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#### Research note

# Oat phenolic content and total antioxidant capacity during grain development



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#### ABSTRACT

Oats (*Avena sativa* L.) were revaluated in recent years as a promising crop for improving the nutritional quality of foods, due to their richness in many bioactive compounds, including phenols. These plant secondary metabolites are useful as radical scavenging, and also possess positive biochemical effects against cardiovascular diseases, cancer growth and age-related diseases. Twenty oat cultivars were analyzed for their soluble phenol content (SPC, ranging 0.78–1.09 g<sub>GAE</sub>/kg d.m.) and total antioxidant capacity (TAC, ranging 13.99–18.84 mmol TE/kg d.m.). In another experiment, the kinetics of SPC accumulation and TAC in the immature grains of five oat cultivars revealed a marked decrease of both parameters (–48.9% and –72.1%, respectively) from ten days after anthesis to maturity. These results could suggest a possible use of immature oat grains in human nutrition, as it was already proposed for other cereals.

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Many compounds generally present in cereals contribute to their antioxidant properties: tocols, carotenoids, polyphenols, flavonoids, anthocyanins, avenanthramides, lignans and sterols. The global action of all antioxidant substances present in a raw material is generally expressed as total antioxidant capacity (TAC) (Re et al., 1999)

Among the molecules that contributed to antioxidant capacity, cereal polyphenols have drawn increasing attention due to marked effects in the prevention of various oxidative stress-associated diseases, like cancer, cardiovascular or age-related diseases (Adom and Liu, 2002).

While oats were previously well characterized for their phenols content (Bryngelsson et al., 2002; Emmons and Peterson, 1999, 2001; Serea and Barna, 2011), few studies have investigated the presence of bioactive compounds in immature oat seeds (Gutierrez-Gonzales et al., 2013). Different authors have described the accumulation of interesting compounds in other cereals and have proposed a possible use of immature cereal grains as ingredients for food products (Lin and Lai, 2011; Paradiso et al., 2006; Xu et al., 2010).

Abbreviation: ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); daa, days after anthesis; SPC, soluble phenolic content; TAC, total antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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In this Research Note, we determine the soluble phenolic content (SPC) and total antioxidant capacity (TAC) in a group of 20 oat cultivars and describe their kinetics in oat kernel during maturation.

Eighteen varieties of *Avena sativa* L. and two varieties of *Avena strigosa* Schreb (Table 1), originating from different countries, were grown in Montanaso Lombardo (45°20′12″N, 9°28′11″E). Seeding (October 18, 2013) was carried out in head-rows, 1 m long and 20 cm apart with no field replications, on a sandy soil pH 5.2, with good levels of K and P; no nitrogen fertilization was carried out. Cultivars were harvested by hand on June 30 and July 1, 2014. The dehulled seeds were ground by a Retsch Zm200 Ultracentrifugal Mill (Retsch GmbH & Co. KG, Haan, Germany), with a 0.5 mm sieve, and stored in darkness at 6–7 °C until analysis.

Two *A. sativa* cultivars (Alcudia and Bionda) and three belonging to *ssp. nudisativa* (Expression, Grafton and Irina) were grown in pots in Bergamo (45°41′42″N, 9°40′12″E); the seeding date was February 21, 2014. Individual florets were taggeted at the onset of anthesis, and developing dehulled seeds were collected at two stages, 10 and 20 days after anthesis (daa), and at maturity (30–35 daa). Two biological replicates were collected for each cultivar. All samples were frozen in liquid nitrogen and stored at  $-80\,^{\circ}\text{C}$  for TAC and SPC analyses. To determine moisture content, additional seed samples for each stage were placed in an oven at 105 °C for 16 h.

Extraction of soluble phenolics was performed following the method described by Tafuri et al. (2014), with minor modifications,

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 Table 1

 List of the oat cultivars grown in Montanaso Lombardo used in this study, their origin, soluble phenolic content (SPC) and total antioxidant capacity (TAC).

	Varieties	Origin	SPCb	TAC <sup>c</sup>
A. sativa	Andrew	USA	$0.84 \pm 0.02$	15.56 ± 0.38
	Bulwark	GBR	$0.82 \pm 0.02$	$14.92 \pm 0.16$
	Dragon	POL	$0.79 \pm 0.01$	$14.92 \pm 0.07$
	Edelprinz	AUT	$0.78 \pm 0.02$	$14.42 \pm 0.95$
	Gorizont	RUS	$0.87 \pm 0.01$	$17.65 \pm 0.74$
	Komes	HUN	$0.78 \pm 0.02$	$13.99 \pm 0.55$
	Irina <sup>a</sup>	ITA	$0.87 \pm 0.00$	$17.50 \pm 0.77$
	Larry	USA	$0.87 \pm 0.00$	$16.65 \pm 0.30$
	Laurent	CAN	$0.93 \pm 0.00$	$16.94 \pm 0.55$
	Luna <sup>a</sup>	ITA	$0.88 \pm 0.01$	$18.08 \pm 0.22$
	Lutz	DEU	$0.87 \pm 0.01$	$15.20 \pm 0.37$
	Nils	SWE	$0.80 \pm 0.02$	$16.45 \pm 0.80$
	Olram	NOR	$0.87 \pm 0.01$	$16.65 \pm 0.23$
	Origine	FRA	$0.86 \pm 0.01$	$14.91 \pm 0.26$
	Perona	NLD	$0.86 \pm 0.04$	$15.47 \pm 0.88$
	Selphin	CZE	$0.86 \pm 0.01$	$17.52 \pm 0.43$
	Stjarn	FIN	$0.87 \pm 0.01$	$17.22 \pm 0.71$
	Ural	RUS	$0.84 \pm 0.01$	$17.36 \pm 0.45$
A. strigosa	Wir 5287	PRT	$1.09 \pm 0.04$	$18.84 \pm 0.32$
	Saia	AUS	$0.98 \pm 0.01$	$17.20 \pm 0.40$
	General mean $\pm$ sd		$0.87 \pm 0.07$	16.37 ± 1.35
	correlation SPC-TAC		$r = 0.69, p \le 0.01$	

