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Alkylresorcinol content in whole grains and pearled fractions of wheat and barley

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Abbreviations:

ANOVA, analysis of variance; ARs, alkylresorcinols; BSA, N,O-bis(trimethylsilyl) acetamide; CV, coefficient of variation; DM, dry matter; GC, gas-chromatography; ISQ, synthetic index of quality; SD, standard deviation; TMCS, trimethylchlorosilane; TMSI; N-trimethylsilylimidazole.

Abstract

The aim of this work was to investigate the content and the composition of alkylresorcinols (ARs) in different wheat and barley cultivars, and in fractions obtained by progressive pearling. Three commercial winter wheat cultivars, characterized by different hardness and technological quality, and three barley cultivars, including hulled and hull-less types, were selected. Two different protocols of sequential pearling were applied, one for wheat and hull-less barley and another one for hulled barley. Pearling of wheat and hull-less barley cultivars gave five fractions (each corresponding to 5% of original grain weight) and 75% of the residue. In the case of hulled barley eight pearled fractions and 60% of inner kernel were obtained. In wheat ARs were prevalently located in the 5-10% intermediate fraction, while for barley results varied depending on the cultivar. In the hull-less cultivar, the AR content progressively decreased from the outermost fraction (0-5%) towards the inner layers, while for hulled barley the highest AR content was observed in the 10–15% fraction, evidencing lower amounts in the coarse hull (included in the 0-5% and 5-10% fractions). Based on the different localization of ARs in the cereal kernel, progressive pearling can be employed to obtain enriched fractions that could be used to enhance ingredients and products rich in these bioactive compounds.

1. Introduction

Resorcinolic lipids, alternatively referred to as 5-n-alkylresorcinols (ARs), are an important group of phenolic compounds that occur in bacteria, algae, fungi, animals and higher plants, consisting of a phenolic ring with two hydroxyl groups in the meta position, and an odd numbered alkyl chain at position 5 (Kozubek and Tyman, 1999).

Due to their amphiphilic properties, ARs and their derivatives were claimed to have a wide range of biological activities, thus contributing to the health benefits of wholegrain cereal intake. Epidemiological studies showed that consumption of wholegrain cereals is linked to a decreased risk of diseases, such as obesity, diabetes, coronary heart disease, stroke, and some cancer typologies (Slavin et al., 2001; Truswell, 2002; Hallmans et al., 2003). ARs are specifically involved in multiple biological activities, including antioxidant (Hladyszowski et al., 1998), antimicrobial (Reiss, 1989), anti-parasitic (Suresh and Raj, 1990), and anti-mutagenic activities (Kenji et al., 2003). It was also demonstrated that dietary ARs regulate γ-tocopherol and cholesterol levels in rat livers, evidencing a significant biological role to the direct modulation of enzymatic activities (Ross et al., 2004b).

Because ARs are prevalently concentrated in the bran fraction of cereals, and are therefore significant components of whole grain–based foods, they were suggested as potential markers for the evaluation of wholegrain cereal (specifically, wheat and rye) intake (Ross et al., 2004a; Landberg et al., 2008a).

Among the cereal grass species, the bran fractions of rye, wheat, triticale and barley contain high levels of saturated AR homologues, including C15:0, C17:0, C19:0, C21:0, C23:0 and C25:0 (Ross et al., 2003).

AR content in wheat has been shown to achieve approximatively 1000 μg/g (dry matter, DM) and in rye up to 3200 μg/g DM. Barley contains in general lower levels of ARs, in the range 42-51 μg/g DM (Ross et al., 2003). Even though the total AR content varies both within and between cereal species, the relative homologue composition in the whole kernel appear in general rather constant within species. The ratio of C17:0 to C21:0 (generally about 0.1 for common wheat, 0.01 for durum wheat, and 1.0 for rye) may be a useful tool to distinguish between individual types of cereals (Chen et al., 2004; Knodler et al., 2010). ARs are located in the intermediate layers between pericarp and testa in the grain and are therefore found in large amounts only in wholegrain and bran products of wheat and rye (Landberg et al., 2008b), and in very small amounts in refined flour or products (Mattila et al., 2005; Ross and Kochhar, 2009). The conventional milling processes lead to a significant loss of these interesting compounds, which are prevalently wasted as by-products; thus alternative strategies, which can lead to decrease of the by-product production and, at the same time, to obtain novel food ingredients rich in bioactive compounds, should be evaluated.

Sequential pearling is an interesting technique useful to separate external bran fractions, which contain coarse fibre and are potentially subjected to safety risks (mycotoxin, pesticides and heavy metal contaminations), from underlying fractions with potential health benefits due to their high content of bioactive compounds (Sovrani et al., 2012). The pearling process could be appropriately modulated in order to obtain intermediate pearled fractions characterized by low safety risk, but high nutritional value and interesting potential health properties related to their composition (Sovrani et al., 2012). These fractions can be efficiently employed as functional ingredients in bakery and particularly, as previously suggested, for bread-making (Blandino et al., 2013; Blandino et al., 2015a; Blandino et al., 2015b).

The aim of this work was to characterize the AR content and homologue composition of different wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) cultivars, and more particularly to investigate how the pearling process can affect the ARs distribution in the different pearled fractions, in order to obtain functional ingredients enriched of these interesting compounds.

2. Materials and methods

2.1 Chemical and reagents

Chromatographic solvents were GC grade, according to their application, and were purchased from Sigma-Aldrich (Milan, Italy). Analytical standard (≥ 95%) 5-*n*-Heptadecylresorcinol ($C_{17}H_{35}$, CAS no 41442-57-3; indicated as C17:0), 5-*n*-Nonadecylresorcinol ($C_{19}H_{39}$, CAS no 35176-46-6; C19:0), 5-*n*-Heneicosylresorcinol ($C_{21}H_{43}$, CAS no 70110-59-7; C21:0), 5-*n*-Tricosylresorcinol ($C_{23}H_{47}$, CAS no 70110-60-0; C23:0), and 5-*n*-Pentacosylresorcinol ($C_{25}H_{51}$, CAS no 70110-61-1; C25:0) were purchased from Sigma-Aldrich; similarly methyl behenate (internal standard (≥ 99%) CAS no 929-77-1) and BSA+TMCS+TMSI (3:2:3), the reagent used to prepare the trimethylsilyl ether derivates.

