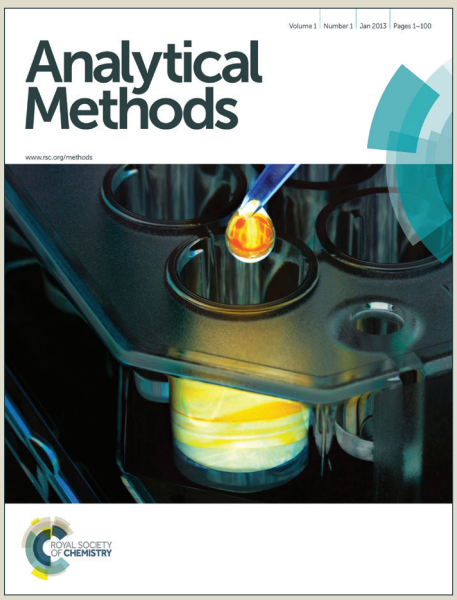
Analytical Methods

Accepted Manuscript



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Study on the role of sugar fatty acids in explaining differences in the malt composition of barley analysed using vibrational spectroscopy and chemometrics

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Abstract

Extensive research has been conducted on the understanding starch structure and composition in relation with grain composition and quality. However, less effort has been dedicated to studying the main role of other non-structural carbohydrates and their interactions with other molecules such as glycolipids and lipopolyscharides. The formation of complexes between amylose and lipids has been also highlighted, indicating that these complexes can modulate different biochemical, chemical and physical properties of the grain of different cereals. Recent studies also suggested that other sugars (e.g. sucrose) can be associated with lipids in the form of lipopolysaccharides, glycol-glycerolipids or sugar esters having an important role on food properties (e.g. gelatinization of the starch, formation of emulsions). The aim of this study was to investigate the presence of sugar fatty esters in barley malt using mid infrared (MIR) spectroscopy. Results from this study indicated the existence of correlations between the area in the MIR related with esters (1800–1600 cm^{-1}) and malt extract, showing statistically significant (p<0.05) correlations (R^2 = 0.36) in barley varieties that yield high malt extract (>83%). The results of this study also indicated that sugar fatty esters or esters might play a role in explaining difference in malt composition between different barley varieties.

Key words: barley, mid infrared, malt, sugar fatty esters

1. Introduction

Starch is known to be the main reserve carbohydrate in barley; however, other non-structural carbohydrates are present in the grain matrix. In particular, monosaccharides such as sucrose, fructose and other minor oligosaccharides are abundant, representing between 1 to 4% of the total dry weight (*dw*) of the grain.[1-2] Extensive research has been conducted on the understanding starch structure and composition in relation with grain composition and quality. However, less effort has been dedicated to study other non-structural carbohydrates and their interactions with other molecules such as glycolipids and lipopolyscharides.[3-4] In recent years, reports on the formation of complexes between amylose and lipids highlighted the importance of different grain components and their relationships in modulating different biochemical, chemical and physical properties in different cereal grains, including barley.[5-6] Recent studies also suggested that the amylose-lipid complex can be associated not only with the direct effect of the lipids in the amylose complex, but also with their interrelations with other molecules such as sucrose esters or lipopolysaccharides that can also penetrate or interlink with the amylose-amylopectin structure.[7-10] It has been reported that triglycerides represent a major fraction of surface lipids of maize and wheat, suggesting that glycolipids and phospholipids could correspond to amyloplast membrane remains.[11] However, the location of such lipids at the surface of the starch granule is still unknown.[11] Glucosyl hydroxyl groups of α[1–4] glucan chains are located on the outer surface of the helix allowing the more hydrophobic inner core to form inclusion complexes with a diversity of ligands.[12] Complexes between amylose and lipids, such as fatty acids, lyso-phospholipids and mono-acylglycerides, have been reported to significantly modify the properties and functionality of the starch.[12] In particular, the presence of lipids during hydrothermal

treatments can decrease the swelling capacity of the starch granules, and complex formation has been shown in many studies to increase gelatinisation temperature, reduce gel rigidity, retard retrogradation and reduce the susceptibility to enzymatic hydrolysis.[12]

It has been reported that sugar fatty esters are important biomolecules as they can carry not only sugars but also long chain fatty acids into the plant cell.[13-18] As sucrose contains eight hydroxyl groups, compounds ranging from sucrose mono- up to octa-fatty acid esters can be produced.[13-18] These compounds can be found to have various degrees of esterification resulting in a similar wide range of properties and including compounds such as glycolipids, glycerolipids, sterols, ceramides, and sphingolipids.[13-18] However, the current state of knowledge of their exact biochemical, chemical and physiological roles and their effects on the bio-physical properties of grain is still incomplete.[13-18] Sucrose serves as one of the main carbon source for the synthesis of membrane lipids, phospholipids, non-storage proteins and enzymes.[13-18] In most cereals, the majority of the sucrose might be converted into starch, where the conversion into lipids accounts for less than 5% of total carbon pool in the plant.[16] In foods, the presence of sugar fatty esters (e.g. sucrose esters, glycolipids) can also determine several rheological properties and in particular they have being used as an emulsifier in ice cream and bread.[19]

