Effect of malting on antioxidant capacity and vitamin E in barley genotypes

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Short title:

Antioxidant capacity and vitamin E during malting.

Abstract

Unprocessed barley is known to contain relatively high levels of antioxidants, which play a critical role in human health and the preservation of food and drink products. However, there are limited data on how the antioxidant levels in barley are affected by malting, and whether the level of antioxidants in the processed malt differs between barley varieties. This study aimed to determine the levels of vitamin E isomers, total vitamin E content and total antioxidant capacity before, during and after malting in twelve covered and two hulless barley genotypes. Vitamin E and antioxidant capacity were determined by high performance liquid chromatography (HPLC) and ability to scavenge DPPH radicals, respectively. The vitamin E content of most genotypes was reduced after steeping, germination and kilning compared to the unprocessed samples. However, the antioxidant capacity in the malt was higher than in the unprocessed samples for the majority of the genotypes. While there was variation in the percentage change in antioxidant capacity between varieties, the antioxidant capacity of samples after malting was directly correlated with their Vitamin E content before processing ($r=0.9$, $n=14$, $p<0.05$). These results indicate that barley varieties which have higher antioxidant capacity at harvest retain their antioxidants after malting. Thus, these varieties are likely to be the most suitable for producing malts with the added health benefits and anti-spoiling properties associated with a higher antioxidant content.

Keywords: Barley; genotypes; vitamin E; antioxidant capacity; malting.

Introduction

Barley *(Hordeum vulgare* L.*)* contains a number of beneficial nutritional components, including β-glucan (4-9%) and the essential amino acid, lysine (0.5%); and has a high fibre content (14-24%) [(1)]. In our previous study, barley also has a relatively high antioxidant content that is genotype dependent (from 57.22 mg ascorbic acid equivalent antioxidant capacity (AEAC)/100 g FW to 158.10 mg AEAC/100 g FW) (Do et al 2014). Antioxidants have a number of important health benefits, which are due to their ability to slow tissue damage by preventing the formation of free radicals, scavenging them, or by promoting their decomposition [(2)]. Antioxidants also play a role in the preservation of foods and beverages, and it has therefore been suggested that these antioxidants may also help to protect foods and beverages containing barley products from spoilage [(3, 4)]. For example, the major antioxidant in barley, Vitamin E, is a lipid phase chain-breaking antioxidant which inhibits lipid peroxidation to limit the formation of trans-2-nonenal, one of the main compounds responsible for the stale taste of beer [(5)]. Therefore, using malts with a higher antioxidant capacity may have commercial benefits for the brewing industry, as well as providing health benefits.

A significant proportion of barley harvested worldwide is used for the production of malt. Malt is a key ingredient in beer, and is now increasingly been used in food products to enhance flavour and provide nutritional benefits, including increased digestibility, making malt an ideal ingredient for baby foods [(6, 7)]. Barley malt is also commonly utilised for bread making, with the further advantage of promoting yeast activity [(7)]. Both the health benefits

and preserving actions of antioxidants mean that higher antioxidant content in malts would be beneficial. To date, however, most of the studies assessing antioxidant content in barley have been conducted in unprocessed samples, and what effect processing has on antioxidant capacity is unclear.

This is particularly important, since malting is a thermal process, and high temperatures can potentially impact on antioxidant capacity [8, 9]. However, the results of previous studies which have investigated changes in the content of the key antioxidant, vitamin E, during malting have been inconsistent. Peterson and colleagues [10] found no significant change in vitamin E content after malting, while other studies have shown both positive and negative effects of malting on levels of vitamin E and individual vitamin E isomers [11, 12]. However, the studies to date have not included all malting stages and have been restricted to some specific vitamin E isomers, such that total vitamin E content, which is calculated by α-T equivalence involving all isomers: α, β, γ, δ-tocopherol (T) and α, β, γ, δ-tocotrienol (T3), could not be assessed. Thus, no previous studies have determined the effect of malting stage on total vitamin E content or antioxidant capacity in barley.

In addition, the studies to date have only included a limited number of genotypes. This is important, since we have previously reported that both Vitamin E content and antioxidant capacity at harvest can vary significantly between barley genotypes (Do et al., 2014). However, whether this variation between genotypes persists after malting and whether any changes that occur during malting is consistent between genotypes are unknown. Expanding the use of hulless malt is of particular interest because it can be directly added to a variety of products after milling without processing the hull disposal or malt extract [13]. Hulless

malt can also benefit to brewing industries with short steeping time and high extract yield due to fine grind of the malt and more fermentable materials [(13)].

The objectives of this study were to determine the impact of malting on the content of vitamin E isomers, total vitamin E and antioxidant capacity in a range of barley genotypes including hulless with different antioxidant capacities at harvest.

MATERIALS AND METHODS

Materials

The barley varieties included in this study were provided by the University of Adelaide Barley Program and consisted of 14 common malting genotypes including 12 covered (Sloop, Flagship, WI2585, Vlamingh, Dhow, Buloke, Harrington, Commander, Alexis, ND24260-1, Chebec and Amaji nijo) and 2 hulless (Sumire mochi and Finniss) genotypes. All 14 genotypes were grown from June 2011 to December 2011 in a single plot in a complete randomised design at Charlick Experimental Research Station, Strathalbyn, South Australia (35°19'46.26" S, 138°52'42.39" E).

Malting

The barley from all 14 genotypes were micromalted in a Phoenix Automatic Micromalting System®, in accordance with the standard protocol used by the Barley Quality Laboratory in The University of Adelaide. The micromalting schedule used comprised three main stages (1) steeping and air rest (7:8:9 h (wet:dry:wet) at 17°C, followed by another air rest, 9:0.5 h (dry:wet)), (2) germination (94.5 h at 17°C) and (3) kilning (50-55°C, for 9 h followed by 55-60°C for 4 h, 60-70°C for 2 h and 70-80°C for 4.5 h). Samples of each genotype, except Harrington, were collected at harvest and after each of the three stages of malting (steeping, germination and kilning) for the measurement of vitamin E content and antioxidant capacity. Samples of Harrington were only collected after kilning due to limited availability of seed. The malts were kept at -20°C until their moisture content, antioxidant capacity and vitamin E content were analysed. At least three biological replicates were analysed for each barley genotype at each malting stage.