2.2 Wheat and barley samples

Three commercial winter wheat cultivars (*Triticum aestivum* L.) were cultivated side by side on the same field in the 2010-2011 growing season at Alessandria (44° 57' N, 8° 29' E; altitude of 121 m; in a deep and acid loamy soil - Aquic Frugiudalf), while three commercial barley cultivars (*Hordeum vulgare vulgare* L.) were cultivated at Carignano, Piedmont, NW Italy (44°53'8.69"N, 7°41'16.75"E, 232 m a.s.l.) during the 2011-12 growing season, according to the ordinary crop management program applied on these crops in the growing areas.

The compared winter common wheat cultivars were:

- Bolero (RV Venturoli, Pianoro, Bologna, Italy), which is classified according to the Italian Synthetic Index of Quality (Indice Sintetico di Qualità, ISQ) (Foca et al.,

2007) as superior bread-making wheat, with soft white kernel and medium-low grain dimension;

- Bologna (S.I.S. Società Italiana Sementi, San Lazzaro di Savena, Bologna, Italy), which is classified as superior bread-making wheat, with medium-hard red kernel and low grain dimension;
- Taylor (Valle Agricola "Tarditi e Ferrando" srl, Cerrina, Alessandria, Italy), which is classified as improver wheat, with hard red kernel and medium grain dimension.

The compared barley cultivars were:

- Mona (S.I.S. Società Italiana Sementi, San Lazzaro di Savena, Bologna, Italy), which is spring, hull-less and two-row cultivar, with medium grain dimension ;
- Trasimeno (Geo Seed, Grinzano di Cervere, Cuneo, Italy), which is winter, hulled and two-row cultivar, with high grain dimension;
- Ketos (Limagrain Italia Spa, Busseto, Parma, Italy), which is a winter, hulled and six-row cultivar, medium-low grain dimension.

Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m^{-2} at the end of October for winter barley and wheat, while cv. Mona was planted in the beginning of March. A total of 130 and 170 kg N ha^{-1} was applied as a granular ammonium nitrate fertilizer for barley and wheat cultivars, respectively. The amount of ammonium nitrate was split equally between tillering and stem elongation stages for each cv.

The considered growing seasons showed different meteorological trends, mainly during the ripening stages: there was very little rainfall as well as high temperature at Alessandria in 2010-2011 growing season, from the stem elongation to anthesis stage, while frequent rainfall occurred at the end of ripening, after the soft dough stage, although grain filling duration was not prolonged.

The precipitation was instead frequent and regular from April to June at Carignano in 2011-2012 growing season, but from the dough stage the average temperature was high leading to quick crop maturation.

Harvest was conducted with a combine-harvester at the end of June and in early-mid July for barley and wheat cultivars, respectively. Grain samples of each cultivar were stored at 4°C until testing.

2.3 Wheat and barley grain pearling

Pearled fractions from wheat and barley kernels were obtained through incremental pearling, as previously described in Sovrani et al. (2012), Blandino et al. (2015a) and Blandino et al. (2015b). The pearling process consisted of consecutive passages of cereal grain and pearled cereal grain, in an abrasive-type grain testing mill (TM-05C model, Satake, Tokyo, Japan) at a constant speed of 55 Hz. The process was monitored by time control. The processed kernel has a moisture content of approximately 12% and was not subjected to conditioning process prior pearling. After each step, the laboratory pearler was thoroughly cleaned by means of dust aspiration and compressed air, to minimize equipment contamination. Initially, a 500 g portion of each unprocessed grain cereal was sub-sampled from a 5 kg sample, and the remaining 4.5 kg was pearled. Starting from unprocessed grain, kernels were initially pearled to remove 5% of the original grain weight, and this resulted in a first fraction (0-5%). The remaining kernels were then pearled to remove a second fraction of 5% w/w (5-10%). The pearling process for the 3 winter wheat cultivars and for hull-less barley cv. Mona was continued until a third, fourth and fifth fraction (designed 10-15%,15-20%, 20-25%, respectively) and the residual 75% w/w of the kernel (25-100% fraction) were collected, thus obtaining a total of seven samples for each cereal.

For the hulled barley cultivars, a different number of bran fractions were obtained, in order to reach a similar level of kernel pearling degree. In this case, the first two passages, each of 5% of the original grain weight, mainly removed the hull fractions (0-5% and 5-10% fractions), while the corresponding fractions of the hull-less barley and wheat were obtained starting from the third pearling passage. A total of ten samples were obtained from each hulled barley cultivars: the whole unprocessed grain and the 0-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40% and 40-100%fractions.

The whole cereal grain samples and the residual kernel fractions were milled using a laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1 mm opening. Then, both the milled and pearled samples (500 g) were ground to pass through a 0.5 mm screen and stored at -25°C before the chemical analyses.

2.4 AR extraction

ARs were extracted with ethyl acetate from pearled fractions (ground samples) and analyzed by gas chromatography (GC) according to Ross et al. (2001). In brief, 200 µL of 0.5 mg/mL methyl behenate solution (C22:0, fatty acid methyl ester, Sigma-Aldrich) was added as an internal standard to each samples (0.5 g) that was extracted with 40 mL of ethyl acetate for 24 h under continuous shaking at 20 °C. The samples were thereafter centrifuged at 20,800 g for 20 min at 4 °C and portions of the extract (4 mL) were evaporated to dryness in vacuum. Ethyl acetate (200 µL) was added, and samples were filtered through 0.45 mm filters before injection into the GC.

2.5 Trimethylsilyl ether derivatives preparation

The alkylresorcinol extract was placed in glass-stoppered test tube. The solvent was removed under nitrogen, and the trimethylsilyl ether derivatives of the alkylresorcinols were prepared by adding 100 µL of BSA+TMCS+TMSI silylating reagent. The tubes were shaken to dissolve the sample in the reagent and then heated at 65 °C for 30 min. Excess reagent was then removed under nitrogen, and the residue was redissolved in hexane (1 mL) and stored at -20 °C for no more than one week.