Malt quality is an economically important character of barley and research in barley breeding, biochemistry and genetics has led to improvements in the understanding of some of the characteristics that determine the properties of the barley grain and corresponding malt.[20-24] Grain biochemical components other than starch such as proteins, non-structural, structural polysaccharides and lipids, influence or modulate the quality of the grain and consequently its malting properties.[4, 20-24] As stated by other authors, malt quality evaluation is approaching a new age beyond the basic quality analyses currently in use.[20, 24] New technologies or methods that measure new aspects of malt quality not considered or understood in the past

will allow a better understanding of the main drivers of composition, to better facilitate product development and to improve efficiencies in the brewing process as well as to develop or improve barley varieties.[20, 24] Therefore, research into new components of the grain is required in order to expand our knowledge of the biochemistry of the grain. Although research in barley biochemistry and chemistry barley breeding has led to considerable improvements in the understanding of starch structure and its implications in explaining malt composition, the potential of obtaining an increase in malt extract appears to be limited not only to the starch content but also to the presence of other sugars and olygosaccharides.[20-24] Malt extract is a measure of the total water soluble materials derived from the barley malt available for brewing.[22] Recently, the presence of functional markers associated with malt extract in the same region associated with glycerol-phospholipids was reported.[25] These authors stated that this glycerol-phospholipid (glycerol-betaine) (localized in the short arm of the chromosome 7H of barley) is also in a region adjacent to the sucrose synthase locus correlated with malt extract.[25] This finding highlights the importance of the interactions between sugars, lipids and malt extract.

Complex biological systems such as grain and malt are very hard to analyse in isolation. Therefore the combination of fingerprint methods such as mid infrared (MIR) spectroscopy with multivariate data analysis has been reported as a powerful analytical tool for the qualitative and quantitative analysis of several biological and food matrices.[26-29]

The aim of this study was to investigate the presence of sugar fatty esters in barley malt using mid infrared (MIR) spectroscopy as a high throughput method. Furthermore, absorption values at specific frequencies in the MIR region associated with the presence of sucrose esters were used to develop correlations with malt quality properties such as hot water extract (HWE).

2. Materials and methods

Barley grain (*Hordeum vulgare L*) and corresponding malt samples were obtained from commercial varieties and experimental lines sourced from The University of Adelaide Barley Breeding Program (Waite Campus, South Australia). Commercial barley varieties analysed in this study included Commander, Navigator, Admiral, Flagship, Schooner and Gairdner, collected at two consecutive harvests (2012 and 2013) and nine localities of South Australia.[29] Malt samples were analysed sequentially using the methodology published elsewhere.[29] For the purpose of this study, samples were taken at 5 min after hot water extraction (65°C ±5) and centrifuged (2500 g).[29] After centrifugation a white creamy layer (emulsion) appeared (see Figure 1). This creamy layer has the characteristics and consistency of an emulsion as reported by other authors.[30-31]

Samples (grain, malt and white creamy layer) were analysed in a platinum diamond attenuated total reflectance (ATR) single reflection cell, mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany). Scanning protocols and set up were given in a previous report.[27-28] Air was used as reference background spectra and the ATR diamond surface was cleaned with ethanol (95% v/v) before each sample was analysed. The absorption region between 2500 and 2000 cm^{-1} due to carbon dioxide and the ATR diamond cell, was discarded prior to the calculation.[27-28] Before univariate and multivariate analysis, the ATR-MIR spectral data were pre-processed using the standard normal variate (SNV) transformation in order to correct for multiplicative interferences and variations in baseline shifts, followed by the second derivative Savitzky-Golay (2nd polynomial order and 40 smoothing data points).[32] The Unscrambler software version X (CAMO ASA, Norway) was used to carry out both the pre-processing and multivariate data analysis. The area in the MIR range and corresponding ratios related with esters and total carbohydrates were calculated

using the infrared absorbance at specific wavenumbers between 1800 to 1600 cm^{-1} and between 1600 and 1000 cm^{-1} for esters and total carbohydrates, respectively. These regions were defined based on published studies by other authors.[35-40] The area and ratios were analysed statistically (Student *t*-test) using GenStat (14th Ed., VSN International, UK, 2011) ($p<0.05$).

3. Results and discussion

Figure 1 shows the white creamy layer in different barley malt samples after 5 minutes of hot water extraction. It can be observed that different barley malt varieties showed a separation in three distinctive phases. An upper liquid phase (the wort was not analysed in this study), the middle emulsified phase or creamy layer, and the bottom phase (having a silky and grist consistency). Similar findings were reported by other authors where artificial or synthetic emulsions of sucrose fatty acids were prepared and extracted using hot water.[30-31, 33]