Grinding

For all measurements, 10 g of barley grains were ground to a fine powder using an IKA Mill (Germany) with running water to avoid overheating during milling.

Moisture content

The moisture content of barley flour was determined in triplicate using near infrared (NIR) spectroscopy (Unity Scientific SpectraStar™ 2500, Unity Scientific Asia Pacific, Australia) according to the methods reported in [(14)].

Determination of Vitamin E content

To extract tocols from barley and avoid degradation of the isomers, an optimized method using hot saponification was adapted from Lampi et al. [(15)]. Briefly, flour (0.1 g) was added to a solution of 1 mL 100% (v/v) ethanol, 0.4 mL water and 20 mg ascorbic acid in a 15 mL Pyrex glass tube with a Teflon screw cap. After the addition of 100 µL 10.7M potassium hydroxide solution and thorough mixing, the tube was capped and transferred to a water bath with temperature of 60°C for Sumire mochi and 80°C for the rest genotypes for 25 min. During saponification, the sample was mixed every 10 min to improve hydrolysis. The tube was cooled in a water bath containing ice for 10 min and 0.5 mL 50% (v/v) ethanol was then added. To extract tocols and other unsaponifiable lipids, three portions (each 2 mL) of n-hexane:ethyl acetate (8:2, v/v) were added to the sample. After shaking the samples and solvent for 10 min and allowing it to separate into phases, the upper organic layers were collected with a disposable glass pipette and transferred to a new glass test tube. This process was repeated three times with n-hexane:ethyl acetate (8:2 v/v), and the extracted samples were then dried under nitrogen. The residue was dissolved in 1mL n-hexane and filtered through a 0.45 µm syringe filter before transfer to a 12x32 GRACE glass HPLC (high-performance liquid chromatography) vial with an amber screw cap for analysis.

The tocols were quantified according to the method reported by [15] with some modification. Tocols were separated by a normal phase HPLC using a GRACE Altima HP Silica 150 x 3 mm, 3 micron column and quantified using a fluorescence detector (NP-HPLC-FLD) with an excitation wavelength of 290 nm and an emission wavelength of 325 nm. The mobile phase was 1,4-dioxane/n-hexane (2:98, v/v) at a flow rate of 1 mL/min. Separation of tocols was based on isocratic elution [15].

The quantity of individual vitamin E isomers in the samples was determined by comparison of the retention time and calibration curves of standard which were prepared in hexane over the concentration range of 1.0-25.0 μg/mL using GenStat 14 (Lawes Agricultural Trust; VSN International, Ltd., Hemel Hempstead, UK). The vitamin E content, expressed in mg of α-tocopherol-equivalents (TE), was calculated according to the method described by [16] using biological activities of 1.0 for α-T, 0.3 for α-T3, 0.4 for β-T, 0.05 for β-T3, 0.1 for γ-T, 0.01 for γ-T3 and 0.01 for δ-T. (α-TE = α-T*1.0 + α-T3*0.3 + β-T*0.4 + β-T3*0.05 + γ-T*0.1 + γ-T3*0.01 + δ-T*0.01).

Determination of antioxidant capacity

Antioxidants were extracted from barley flour (1 g) with 20 mL of 80% ethanol, which was placed in a flask in a 200 rpm shaking water bath for 4 h in the dark at 45ºC. Vacuum filtration was used to separate the supernatant, which was stored in the dark at -20ºC and analysed within 24 h [17]. Antioxidant capacity was measured using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical method [17]. Briefly, barley flour extract (0.1 mL) was added to 2.9 mL of DPPH (112 μmol L^{-1}). After mixing, the sample was allowed to stand at 23°C in the dark for 20 min. Reduction in absorbance was measured at 517 nm after 20 min using a spectrophotometer (UV/VIS. SP 8001, Metertech, Taiwan). Antioxidant activity was then

determined using a standard curve for ascorbic acid and prediction models provided by Genstat 14 and expressed as mg ascorbic acid equivalent antioxidant capacity per 100 g of fresh weight of grain.

Middle Infrared (MIR) measurement

MIR analysis was applied to determine whether there were any identifiable biochemical differences between samples from the different malting stages. Barley flour of all genotypes in each stage was scanned using a platinum diamond ATR single reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany) and spectra were recorded on OPUS software version 7.0 provided by Bruker Optics (average of 64 scans at a resolution of 8 cm^{-1}, between 4000 and 375 cm^{-1}) [(18)]. The samples were held against the ATR crystal using the pressure applicator or sample clamp mechanism supplied by the instrument manufacturer to ensure that the pressure applied was constant for all replicates.

The MIR spectra was exported in csv format into The Unscrambler X software (v 10.1, CAMO ASA, Oslo, Norway) for principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Full cross validation (leave-one-out) was used to validate both PCA and PLS-DA models [(19)]. In order to avoid over fitting in the models, the optimum number of terms in both PCA and PLS-DA models were indicated by the lowest number of factors that gave the minimum value for the predicted residual error sum of squares (PRESS) in cross validation.

Discriminant models were developed using PLS-DA regression as described elsewhere [(20)]. In this pattern recognition technique, each sample is assigned with a dummy variable as

reference value, which is an arbitrary number designating whether the sample belongs to a particular group or not; in this case barley samples sourced before malting were assigned a numeric value of 4, after kilning with a value of 3, germination with a value of 2 and samples sourced from steeping with a value of 1.