2.6 Gas chromatographic analysis

The qualitative/quantitative AR composition of the samples was determined using a GC-17A Shimadzu gas chromatograph coupled to a flame ionization detector. The separation was performed on a TR-5MS capillary column (5% Phenyl Polysilphenylene-siloxane; length 15 m, inner diameter 0.25 mm, film thickness 0.25 μm; Thermo Fisher Scientific) with the following temperature program: 50 °C (0 min), raised by 10 °C min^{-1} to 300 °C, held for 20 min at 300 °C. H_2 was used as carrier gas at an inlet pressure of 0.7 bar and with a constant column flow rate of 1.0 ml min^{-1}. The injector and detector temperatures were 250 and 350 °C respectively. The apparatus used was equipped with split/splitless injector. Peak identifications were based on the comparison of retention times with those of pure standards. Individual compounds were quantified against the internal standard by automatically integrating peak areas.

All the working standard solutions were freshly prepared daily prior to use.

Values were reported on a dry matter (DM) basis. DM was determined using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). All analyses were carried out in triplicate.

2.7 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by analysis of variance (ANOVA) followed by Tukey’s ‘‘Honest Significant Difference” test. The statistical significance level was set to

0.05. Statistical analyses were performed using the free statistical software R 2.15.2 version (http://www.R-project.org).

3. Results and discussion

3.1 AR composition of wheat and barley

The gas chromatographic method permitted to identify and quantify saturated homologues of ARs (C17:0, C19:0, C21:0, C23:0 and C25:0). Both commercial reference compounds and literature information were employed for the determination of their retention times. The quantification of ARs was obtained using methyl behenate as internal standard. In Table 1 is reported the AR composition of the different cultivars of wheat and barley analyzed. Wheat showed much higher values than barley, showing mean values of 839 and 75.3 µg/g DM, respectively. In a general way, the total AR content of both cereals is in the range of the data previously reported in literature, even if for wheat we obtained values slightly higher. Chen et al. (2004) reported for 32 samples of Swedish spring and winter wheats AR content of 412 µg/g (ranging between 227 and 639 µg/g). Andersson et al. (2008a) analyzed a total of 131 winter and 20 soft wheats, obtaining values in the range of 220-652 µg/g and of 254-537 µg/g, respectively. All these values are lower than those observed for the cultivar analyzed in the present work. Ross et al. (2003) compared the AR content of 13 *Triticum* species. Their results evidenced a large variation among different species (200-1489 µg/g), and showed for *Triticum aestivum* a total AR content of 916 µg/g, which is very close to the value obtained in this work for the Bologna cv.

Among the wheat cultivars analyzed in this work, Bologna showed the highest total AR content, also considering the individual homologues. Eventualmente dire qui che ha la granalla più piccolo, citando il lavoro suggerito da revisore. In a general way, Bolero and Taylor presented a similar composition, even if the most abundant homologue (C21:0) was higher in the Bolero cultivar. Considering the ratio C17:0 to C21:0, which was suggested as a tool to differentiate cereals, the three cultivars presented values in accord with that proposed for the common wheat (Chen et al., 2004), having observed values of 0.07, 0.1 and 0.08 for Bologna, Taylor and Bolero, respectively.

Concerning the barley samples, the hull-less cultivar Mona showed a remarkably higher content of ARs (total content 98.2 μg/g DM) than the hulled cultivars (55.8 and 65.7 μg/g DM for Trasimeno and Ketos, respectively). Also individual resorcinols presented higher concentrations in cv. Mona, except for C17:0, which was similar in all the cultivars considered. Comparing the hulled cultivars, a slightly different relative composition of the individual homologues was evidenced. In particular, the two-row Trasimeno cv., which presented the lowest total AR content, showed higher C17:0 and C19:0 content than Ketos cv. (six-row), and lower content for C21:0 and C25:0 homologues. The C23:0 content was similar for the two hulled cultivar, and anyway lower in respect to the value observed for the hull-less cultivar Mona.

Previously, Andersson et al. (2008b) characterized the phytochemical components, and particularly ARs, in 10 barley cultivars, including both spring and winter types, as well as two-rowed and six-rowed types, from different origins. The AR content ranged from 32 to 103 μg/g DM, with an average of 55 μg/g. The highest content was found in a hulled barley type with waxy starch, but no clear trend in the content of ARs was evidenced in function of type of cultivars. Zarnowski et al. (2002) analyzed the composition of resorcinolic lipids of five different cultivars of two-row barley, obtaining total AR content in the range of 41-210 μg/g. The highest value (210 μg/g) was obtained for milled grain of the cv. Rudzik.

In Figure 1 is reported the relative composition of ARs in both wheat and barley samples. The dominant AR in wheat was C21:0, with a mean value among the three cultivars of 50%, followed by C19:0 (31%) and C23:0 (11%). C17:0 and C25:0 accounted only for a small part of the total content (4 and 5%, respectively). Concerning barley, the distribution of individual homologues was in the order C25:0 (36%), C21:0 (29%), C23:0 (21%), C19:0

(13%) and C17:0 (1%). These results are in accord with the evidence that the relative composition of ARs is characteristic of different cereals, but is rather constant within species. In fact we observed only minor differences among different cultivars of the same cereal. In particular, for wheat cultivars we observed a coefficient of variation (CV) lower than 9% for all the resorcinolic compounds identified, thus indicating a high homogeneity among cultivars. Conversely, for barley differences were more evident, especially for C17:0 (CV=43%) and C19:0 (CV=24%); in fact, for both these compounds, the Trasimeno cv showed significantly higher percentage than the other cultivars.

