 ab |
| AVN 2f | 3B | 27 ± 1 a | 222 ± 11 a |
| | 4B | 29 ± 1 b | 289 ± 14 b |
| | 5B | 32 ± 1 c | 350 ± 17 c |
| AVN 2s | 3B | 33 ± 2 a | 96 ± 5 a |
| | 4B | 47 ± 2 b | 164 ± 8 b |
| | 5B | 50 ± 2 c | 235 ± 12 c |
| AVN $2p_d$ | 3B | 35 ± 2 a | 353 ± 18 a |
| | 4B | 38 ± 2 b | 404 ± 20 b |
| | 5B | 44 ± 2 c | 455 ± 23 c |
| AVN $2f_d$ | 3B | 38 ± 2 a | 135 ± 7 a |
| | 4B | 41 ± 2 b | 176 ± 9 b |
| | 5B | 50 ± 3 c | 221 ± 11 c |
| Total AVNs | 3B | 28 ± 1 a | 1190 ± 60 a |
| | 4B | 29 ± 1 b | 1446 ± 62 b |
| | 5B | 35 ± 2 c | 1703 ± 85 c |
| SPs | 3B | 41 ± 2 c | 21 000 ± 1470 c |
| | 4B | 32 ± 2 b | 17 500 ± 1050 b |
| | 5B | 21 ± 1 a | 14 500 ± 725 a |
| ORAC | 3B | 62 ± 4 c | 1000 ± 60 c |
| | 4B | 42 ± 2 b | 800 ± 48 b |
| | 5B | 29 ± 2 a | 650 ± 39 a |

Values of the absolute bioaccessibility are given as 1g of soluble phenols (SPs) and avenanthramides (AVNs) per 50 g portion and as 1mol of Trolox Equivalents (ORAC) per 50 g portion. Values are the mean SD of three determinations for SPs and AVNs. ORAC values are the mean SD of eight different analysis. [a,b]Different letters indicate statistically significant differences among samples, for each parameter ($P < 0.05$, one-way ANOVA).

- Effect of the prolonged malting process on milling. The malting process may affect the milling of oats, producing flours at smaller particle size which makes AVNs more accessible and extractable (Wang *et al.*, 2014). It may be therefore that, although the flours were milled using the maximal dimension of 0.5 mm ring sieve, those used for the formulation of 5B cookies were characterised by smaller particle size than the flours used for the formulation of 4B and 3B cookies. In this regard, an interesting area of research involves developing methods to increase AVN bioaccessibility through pre-processing technique like dry-fractionation of the bran, following ultrafine grinding and bran electrostatic separation, as reported (Hemery *et al.*, 2010). The additional technological step may increase the cost of the products, but these higher costs would be justified if AVNs intake and bioaccessibility are substantially enhanced.

Overall, the 5B cookies showed 35% total AVN relative bioaccessibility. Considering a 50 g serving size, the absolute bioaccessibility of AVNs corresponded to 1703 85 lg (Table 3). The CTRL, made with non-malted oat flour, yielded an absolute bioaccessibility of 135 8 lg of AVNs per portion, thus confirming that the malting process greatly increased the AVN concentration and that AVNs were not lost in the bakery preparation process (Dimberg *et al.*, 2001).

A portion of 5B cookies provides 4.8 mg of AVNs, which is more than the double of the maximal daily AVN intake in oat consumers (Pridal *et al.*, 2018). The few data available on *in vivo* studies showed that the consumption of 9.2 mg $day^{-1}$ AVNs are sufficient to provide effect on exercise induced inflammation (Koenig *et al.*, 2016). A double serving size (100 g) of 5B cookies, administered daily, reaches the above value, recognised to provide anti-inflammatory activity.

## Conclusions

Malting procedure significantly increases the soluble polyphenols, especially AVN forms, and the antioxidant capacity of raw oat grains. Using 27% malted oat flours, in combination with wheat flour and other conventional ingredients, it is possible to produce cookies with high AVN content and satisfactory technological properties. Sensorial characteristics were verified by the authors with a preliminary test indicating that the acceptance level of malted oat cookies was similar to CTR cookies. However, analytical descriptive and hedonic sensorial tests would be necessary for marketing of cookies with high AVN levels. From the *in vitro* digestion process, it emerges that cookies, formulated with oat grains malted for 5 days, release a higher AVN content, thus suggesting that malting contribute in making the AVNs, especially the minor forms, potentially more bioaccessible. Hence, these cookies have potential applications as functional foods with prompting health effects, in terms of antioxidant and anti-inflammatory actions.

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## Conflict of interest

There are no conflicts to declare.

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