Statistical analysis

In order to determine the differences between means using the Least Significant Difference (LSD) at $P<0.05$, the one-way and two-way Analysis of Variance (ANOVA) was performed using Genstat 14. The correlation test was performed using Microsoft Excel. Spectra were exported from the OPUS software into The Unscrambler software (version X, CAMO ASA, Oslo, Norway) for chemometric analysis. Principal component analysis (PCA) was performed to determine relevant and interpretable structure in the ATR-MIR data.

RESULTS AND DISCUSSIONS

Vitamin E content during malting

Both total vitamin E content and that of its main isomer, α-T, decreased significantly from that seen at harvest (between 57-79% and 49-66%, respectively) after steeping in all barley genotypes (Fig. 1B). The same pattern was seen for the other vitamin E isomers, however, the degree of loss varied, with decreases in the content of 54-72% for α-T3, 34-61% for β-T3, 30-87% for γ-T and 42-82% for γ-T3. In the case of β-T, the levels after steeping were below the limit of detection in all genotypes.

The percentage decrease in both the total vitamin E content and the content of individual vitamin E isomers after steeping also varied between genotypes. Sumire mochi, which had the lowest vitamin E content prior to steeping, also exhibited the greatest percentage loss in total vitamin E content and the content of all isomers with the exception of α-T (100% for α -T3, β-T3, γ-T and 82% for γ-T3). Amaji nijo exhibited the next most substantial decline in vitamin E content after steeping (70%), and the greatest percentage decline in content of the main vitamin E isomer, α-T (66%). Consequently, this genotype had one of the lowest vitamin E contents after steeping, despite being among the genotypes with the highest vitamin E content at harvest.

In contrast, the reduction in both total vitamin E content and the content of the individual isomers after steeping was lowest in Sloop (58% for total vitamin E, 60%, 55%, 58%, 38% and 51% for α-T, α-T3, β-T3, γ-T and γ-T3 respectively). This is likely to be a result of the lower water sensitivity of Sloop in comparison to all other genotypes included in this study (data not shown). This is due to the fact that, during steeping, the moisture content of barley increases to a level (~45%) at which the enzymes produced during germination can diffuse

throughout the starchy endosperm and vitamin E can leach from the grain into the water [21]. As a result, barley varieties which are less sensitive to water, such as Sloop, would be expected to exhibit a lower increase in their moisture content during steeping, and therefore retain a greater proportion of their vitamin E content.

Similar to the effect of steeping, the content of vitamin E and its isomers was lower after germination compared to after steeping for most of the genotypes (Fig. 1C). However, there were some exceptions, with increases in the content of specific isomers observed for α-T in Finniss (7% increase), β-T3 in Alexis, ND14260-1, Flagship and Amji nijo (91%, 48%, 30% and 3% increase, respectively). The content of γ-T increased during the germination stage for the majority of genotypes, from 13% (Buloke) to 190% (Finniss). Again, however, the change in γ-T content was not consistent across genotypes, with Sloop and Chebec exhibiting no change in γ-T content, and levels being lower after germination in the Vlamingh (24%) and Dhow (78%).

There have been limited previous studies evalulating the changes in the content of either total vitamin E or specific vitamin E isomers during malting, and these have produced conflicting results. In the only prior study to date which has been conducted in barley, Ilona et al. [22] found that the vitamin E content was increased by 4.1 and 4.5 fold after germination in the two genotypes they studied, which they suggested was due to the beginning of biochemical reactions in the grains during the germination stage. However, this was limited to three genotypes and the method used to assess vitamin E content, the AOAC Official Method 971.30 "α-tocopherol and α-tocopheryl acetate in foods and feeds" standard colorimetric method (1971-1972), is no longer considered optimal and has been

replaced by other much more sensitive methods [23, 24]. Thus, whether these results are reliable is not clear.

Other studies, conducted in wheat [25], legumes [26] and lupins [27, 28] have also reported an increase in the content of specific vitamin E isomers after germination. Koga and Terao [28] suggested that the increase in vitamin E content of lupin grains during germination could be related to the degradation of liposome membranes releasing vitamin E with various isomers being consumed to scarvenge phospholipid peroxyl radicals. In wheat, for example, the contents of specific vitamin E isomers, including α-T and γ-T, were reported to increase from 4.37 µg/g to 10.92 µg/g and from 0.91 µg/g to 1.50 µg/g, respectively, after 7 days of germination [25]. However, there were only two isomers analysed in this study while other isomers such as α-T3, which is considered to be the dominant Vitamin E isomer, were not included. A similar study on lupin [27] showed that germination in general brought about an increase in the content of α-T which resulted in an increment in the vitamin E activity by 238% after 9 days of germination. This same study also reported, however, that germination caused a decrease in the content of γ-T and did not affect the content of δ-T in the lupin. Significant differences in the effect of germination on the content of different Vitamin E isomers has also been reported in studies in mung-beans and cowpeas [26], which showed an increase in α-T content, but decrease in γ-T content after germination.

In the current study, the decline of isomer contents was observed in α-T from 4% (Buloke) to 19% (WI2585), in α-T3 from 23% (Buloke) to 78% (Amaji nijo), in β-T3 from 19% (Buloke) to 42% (Finniss), in γ-T3 from 40% (Buloke) to 91% (WI2585). In addition, the presence of β-T was not detected after germination which resulted in a decline in the vitamin E content from 14% - 47% and 65% - 80% in comparison to steeping and

harvesting, respectively. Differences in the finding between this study and previous studies may relate to the shorter period time of germination (4 days and 7-9 days, respectively). According to Yang et al. [25], the α-T content of wheat increased after four days of germination, while γ-T content increased after four or five days germination, depending on genotypes, and reached a peak on day 8. This finding is in agreement with study in lupin which showed a significant decrease in vitamin E content of samples in the first two days of germination compared with steeping, followed by gradual increase up to day 9 [27]. Thus, the determination of changes in all vitamin E isomers, as well as total vitamin E content, across the germination period is interesting for future studies.