<sup>&</sup>lt;sup>a</sup> Naked oats.

whereas quantification of these compounds was performed with the Folin–Ciocalteu method modified from Singleton et al. (1999). Data are the average of three different readings and are expressed as grams of gallic acid equivalents per kilogram of dried matter ( $g_{GAE}/kg_{dm}$ ), based on a gallic acid dose-response curve.

Total antioxidant capacity was determined by the direct ABTS assay described by Serpen et al. (2007), with minor modifications. TAC was expressed as mmol of Trolox equivalents (TE) for kg of dry matter by means of a Trolox dose-response curve. Each sample was analysed in three replicates and all measurements were performed twice: the results are presented as averages of the measurements.

SPC in 20 oat cultivars showed a mean value of  $0.87 \pm 0.07 \, g_{GAE}$ kg d.m. (Table 1); the two A. strigosa varieties had the highest values. As compared to other cereals, oats were shown to have SPC intermediate between corn and rice (Adom and Liu, 2002). Ranges of soluble phenolic content between 0.2 and 0.7 g<sub>GAE</sub>/kg d.m. were reported by Emmons and Peterson (2001) and Brindzová et al. (2008) for different oat cultivars. On the contrary, higher values, up to 1.5 g<sub>GAE</sub>/kg d.m, were found in oat grains by Adom and Liu (2002), and a mean of 2.1 g<sub>GAE</sub>/kg d.m. was reported by Menga et al. (2010) in a study about the effects of genotype and location on SPC. Total antioxidant capacity showed a mean value of  $16.37 \pm 1.35$  mmol TE/kg d.m. Irina and Luna, the two naked oats, showed TAC values higher than the others A. sativa cultivars  $(17.50 \pm 0.77 \text{ and } 18.08 \pm 0.22 \text{ mmol TE/kg d.m., respectively})$  and quite close to those observed in A. strigosa (Table 1). Other authors had previously analysed TAC in oats (Menga et al., 2010; Serea and Barna, 2011; Zilic et al., 2011). Serpen et al. (2008), using the same method here described, found in one oat sample TAC values of about 30 mmol TE/kg d.m. Literature data indicated that both the environment and genotype had a significant influence on the accumulation of phenolic compounds and antioxidant activity (Emmons and Peterson, 2001; Menga et al., 2010; Martini et al., 2014).

A significant positive correlation was found between TAC and SPC values of these 20 varieties (r = 0.69, p  $\leq$  0.01, Table 1). Total phenolic content has been previously related to total antioxidant

activity (Adom and Liu, 2002; Serea and Barna, 2011). On the other hand, oats are a source of many compounds that exhibit antioxidant activity as tocols, phenolic compounds, phytic acid and avenanthramides (Peterson, 2001); therefore, further investigation is needed to identify the secondary metabolites that, together with the phenols, contributes to the total antioxidant capacity of the oat samples.

The results obtained has revealed that these cultivars have a high antioxidant capacity and a rather low SPC, as compared with other cereals. Moreover, it was possible to see that the varieties belonging to cultivated diploid specie *A. strigosa* have a good content of these bioactive compounds.

Immature whole seeds of five oat cultivars were collected at two stages (10 and 20 daa) and at maturity and analysed for SPC and TAC. A marked decrease in SPC, expressed as the mean of the five cultivars, was observed from 10 daa to maturity (Fig. 1A); this decrease reached 48.9% (data not shown). Some differences were observed among the five varieties (Fig. 1B—F). A higher decrease of SPC in cultivars Alcudia, Bionda and Irina occurred between 20 daa and maturity; on the contrary, cultivar Expression showed an opposite pattern, with a greater decrease from 10 to 20 daa. Finally, in cultivar Grafton the value at maturity was higher than at 20 daa.

On average, also TAC decreased by 72.1% (*data not shown*) from 10 daa to maturity (Fig. 1A). The trend of this parameter was similar in all varieties, and the larger decrease occurred between 10 and 20 daa (Fig. 1B–F).

Changes of different phenolic compounds during grain development were found to be related to plant species and varieties (Xu et al., 2010). For example, in a recent work about rice grains (Lin and Lai, 2011) the fraction of free phenols decreased with the seed maturation for cv Taikeng 16, whereas it first increased and then decreased in cv Kuang-fu-shiang-waxy. Moreover, total phenolic content and total antioxidant activity decreased also during grain development of yellow corn grains (Xu et al., 2010).

These preliminary experiments related to accumulation of SPC and TAC in five *A. sativa* varieties showed that antioxidant

b g<sub>GAE</sub>/kg d.m.

c mmol TE/kg d.m.

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