The relative homologue composition of ARs in wheat has been shown to be an average of 5% C17:0, 38% C19:0, 47% C21:0, 8% C23:0, and 2% C25:0 (Chen et al., 2004; Andersson et al., 2008a). Concerning barley, Andersson et al. (2008b) reported that the dominant AR homologue is C25:0 (ranging from 35-48% depending on barley cultivar), followed in the order by C21:0 (23-33%) and C23:0 (12-19%). The relative content of C17:0 and C19:0 is generally lower, and greatly variable between genotypes. The same results were obtained by Ross et al. (2003) for Swedish barleys. On the contrary, results obtained for barleys cultivated in Poland showed as dominant AR homologue C21:0 (34-43%), followed by C19:0 (27-37%) and C25:0 (15-25%) (Zarnowski et al., 2002).

AR content and the homologue composition in cereal grains have been demonstrated to be highly variable and dependent on both cultivar and environmental conditions. Andersson et al. (2010) observed a significant effect of year, location, and cultivar on both total AR and individual AR homologue content in wheat. Also the AR composition of barley is strongly influenced by environmental conditions: grains of the same cultivar harvested at two different distant field locations showed different predominant compounds, being C21:0 or C25:0 depending on the field location (Zarnowski et al., 2004).

3.2 AR composition of wheat pearled fractions

The AR content in the fractions obtained from the pearling process of wheats is reported in Table 2. AR concentration in the wheat kernel tend to decrease from the outer fractions to the endosperm, but with a slightly different behavior depending on wheat cultivar. In the Bologna cv. the AR content was similar in 0-5% and 5-10% fractions and then significantly decreased at each successive pearling passage towards the inner layers. On the contrary, for Bolero and Taylor cultivars the highest AR content was observed for the 5–10% fraction, while the more external layer (0–5%) presented lower values.

In a previous work, Landberg et al. (2008b) prepared seven wheat fractions by sequentially pearling common wheat at fixed and constant times, until about 10% by weight of the starting material was abraded. The AR content strongly increased during the first step of pearling, reaching the maximum values in correspondence of the third and fourth fractions (when the cumulative yield was about 2-4%), then a progressive decrease was observed toward the inner layers; the lowest value was registered for the first fraction, corresponding to about 1% of total wholegrain. Analyzing hand-dissected botanical fractions, the same authors also observed that more than 99% of ARs was found in the intermediate layer (inner pericarp, hyaline layer and testa), while there were no or very low levels of AR in the aleurone layer. Shetlar et al. (1947) reported that the outer pericarp, the inner pericarp, the testa and the aleurone layer, represent 3.9%, 0.9%, 0.7%, and 9.0% of the kernel weight, respectively. Thus, although ARs are prevalently concentrated in the bran fraction of cereals (Ross et al., 2004a), the outermost layers are not so rich of these compounds. The highest AR content should be approximatively obtained in a pearled fraction equivalent to a cumulative yield of 4-6%, which is straddling the two first fractions (0-5% and 5-10%) analyzed in the present work. Consequently, although pearling fractions are not necessarily homogenous in terms of tissue and biochemical composition, the results presented in Table 2 seem to be consistent with anatomical structure of the kernel.

The relative composition of AR homologues in progressive pearling fractions was quite constant and similar to that observed for the corresponding wholegrains, presenting values in accord with the literature data for wheat (Ross et al. 2003). However, minor but significant differences were observed among the fractions in relation with the pearling degree (Figure 2, values are the means of the three different wheat cultivars). In particular, the most abundant C21:0 homologue showed an higher relative content in the inner fraction, ranging from 49% in the outermost fraction to 52% in the residual kernel, while the relative content of C19:0 progressively decreased (from 34% to 30%, following the increase of the pearling degree). This trend was common to all the cultivars considered.

Results on ARs confirm our previous evidences on the potential health and nutritional value of selected wheat flours (ground fractions) obtained by progressive pearling. In fact, the AR distribution suits with that of other bioactive compounds previously quantified in wheat pearled fractions. In particular, β-glucans and proteins showed the same behavior than ARs, while dietary fiber, phenolic acids and antioxidant compounds were mainly concentrated in the outermost layers, progressively decreasing toward the inner of the kernel (Sovrani et al., 2012). On the other hand, the external coatings of wheat kernel are potentially subjected to contamination (e.g. mycotoxins and heavy metals) (Sovrani et al., 2012), thus the progressive pearling would permit to discard these most external layers, reducing the contamination risk, but obtaining selected fractions enriched of bioactive compounds, among which also ARs. Removing the 0-5% fraction would preserve the most part of ARs, because they are prevalently concentrated in the 5-10% fraction.

3.3 AR composition of barley pearled fractions

As for wheat, barley samples were subjected to the pearling process, then the AR content of the different pearled fractions was determined (Table 3). Two different pearling protocols were applied to hulled and hull-less cultivars, in order to reach a similar level of kernel pearling degree. Thus, a different number of bran fractions was obtained, six for the hull-less cultivar Mona and nine for the hulled cultivars Trasimeno and Ketos. According to our previous work (Blandino et al., 2015b), the two first pearling steps (0−5 and 5−10%) of the hulled cultivars led to an almost complete dehulling of the kernel.

For the cv. Mona, the AR content significantly decreased at each successive pearling step from the outermost fraction (0-5%) towards the inner layers, while for Ketos and Trasimeno cultivars the highest AR content was observed in the 10–15% fraction; for these cultivars, the 0-5% and 5-10% fractions resulted in a lower concentration. As reported in Blandino et al. (2015b), these initial surface removal layers presented higher content of dietary fiber (in the range 79-64%, depending on fraction and cultivar considered), more than 97% of which as insoluble fiber, thus confirming that they mainly correspond to the coarse hull fraction. Starting from the third fraction (10-15%), also for the hulled barley cultivars a progressive decrease from the external to the internal layers was observed, having registered the lowest value in the residual 40-100% kernel.

The individual resorcinolic compounds identified followed the trend described above, showing only minor differences depending on the molecule and the different barley cultivars.