Kilning, the final stage of the malting process, was associated with a significant increase in both total vitamin E content, and the content of individual isomers, for most genotypes compared with levels at the completion of the germination stage (Fig. 1D). This resulted in substantially higher vitamin E contents at the end of the malting process compared to the steeping and germination stages (from 164% in Finniss to 354% in Amaji nijo). The differences in the content of vitamin E and its isomer contents between germination and kilning varied both between isomers and between the genotypes. The most substantial changes in isomer content were observed for γ-T3, followed by γ-T, α-T3, α-T and β-T3. Interestingly, β-T was not detected in any genotypes at the completion of the steeping and germination stages, but returned to levels comparable to those in unprocessed barley after kilning. Biosynthesis of β-T in seeds may therefore take place during kilning.

While there was a sharp rise in contents of vitamin E and its isomers after kilning compared to germination, the levels attained in malts were still lower than those in unprocessed barley for the majority of genotypes (Fig. 2). α-T content was significantly lower after malting

compared to before processing in 8 genotypes (Dow, Vlamingh, Amaji nijo, ND24260-1, Flagship, Sloop and Alexis), but was not different between unprocessed barley and the malt for the remaining genotypes (Fig. 2A). In the case of α-T3 content, all genotypes, with the exception of Chebec and Sumire mochi, exhibited a significant decrease after malting compared to before processed (Fig. 2B). All genotypes, except for WI2585, also had significantly lower levels of β-T3 after malting (Fig. 2D). In contrast, most genotypes had higher levels of β-T in malt than in the unprocessed samples, but this was only significant for Amaji nijo, Sloop, WI2585 and Finniss (Fig. 2C). Interestingly, malting seemed to have little or no effect on γ-T (Figure 2E) and γ-T3 (Fig. 2F) in most genotypes, with the exception of Finniss which exhibited a considerable increase in γ-T content after malting (656%). This was not, however, sufficient to increase to total Vitamin E content to levels above those in unprocessed Finniss.

In relation to total vitamin E content, levels were lower in the malt than in the unprocessed barley for the majority of the genotypes, although this difference was relatively small (between 6% (Finniss) and 22% (Alexis), and was not significantly different for Sumire mochi, Chebec, WI2585 and Buloke (Fig. 2G). Vitamin E is known to be unstable in the presence of light, water, oxygen and heat, all of which are applied to the samples during processing, and this may explain the decline in its content during malting [(29)]. However, both the levels of vitamin E in the unprocessed samples, and the stability of this compound during malting, clearly varied between genotypes (Do et al., 2014). Therefore, in order to obtain malts with higher vitamin E contents, genotypes containing higher levels of vitamin E before processing, and in which this compound remains relatively stable during malting (such as Chebec, WI2585 and Commander) are likely to be more suitable than other varieties.

Antioxidant capacity during malting

Similar to vitamin E, antioxidant capacity was reduced after steeping by between 49% (WI2585) and 74% (Sumire mochi) (Fig. 3B). Importantly, the decline in antioxidant capacity was directly correlated (r=0.7, n=14, $p<0.05$) with the decrease in total vitamin E content after steeping. This suggests that the loss of antioxidant capacity after steeping is likely to be largely ascribed to the loss of vitamin E. However, since the loss of vitamin E did not explain 100% of the reduction in total antioxidant capacity after steeping. Compounds, particular phenolic compounds such as caffeic acid, vanillic acid and gallic acid, may have leached from the pericarp and testa of the barley or formed insoluble complexes with proteins during steeping [(5)].

Interestingly, while the vitamin E was generally reduced after malting compared to before processing, antioxidant capacity was increased in the majority of genotypes, with the exception of Sloop, Vlamingh, Flagship and WI2585 (Fig. 3C). This finding is in accordance with a previous report in barley that polyphenol, β-carotenoid and vitamin C increased after germination, which was explained by the synthesis of these compounds in the seeds [(22)], and suggests that the vitamin E content of the malt may not be a reliable indicator of its overall antioxidant capacity at this stage.

Malting was associated with an increase in antioxidant capacity in comparison with before processing in most genotypes, which was significant in the case of Alexis, Flagship, Sloop and Amaji nijo (8%, 12%, 13% and 22%, respectively) (Fig. 4). The increase in antioxidant capacity after malting is likely to be due to the collective influence of three processes, (i) release of phenolic compounds bound to cellular structures, (ii) better extraction and (iii) the

formation of Maillard reaction products [30]. The phenolic compounds, mainly phenolic acids, which were bound to lignin and arabinoxylans in the unprocessed samples would be expected to be released in the presence of enzymes which are synthesised and/or activated during either the final stages of germination or the early stages of kilning [30]. The change in tissue structure caused by kilning, in particular the increased friability of the grain, has been shown to increase the ease of extracting compounds located in the outer layers of the grain, such as the phenolic compounds, resulting in improved efficiency of extraction of antioxidant compounds [30]. In addition, reducing sugars and amino acids which are reported to be released during germination could react to produce Maillard products such as melanoidins which also have antioxidant capacity [30, 31]. Interestingly, whilst almost all genotypes exhibited a significant increase in antioxidant content, Sumire mochi exhibited a significant decrease of 12.8%.

As free radicals have been widely indicated as the major cause of seed deterioration [32], those genotypes with higher antioxidant capacity may be more likely to be preserved during processing. In support of this, we found significant correlations between the antioxidant capacity in unprocessed barley and that after kilning when data from all genotypes were combined ($r=0.9$, $n=14$, $p<0.05$). Similar correlations were present for all vitamin E isomers and total vitamin E content, $r=0.9$ (for α-T, β-T3, γ-T, γ-T3), 0.7 (for β-T and total vitamin E), 0.5 (for α-T3) all with ($n=14$, $p<0.05$). These findings therefore suggest that a higher antioxidant capacity and/or vitamin E content in unprocessed barley may protect the samples from losses in antioxidant capacity and/or vitamin E during processing. Furthermore, these data suggest barley genotypes which are naturally high in antioxidant capacity and/or vitamin E are likely to be the most suitable for producing malt which also

has a high antioxidant capacity, and therefore providing potential associated health and anti-spoiling/staling benefits.

Expanding the use of hulless barley in the production of malt is a particular interest, since malts produced from hulless varieties can be directly added to a variety of products after milling without the need for hull disposal or malt extraction and processing times are generally shorter than for covered genotypes [(13)]. Of the two hulless genotypes investigated in this study, the malt produced from one, Sumire mochi, was lowest in both vitamin E content and antioxidant capacity. Finniss malt was the second lowest in vitamin E content, however its antioxidant capacity was ranked sixth among 14 genotypes indicating that this attribute was comparable to that of the hulled covered genotypes. This suggests that the Finniss hulless barley may have some potential for use as a variety for malt production. We are currently investigating the potential for Finniss malt to be used in food products.

Investigating compositional changes during processing by MIR

In an attempt to gain further insights into the compositional changes which occurred within the different barley genotypes during processing, samples were analysed by MIR before and after each of the malting stages (Fig. 5A). PCA analysis of the data acquired from the MIR spectra produced clear clustering of the samples according to the processing stage. The distribution pattern of the genotypes, however, varied according to the processing stage, and the different genotypes were much more tightly clustered before processing and after kilning than after the steeping and germination stages. The tight clustering of the unprocessed barley samples is expected given that they had not yet gone through any processing thus no reaction had occurred. Similarly, the close clustering of the genotypes after malting likely due to the fact that the chemical reactions which would have occurred

during processing were stopped by the high temperatures applied during kilning, since previous studies have demonstrated that enzymes in grains were inactivated and moisture content reduced to 2-6% after the kilning stages [(31)].

In contrast, there was considerably wider spread of the genotypes for the samples collected at the completion of the steeping and germination stages. This is likely to be due to variation between the genotypes in their moisture content and/or water sensitivity (data not show), both of which would affect the rate at which biochemical reactions stimulated by the increasing oxygen/water content of the grains, or by swelling of grains, proceeded during the steeping stage [(21)]. Similarly, the rate and extent of the biosynthesis of new enzymes/compounds that occurred in the germination stage is also likely to be affected by the water content and physical characteristics of the grain, and therefore to vary between genotypes. In both the steeping and germination stages, the two hulless varieties, Finniss and Sumire mochi, were clustered closely together, and at a distance from the covered varieties, likely to be indicative of differences in composition/physical characteristics between covered and hulless varieties of barley.

The ability of the classification models based on MIR to identify the process is based on the molecular information provided by the MIR spectra, and the greater the variability in composition between the various malting stages (kilning, germination, etc.), the better the accuracy of the model. The combination and interactions of several compositional characteristics of the sample therefore provides the necessary information for discrimination. In this study, the combination of spectroscopy and chemometrics as an analytical tool gave the advantage of being able to rapidly visualise the changes occurring during the processing of barley without the need for quantitative data.

Similar eigenvectors or loadings as described for the PLS-DA (data not shown) were observed. The classification statistics obtained when the PLS-DA classification model was developed using the ATR-MIR spectra are shown in (Fig. 5B). The coefficients of determination (R2) obtained for the PLS-DA models were 0.9 (standard error of cross validation 0.3), indicating that these models accounted for 90% of the variability in composition related with processing stage. The number of PLS loadings used to develop the models were 5 where the optimal PLS-DA loadings derived from the calibration model are shown in (Table 1). The analysis of the optimal loadings indicated that MIR regions corresponded to compounds containing nitrogen (e.g. proteins), carbohydrates and lipids explain the variability observed between the barley samples related to processing stage. These findings are consistent with existing knowledge of the changes which occur during the malting process, which include (i) the formation of insoluble phenolic complexes with proteins [(5)], (ii) the synthesis of amylases, proteases and β-glucanases, causing polymer degradation [(33)], (iii) degradation of lipids due to the appearance of water and oxygen and high temperature [(34)]. The highest and negative loadings were observed at 2939, 1716, 1427 and 1396 cm^{-1}, corresponding to lipids and carbohydrates [(35)]. Specifically, the 2939 and 1427 cm^{-1} bands are associated with CH stretching and bending respectively, while the 1716 cm^{-1} band corresponds to the carbonyl group. These bands indicated the presence of triglycerides, diglycerides, acyl group carbonyls, and hydrocarbon chains in the barley samples and they change as a consequence of the processing. The diethyl ether soluble polar fractions around 1267 cm^{-1} might be also associated with the P=O band of phospholipids. In addition, the 985 and 771 cm^{-1} regions have been reported by other authors to correspond to trans fatty acids in cereals products (Pomeranz 1973). While the unprocessed and processed samples were clearly distinct from all other malting stages in the

PLS-DA and PCA analyses, there was, some overlap in the models for the steeping and germination stages. This can be explained by overlap in the lipids composition in some of the varieties at these two stages.

CONCLUSIONS

There have been significant correlations between unprocessed barley and malt in terms of the antioxidant capacity and vitamin E. The change of antioxidant capacities after malting also corresponded with that of vitamin E and both were genotypic dependant. These findings will allow genotypes remaining in high antioxidant capacity and/or vitamin E content to be chosen for malting. Additionally, Finniss has comparable antioxidant capacity to that of other genotypes and currently investigated the potential use as hulless malt in food products.