Concerning the AR relative composition, the progressive pearling fractions showed similar values, and in accord with the typical composition observed for barley (Ross et al. 2003; Andersson et al., 2008b). C25:0 was predominant followed by C21:0, accounting together for about 65% of the total AR content (on average about 62% and 69% for hulled and hull-less cultivars, respectively). The other AR homologues identified were present in minor concentrations; among them C17:0 accounted for less than 1%. Analyzing more specifically the composition of the individual resorcinolic homologues, we observed that in the pearled fractions of hulled cultivars the minor compounds C17:0, C19:0 and C23:0 did not significantly varied, while significant differences were registered for C25:0 and C21:0

homologues (Figure 3A). Significant differences were observed also comparing the composition of the Mona (hull-less cultivar) pearled fractions, but we were not able to identify a specific trend related to the pearling degree (Figure 3B). Thus, these differences could be principally correlated to analytical variability and not to intrinsic characteristics of the fractions.

In a previous work, the AR localization in cereal grains was studied on hand-dissected botanical fractions by color reaction with Fast Blue B dye. None of the Fast Blue B soaked barley samples showed staining, probably because of the small AR content in barley (lower amounts than other cereals as wheat and rye) and the low sensitivity of the method employed (Landberg et al., 2008b). In the present work, the use of the pearling process, and the successive gas-chromatographic determination of individual AR homologues, was useful to identify the AR localization toward barley kernel in both hull-less and hulled cultivars, having observed the lowest values in the inner part of the kernel and, in the case of the hulled cultivars, in the outermost hull fractions.

In addition, the progressive pearling was successfully employed to obtain AR enriched fractions that can be used as functional ingredients. In particular, for Mona cv. the 0-5% fraction presented a 2.5 times higher content than the corresponding whole kernel, while for the hulled cultivar the major increase was observed in the 10-15% fraction, reaching values 5 and 3.6 times higher than the corresponding wholegrains for Ketos and Trasimeno cv, respectively. Therefore, the best performances were obtained by processing hulled barleys, so much that although the highest amount of ARs was registered in the cultivar Mona (98.2 µg/g in the wholegrain), the richest fraction was obtained from Ketos (10-15% fraction, 328.9 µg/g).

As previously stated, for hulled cultivars the higher AR content was observed in the 10-15% fraction. This fact is advantageous because in order to prepare the 10-15% fraction, the hull portions, which are majorly subjected to natural and/or synthetic contamination (mycotoxins, heavy metals, pesticides), are removed, thus obtaining an intermediate fraction rich in bioactive compounds and characterized by low safety risk. In fact, we previously demonstrated that the 10-15% fraction is also rich of minerals, proteins, dietary fiber and antioxidant compounds, and presents a β-glucan content similar to that of whole kernel (β-glucans are prevalently concentrated in the inner part of the barley kernel, presenting lower value in external layers) (Blandino et al., 2015b). On the other hand, this intermediate fraction presented lower DON levels than the outermost layers (hull), resulting in a low contamination risk (Blandino et al., 2015b).

In a recent paper, Gómez-Caravaca et al. (2015) proposed the air classification technology as a green approach to prepare barley flours rich in alkylresorcinols, β-glucans and phenolic compounds. Starting from de-hulled barley whole meal, they obtained two fractions (coarse and fine), characterized by different particle sizes and chemical composition. The coarse fraction presented a higher content of both β-glucans, free and bound phenolic compounds, and ARs than whole flour. Specifically, the AR content of coarse fraction increased 1.2–1.4 times (depending on different barley cultivars considered) in respect to whole meal. Compared to this approach, the pearling process seems to be more efficient to enrich barley flours in ARs, obtaining fractions up to 5 times richer than the corresponding whole kernel.

4. Conclusions

Our results confirmed previous evidences that ARs are prevalently located in the bran portion of cereals. The application of progressive pearling permitted to obtain additional information on their specific distribution in different cultivars of both wheat and barley. In wheat ARs are concentrated in an intermediate fraction corresponding to 5-10% of the whole grain weight (even though in Bologna cv. similar amounts has been observed in the first 0-5% pearling fraction), while for barley results varied depending on hull-less and hulled cultivars. In particular, for the hull-less cultivar Mona, the AR content progressively decreased from the outermost fraction (0-5%) towards the inner layers, while for Ketos and Trasimeno cultivars (both hulled) the highest AR content was observed in the 10–15% fraction; for these cultivars, the coarse hull, which was included in the 0-5% and 5-10% fractions, resulted in a lower AR concentration.

The progressive pearling has also been confirmed as a useful strategy to obtain functional ingredients, valorizing kernel portions normally classified as by-products, but rich of interesting compounds with potential health benefits, such as ARs. The knowledge of the distribution of ARs, as well as of the other bioactive components and contaminants (both natural and synthetic) previously quantified in the same wheat and barley pearled fractions (Sovrani et al., 2012; Blandino et al 2015a; Blandino et al., 2015b), could be efficiently employed to modulate the pearling process in order to select the kernel fractions with major health and nutritional value, removing the most external layers characterized by higher safety risk.

Starting from significantly higher content in the whole kernel, wheat is confirmed as more suitable than barley to obtain fractions rich in ARs; however, in both the cereals the pearling process permitted to prepare enriched fractions, which can be employed as functional food ingredients for their potential health benefits.

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Table 1 AR content of different cultivars of common wheat and barley.

Cereal	***Variety***	**ARs (µg/g, d.m.)**					
		C17:0	***C19:0***	***C21:0***	***C23:0***	***C25:0***	***Total***[#]
Wheat	Bolero	32±1 a	267±1 ab	426±5 b	88±1 b	26±0.3 b	***839 b***
	Bologna	34±2 a	272±14 a	474±26 a	108±6 a	33±2 a	***921 a***
	Taylor	33±1 a	250±7 b	368±13 c	84±2 b	26±0.2 b	***761 b***
Barley	Mona	0.5±0.05 a	12.4±0.1 a	29.7±0.9 a	22.3±0.7 a	33.3±1.8 a	***98.2 a***
	Trasimeno	0.5±0.02 a	9.4±0.3 b	15.4±0.4 c	12.0±0.4 b	18.5±1.3 c	***55.8 c***
	Ketos	0.3±0.04 b	7.0±0.2 c	19.7±0.7 b	12.4±0.3 b	26.3±0.6 b	***65.7 b***

Statistical significance was evaluated separately for wheat and barley. Values followed by different letter, within a column, are significantly different ($p<0.05$).

[#] Sum of the individual ARs identified.