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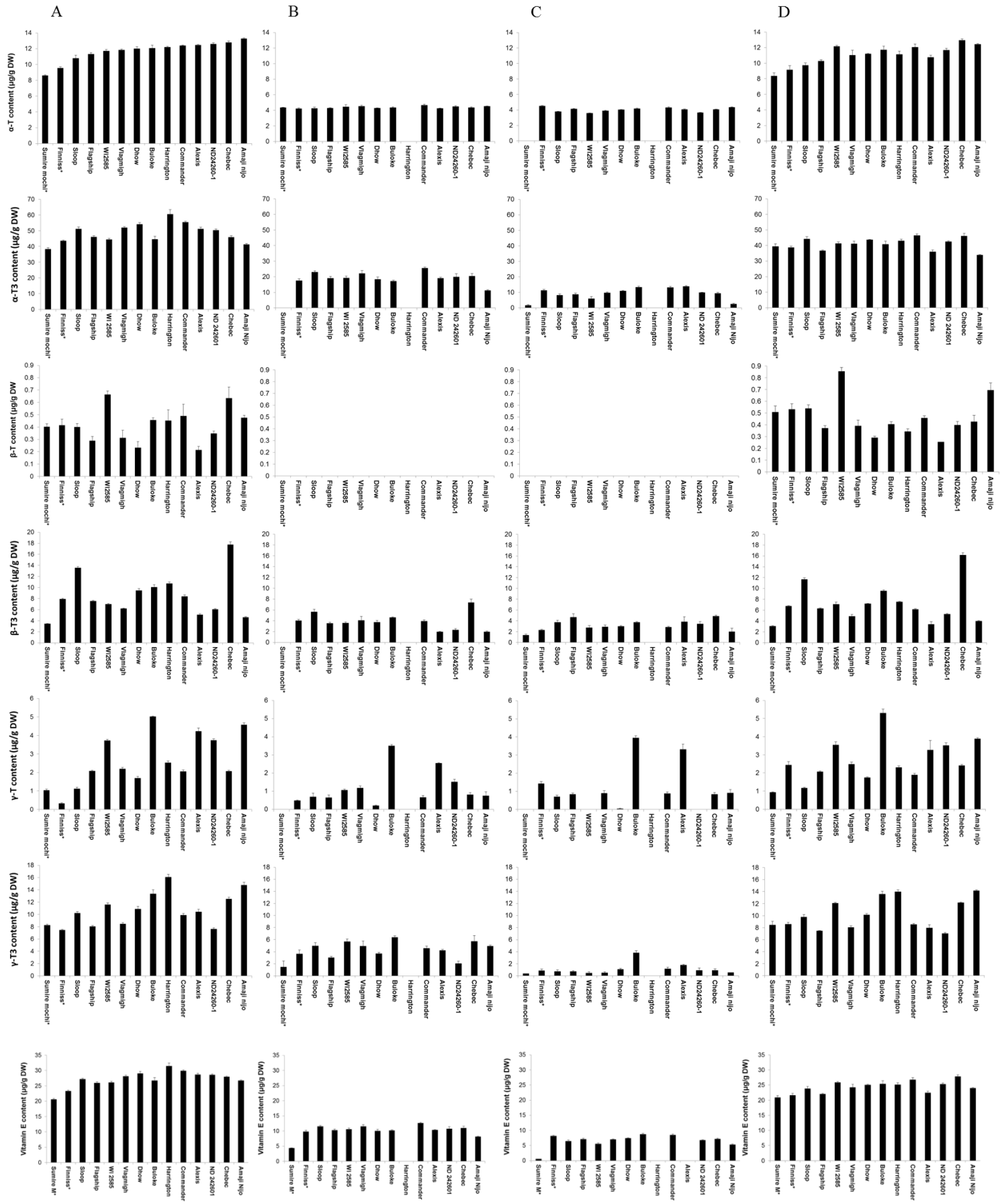
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Finniss*
Sloop
Flagship
Wi2585
Vitagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo

Figure 1 The content of isomers and vitamin E in 14 genotypes during malting. The genotypes marked with * are hulless genotypes. The others are covered genotypes. Vitamin E is expressed in mg of α-tocopherol-equivalents (TE). Bars represent the mean ± SE. A, before processing, n=6 except n=3 for Buloke. B, after steeping, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis and Amaji Nijo. C, after germination, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis and Amaji Nijo. D, after kilning, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis, Amaji Nijo, and Harrington. Harrington was only sampled before processing (A) and after kilning (D) due to limited availability of seed.

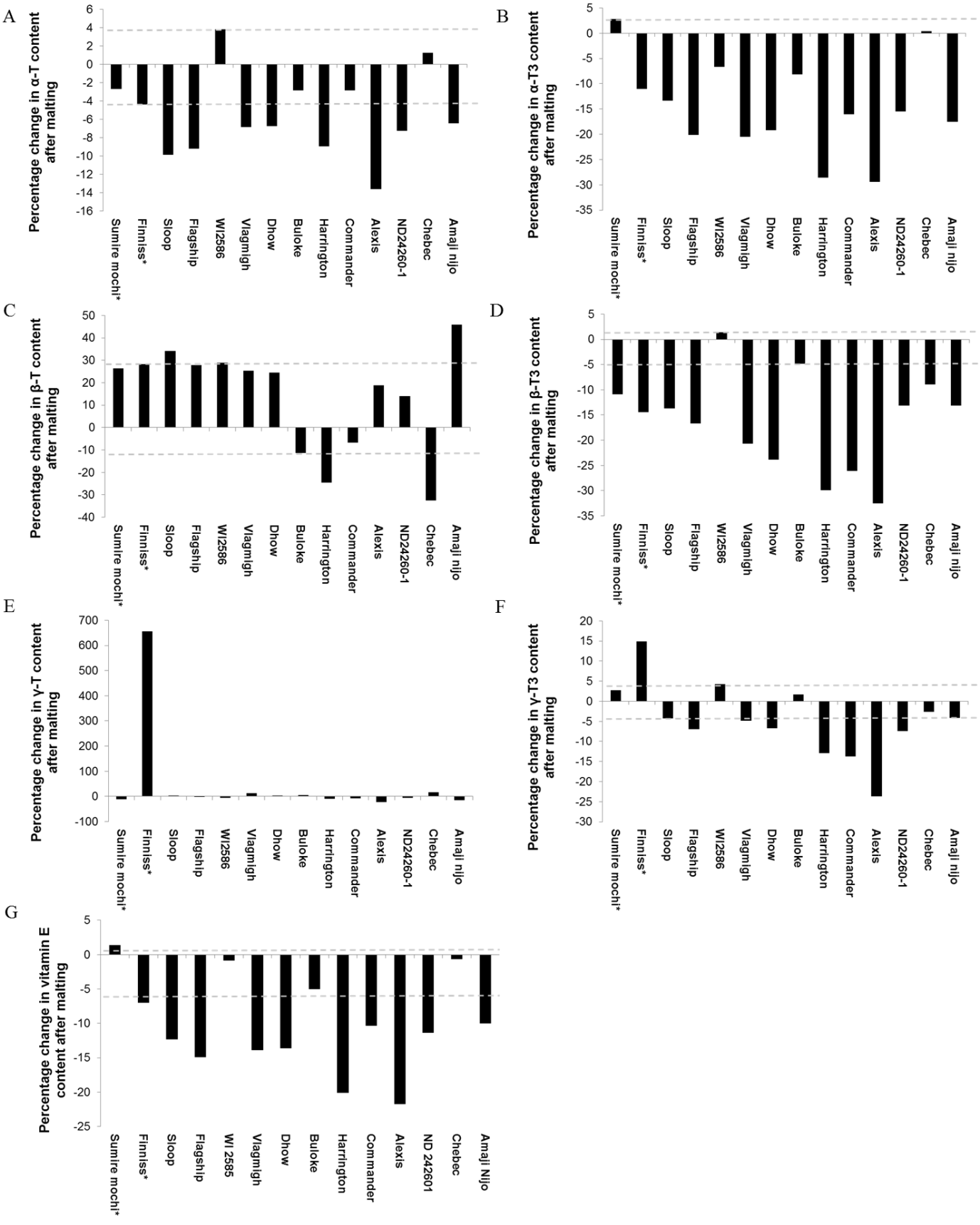
A
Percentage change in α-T content after malting
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
B
Percentage change in α-T3 content after malting
5
0
-5
-10
-15
-20
-25
-30
-35
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
C
Percentage change in β-T content after malting
50
40
30
20
10
0
-10
-20
-30
-40
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
D
Percentage change in β-T3 content after malting
5
0
-5
-10
-15
-20
-25
-30
-35
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
E
Percentage change in γ-T content after malting
700
600
500
400
300
200
100
0
-100
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
F
Percentage change in γ-T3 content after malting
20
15
10
5
0
-5
-10
-15
-20
-25
-30
Sumire mochi*
Finniss*
Sloop
Flagship
WI2586
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND24260-1
Chebec
Amaji nijo
G
Percentage change in vitamin E content after malting
5
0
-5
-10
-15
-20
-25
Sumire mochi*
Finniss*
Sloop
Flagship
WI 2585
Vlagmigh
Dhow
Buloke
Harrington
Commander
Alexis
ND 242601
Chebec
Amaji Nijo

Figure 2 The percentage change after malting in content of α-T (A), α-T3 (B), β-T (C), β-T3 (D), γ-T (E), γ-T (F) and vitamin E (G) in different barley genotypes. The genotypes marked with * are hulless genotypes. The others are covered genotypes. Vitamin E is expressed in mg of α-tocopherol-equivalents (TE). Bars present the mean ± SE. The Least Significant Difference ($LSD_{sample.time}$) ($P<0.05$) for α-T=0.81; $LSD_{sample.time}$ for α-T3=2.92; $LSD_{sample.time}$ for β-T=0.10; $LSD_{sample.time}$ for β-T3=0.49; $LSD_{sample.time}$ for γ -T=0.25; $LSD_{sample.time}$ for γ-T3=0.72; $LSD_{sample.time}$ for vitamin E=1.50. Bars not within dotted lines represent genotypes with significantly different percentage change of antioxidant capacity after kilning.

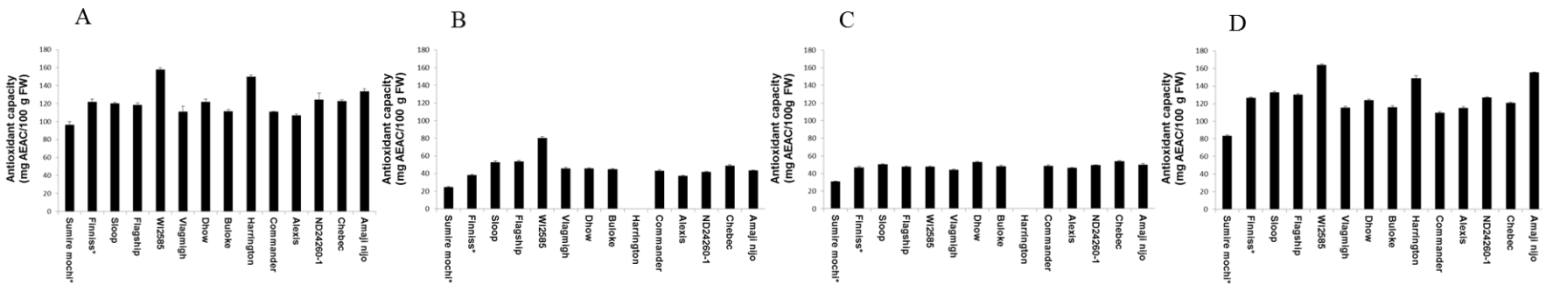

Figure 3 The antioxidant capacity in 14 genotypes during malting. The genotypes marked with * are hulless genotypes. The others are covered genotypes. Antioxidant capacity is expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh weight (FW) of grain. Bars present the mean ± SE. A, before processing, n=6 except n=3 for Buloke. B, after steeping, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis and Amaji Nijo. C, after germination, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis and Amaji Nijo. D, after kilning, n=9 except n=3 for Chebec, Dhow, Sumire mochi, ND24260-1, WI2585, Alexis, Amaji Nijo and Harington. Harrington was only sampled before processing (A) and after kilning (D) due to limited availability of seed.