Table 2. ARs content of fractions obtained by sequential pearling of common wheat.

Variety1	*Fraction*	*ARs (µg/g, d.m.)*					
		C17:0	*C19:0*	*C21:0*	*C23:0*	*C25:0*	*Total[#]*
Bolero	0-5%	86±8 b	780±16 b	1124±24 b	204±4 b	63±1 b	***2257 b***
soft	5-10%	124±4 a	1093±50 a	1558±71 a	288±12 a	74±3 a	***3137 a***
white kernel	10-15%	82±1 b	730±8 b	1093±4 b	213±2 b	56±1 c	***2174 b***
*SBW**	15-20%	59±1 c	516±11 c	766±18 c	144±4 c	45±1 d	***1530 c***
	20-25%	37±2 d	322±12 d	502±16 d	97±1 d	29±1 e	***987 d***
	25-100%	16±2 e	121±6 e	214±14 e	45±3 e	11±1 f	***407 e***
Bologna	0-5%	164±31 a	1411±71 a	2191±20 a	509±14 a	129±4 a	***4404 a***
medium-hard	5-10%	180±2 a	1400±22 a	2093±38 a	462±15 b	112±4 b	***4247 a***
red kernel	10-15%	115±10 b	926±74 b	1529±96 b	330±16 c	92±5 c	***2992 b***
*SBW**	15-20%	73±1 c	574±10 c	939±14 c	202±2 d	53±0.4 d	***1841 c***
	20-25%	54±1 c	428±17 d	743±10 d	158±5 e	40±3 e	***1423 d***
	25-100%	14±0.4 d	111±6 e	206±12 e	46±2 f	12±0.4 f	***389 e***
Taylor	0-5%	110±22 a	1023±40 b	1362±55 b	259±14 b	68±5 a	***2822 b***
hard	5-10%	129±15 a	1185±29 a	1635±28 a	305±6 a	74±2 a	***3328 a***
red kernel	10-15%	108±11 a	874±22 c	1208 ±17 c	227±2 c	55±1 b	***2472c***
*IW**	15-20%	41±6 bc	555 ±6 d	819±12 d	167±5 d	43±1 c	***1625 d***
	20-25%	55±0.2 b	432±6 e	624±2 e	127±1 e	34±1 d	***1272 e***
	25-100%	17±1 c	132±7 f	202±14 f	48±3 f	13±1 e	***412 f***

Statistical significance was evaluated separately for each wheat variety. Values followed by different letter, within a column, are significantly different ($p<0.05$).
[#] Sum of the individual ARs identified.
* Italian ISQ classification (SBW: superior bread-making wheat, IW: improver wheat)

Table 3. ARs content of fractions obtained by sequential pearling of barley.

Variety1	*Fraction*	*ARs (μg/g, d.m.)*					
		C17:0	*C19:0*	*C21:0*	*C23:0*	*C25:0*	*Total*[#]
Mona	0-5%	2.52±0.23 a	41.3±0.6 a	72.7±1.9 a	52.4±0.3 a	76.8±3.6 a	***245.7 a***
hull-less	5-10%	1.49±0.07 b	34.3±3.3 b	61.5±0.3 b	45.8±3.6 b	68.0±0.2 b	***211.1 b***
two-row	10-15%	1.10±0.01 c	26.6±0.8 c	48.2±0.9 c	40.5±0.3 c	52.2±0.5 c	***168.6 c***
	15-20%	0.70±0.02 d	15.0±0.9 d	24.1±0.5 d	18.7±1.0 d	28.8±0.7 d	***87.4 d***
	20-25%	0.36±0.06 e	9.9±1.4 e	17.5±0.5 e	13.7±0.1 e	23.2±1.7 e	***64.7 e***
	25-100%	0.12±0.00 e	1.9±0.3 f	6.1±0.8 f	3.8±0.6 f	7.2±0.7 f	***19.1 f***
Trasimeno	0-5%(hull)	0.48±0.04 de	10.0±0.3 d	16.8±0.1 e	12.6±0.2 e	19.5±0.7 d	***59.5 e***
hulled	5-10% (hull)	1.40±0.04 b	12.8±0.5 c	24.1±0.9 d	17.0±0.4 d	35.2±0.7 c	***90.5 d***
two-row	10-15%	3.31±0.08 a	32.6±1.0 a	51.3±0.6 a	41.1±0.4 a	71.3±1.0 a	***199.6 a***
	15-20%	1.53±0.11 b	22.2±1.1 b	41.4±1.1 b	28.6±0.5 b	50.6±1.8 b	***144.3 b***
	20-25%	1.51±0.03 b	14.4±0.6 c	31.9±0.7 c	23.0±0.6 c	37.2±0.6 c	***107.9 c***
	25-30%	0.69±0.04 c	9.3±0.4 d	15.6±1.4 e	11.3±0.3 f	21.3±0.4 d	***58.2 e***
	30-35%	0.59±0.05 cd	5.8±0.1 e	9.4±0.1 f	7.4±0.3 g	14.0±0.1 e	***37.2 f***
	35-40%	0.37±0.04 e	3.5±0.2 f	6.4±0.3 g	4.2±0.2 h	9.4±0.6 f	***23.9 g***
	40-100%	0.09±0.01 f	1.6±0.1 g	2.1±0.2 h	1.8±0.2 i	2.3±0.2 g	***7.9 h***
Ketos	0-5% (hull)	0.19±0.03 e	6.1±0.3 f	21.2±0.3 f	13.6±1.3 ef	24.2±0.5 f	***65.3 f***
hulled	5-10% (hull)	0.35±0.03 de	25.5±0.8 c	68.5±1.0 b	42.1±2.3 b	93.2±2.2 b	***229.6 b***
six-row	10-15%	2.56±0.29 a	47.6±0.4 a	93.9±5.1 a	61.0±0.9 a	123.9±2.6 a	***328.9 a***
	15-20%	1.78±0.08 b	28.3±1.4 b	52.5±0.7 c	36.6±0.9 c	75.5±1.2 c	***194.7 c***
	20-25%	0.69±0.03 c	14.3±1.1 d	37.4±0.3 d	21.0±1.5 d	51.6±1.4 d	***125.1 d***
	25-30%	0.59±0.04 cd	10.8±0.2 e	28.0±0.6 e	16.6±0.3 e	36.5±0.6 e	***92.5 e***
	30-35%	0.24±0.05 e	6.1±0.4 f	16.4±1.0 fg	11.8±1.1 fg	27.6±0.4 f	***62.1 f***
	35-40%	0.18±0.03 e	3.8±0.1 g	11.3±0.3 g	8.8±0.4 g	17.9±0.3 g	***42.0 g***
	40-100%	0.08±0.01 e	1.0±0.03 h	2.3±0.1 h	1.3±0.04 h	3.0±0.08 h	***7.6 h***

Statistical significance was evaluated separately for each barley variety. Values followed by different letter, within a column, are significantly different ($p<0.05$).
[#] Sum of the individual ARs identified.

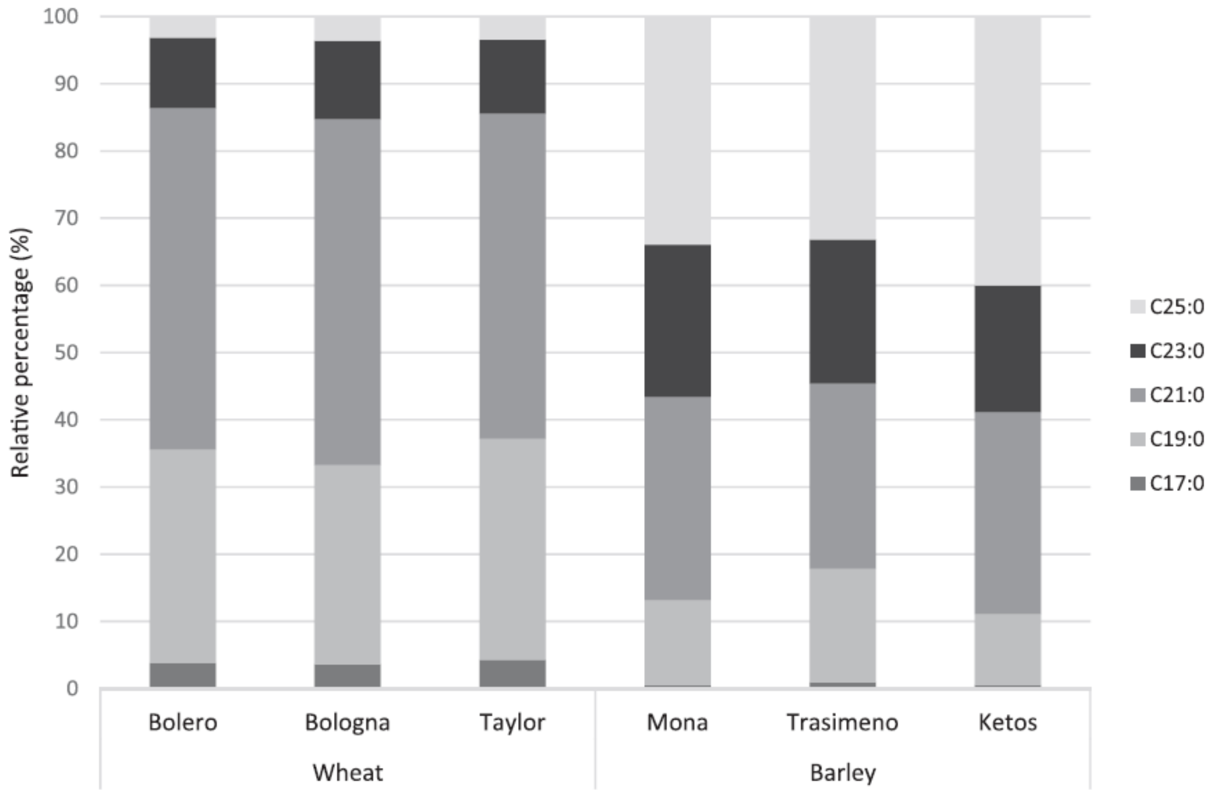

Figure 1.

AR composition, expressed as relative percentage of AR homologues, of different wheat and barley cultivars.