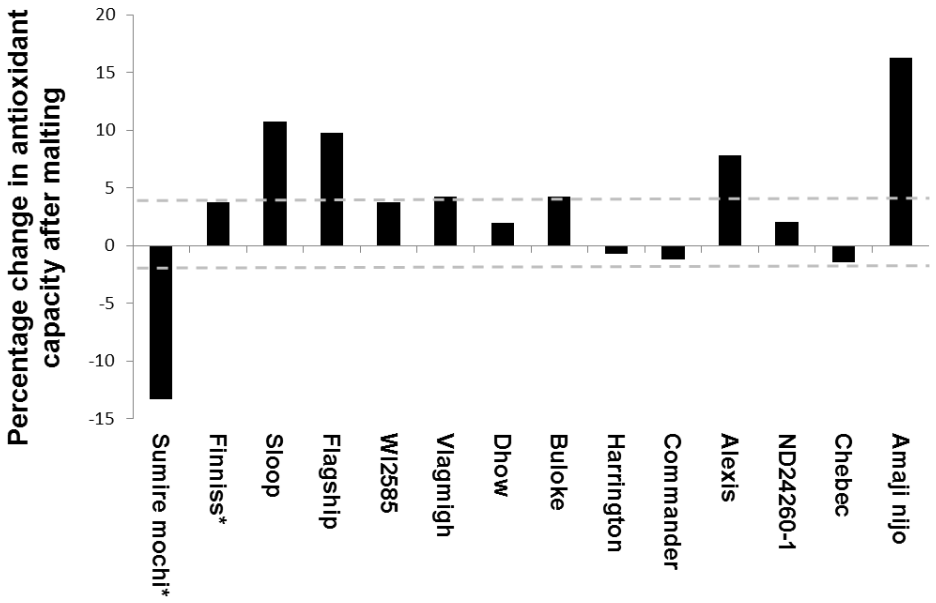

Figure 4 The percentage change after malting in antioxidant capacity in different barley genotypes. The genotypes marked with * are hulless genotypes. The others are covered genotypes. Antioxidant capacity is expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh weight (FW) of grain. The Least Significant Difference ($LSD_{sample.time}$) ($P<0.05$) =4.91. Bars not within dotted lines represent genotypes with significantly different percentage change of antioxidant capacity after kilning.

A B

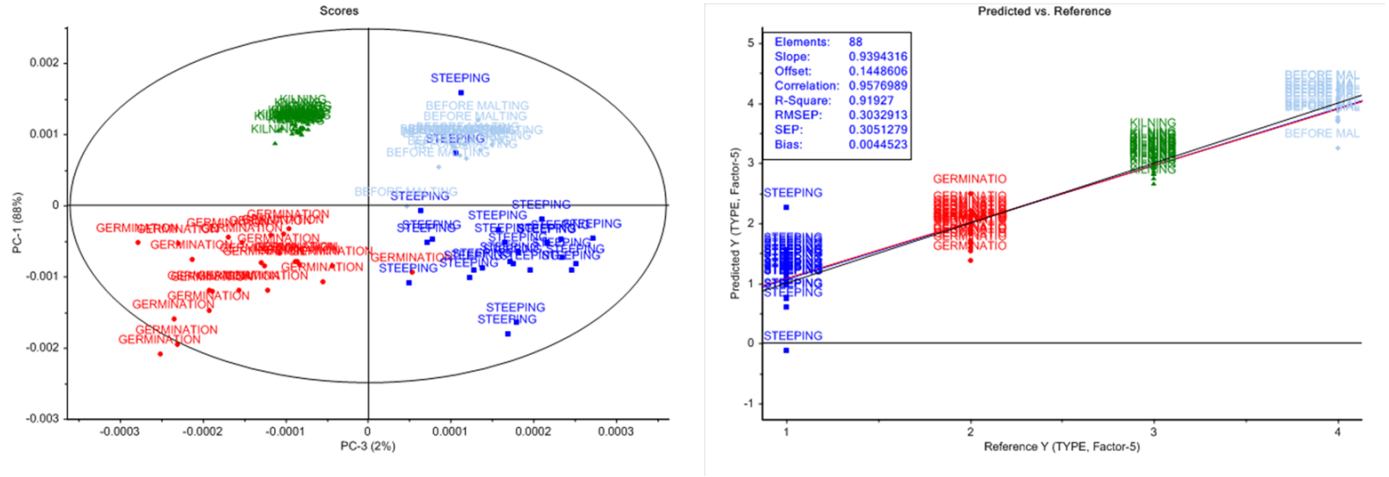

Figure 5 Score plot of the two principal components of barley genotypes during malting. A, classification using Partial Least Squares Discriminant Analysis (PLS-DA). B, using score plot of the two principal components based on the attenuated total reflectance and mid infrared spectroscopy (ATR-MIR). PC1, principal component 1; PC3, principal component 3.

Table 1 Optimal loadings derived from the PLS-DA analysis at wavenumbers associated with certain molecules. +, positive; -, negative.

Wavenumbers (cm^{-1})	Molecules	Effect
2939	Lipids	-
1716	Lipids	-
1636	Amide group and water	+
1542-1539	Amide groups/protein	-
1427		-
1396		+
1267	P=O phospholipids	-
985	Trans fatty acids	+
771	Trans fatty acids	-

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