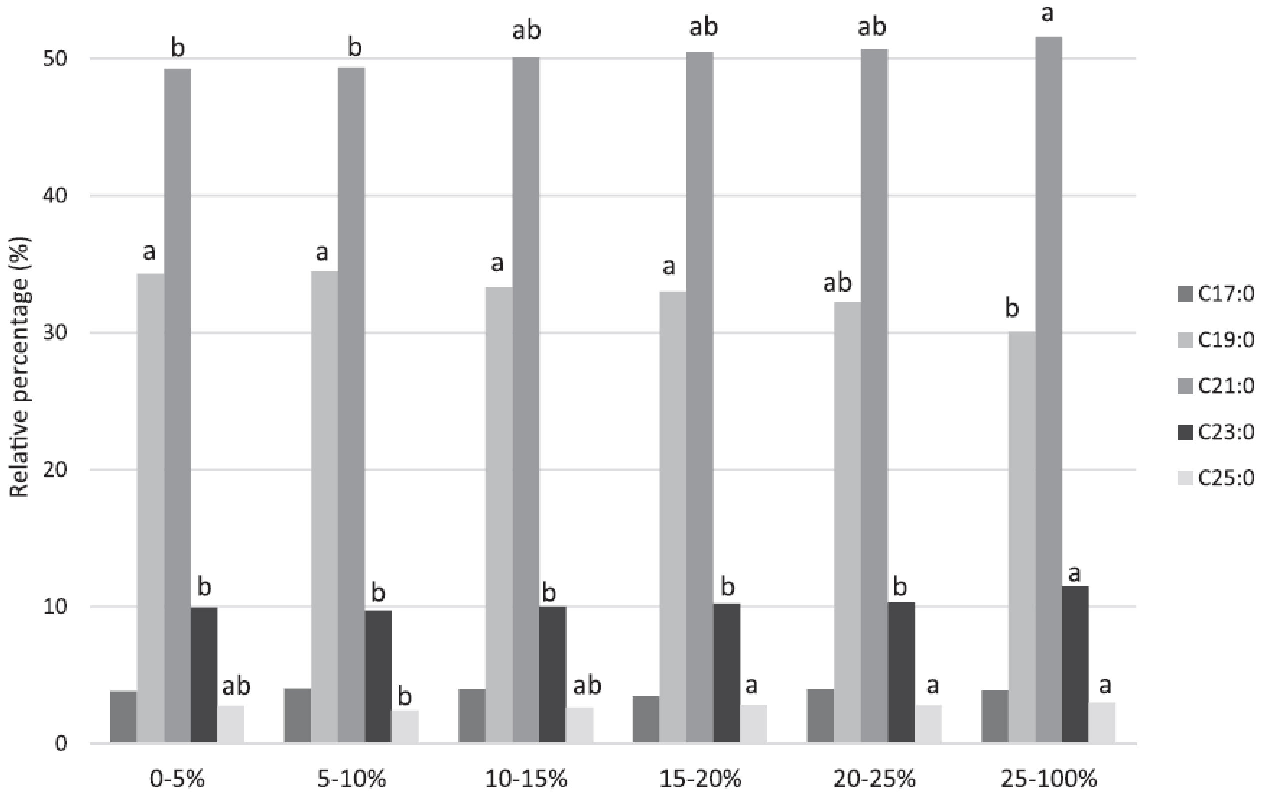

Figure 2.

AR composition, expressed as relative percentage of AR homologues, of wheat pearled fraction (values are expressed as mea of the three varieties) significant differences within each AR homologue are identified using different letter (P< 0.05).

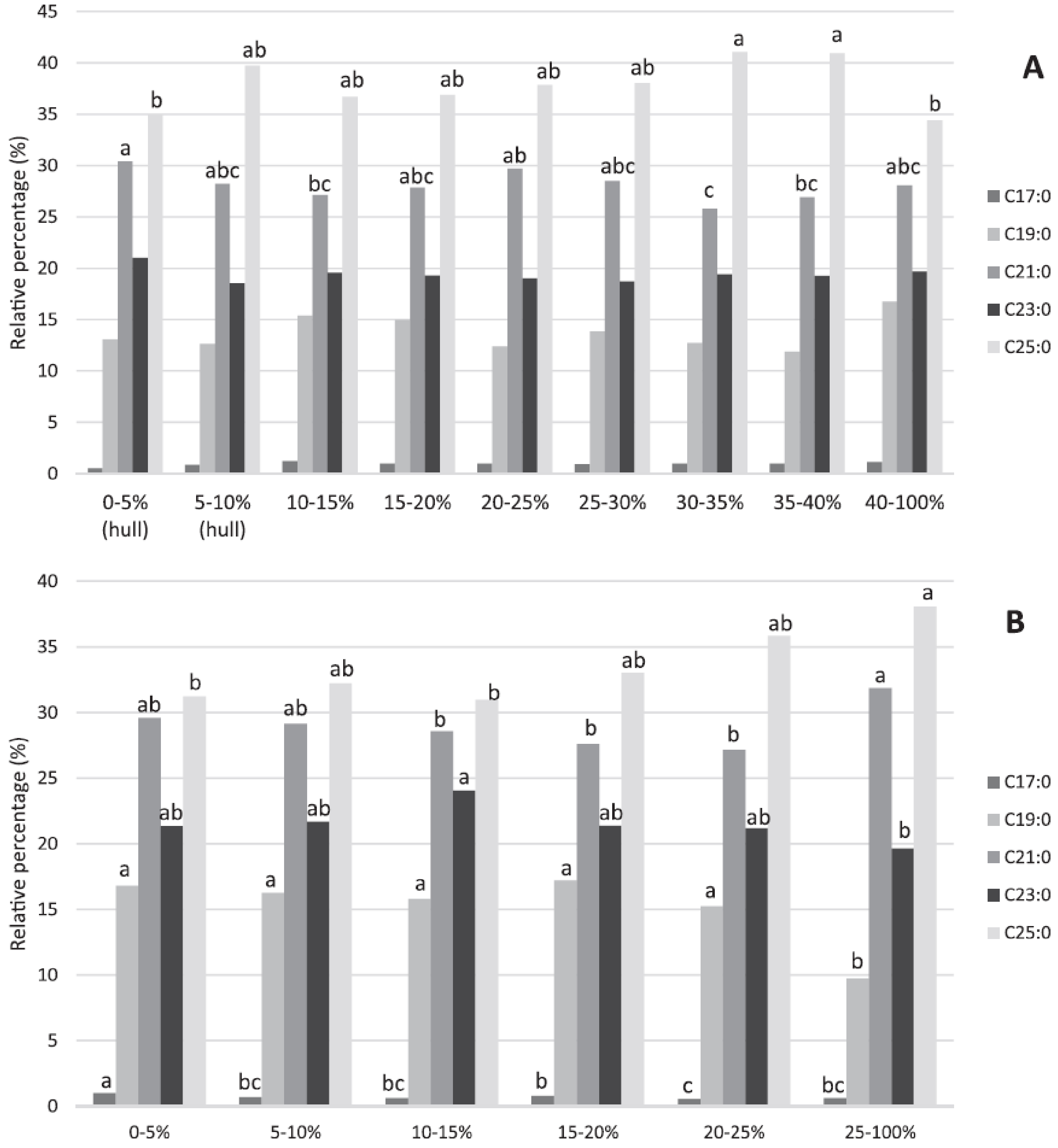

Fig. 3. AR composition, expressed as relative percentage of AR homologues, of barley pearled fractions (panel A: values are means of the hulled varieties Trasimeno and Ketos; panel B: hull-less variety Mona). Significant differences within each AR homologue are identified using different letters ($p < 0.05$).

Figure 2.

AR composition, expressed as relative percentage of AR homologues, of barley pearled fraction (panel A: values are means of the hulled varieties Trasimeno and Ketos; panel B: hull-less variety Mona). Significant differences within each AR homologue are identified using different letter ($P< 0.05$).