It has been reported that for the physical characterisation of sugar lipids and glycolipids MIR spectroscopy is a suitable methodology and in particular to study the properties of molecules that form polymeric aggregates.[34] Therefore, in order to investigate the composition of the creamy fraction in each of the barley malt samples, the layer phase was analysed. Figure 2 shows the second derivative of the MIR spectrum of the creamy layer for each of the malt samples analysed. The second derivative showed absorptions at 3369 cm^{-1} (O-H, alcohols), at 2923 cm^{-1} (C-H, aldehydes) (not shown), 1646 cm^{-1} C-O (carbonyl group, aldehydes), and as 1259 cm^{-1} C-O (alcohols).[35-40] Intense absorptions were also observed around 1030, 1070, 1160 and 1630 cm^{-1} related to water, sugars and compounds containing nitrogen (**Panel A**).[35-40] Absorptions related to the CH-OH and alkyl frequencies

for sugars mainly associated with glucose and fructose, were found between 1000 and 1200 cm^{-1}, where absorptions associated with sucrose were found around the region between 1130 and 1025 cm^{-1} (specific peaks at 926, 1005, 1048, 1124 cm^{-1}).[35-40] Sucrose is characterized with an absorption at frequencies around 1005 cm^{-1} due to the presence of the glycosidic links.[41-42] It has been reported by other authors that the MIR range between 1150 and 1024 cm^{-1} might be also associated with the presence of glycolipids, and around 1740 and 1469 cm^{-1} with phosphodiesters (**Panel A**).[35-40] In addition, it has been reported that in the MIR region frequencies around 1740 cm^{-1} might be characteristic of sucrose esters (Panel A) as reported by other authors.[35-40] Other regions that were reported to be associated with the presence of ester linkages showed characteristic functional groups around 3420−3500 cm^{-1} (O−H stretch of free hydroxyl in sucrose), 1740−1750 cm^{-1} (ester C-O), 1056, 1107 cm^{-1} (C−O stretch of C−O−C), 995 cm^{-1} (glycosidic bond stretch of sucrose), and 2847−2860, 2904−2945, 1460−1470 cm^{-1} (C−H stretch in CH3 and/or CH2).[35-40] In this study, frequencies at 1747 and 995 cm^{-1} were associated with the white creamy layer and have been reported to be associated with sucrose esters.[35-40] The reduction in intensity of the hydroxyl band and the formation of strong bands corresponding to ester carbonyl and C-H stretch in CH_3 and/or CH_2 (between 1700−1750 cm^{-1}) has been reported by other authors to be associated with sucrose esters that exhibit a maximum carbonyl band intensity (**Panel B**).[40-42]

Figure 3 shows the carbohydrate to esters ratio calculated in the different malt samples analysed. Statistically significant ($p<0.05$) differences in the area of the MIR and the corresponding ratios between the malt samples analysed were observed. In barley varieties that tend to yield high malt extract (>83%) a high content of esters (measured as the esters area) in relation to the carbohydrates (starch content) was observed. It is important to note that the current available laboratory methods used to measure malt extract determine the specific gravity of the wort, relating the strengths of sucrose solutions with their specific

gravities, assuming that the dissolved changes in the extract solids measured as specific gravity are to the same extent related to sucrose.[29] However, the specific density of the wort might not be exclusively related with sugars in order to explain the observed differences in malt between barley.[29]

In order to further establish that sucrose fatty esters are present in the malt samples and they might be associated in explaining malting quality characteristics, correlations between HWE and the area in the MIR range related with esters (1800-1600 cm^{-1}) were reported (see Figure 4). Statistically significant ($p<0.05$) correlation between the MIR area and hot water extract (HWE) ($R^2 = 0.36$) was observed in the malt barley varieties having high malt extract (>83%). The coefficient of determination obtained indicated that almost 40% of the variation in HWE can be explained by the MIR region associated with esters groups.

The ratio between carbohydrates to esters was calculated in the malt samples sourced from different harvests, localities and crosses (Figures 5 and 6). Figure 5 shows the calculated ratio for two malt varieties sourced from two localities and three consecutive harvests. The trend in the relationship between carbohydrates to esters was consistent in each of the varieties independent of the year of harvest. Figure 6 shows the results of the ratio between carbohydrates to esters in a validation set using a cross between the variety Canada with Navigator and Admiral (see Figure 5). The most important outcome from the validation was that lines or crosses having either Navigator or Admiral as one of the parents showed similar trends for the ratios of starch to esters.

The results of this study indicated that sucrose esters or esters might play a role in explaining differences in malt properties in different barley varieties. These results also indicated that the MIR spectrum of the barley endosperm contains the fingerprint of the main

chemical, biochemical or biophysical characteristics related to an individual genotype. However, more studies need to be carried out in order to extend the use of this approach to other varieties or breeding lines.

4. Conclusion

The results of this study indicated that sucrose esters or esters play a role in explaining differences in malt properties in different barley varieties. These results also indicated that the MIR spectrum provides with the fingerprint of the main chemical, biochemical or biophysical characteristics related to an individual genotype and this is not related exclusively with starch content (amylose or amylopectin). However, more studies need to be carried out in order to extend the use of this approach to other varieties or breeding lines.

Acknowledgments

The authors thank technical staff of the Barley Quality Laboratory and the Barley Breeding Program, The University of Adelaide. This project (UA00126) is supported by Australia's grain growers through their investment body the Grain Research and Development Corporation (GRDC), with matching funds from the Australian government. The authors declare no conflict of interest.

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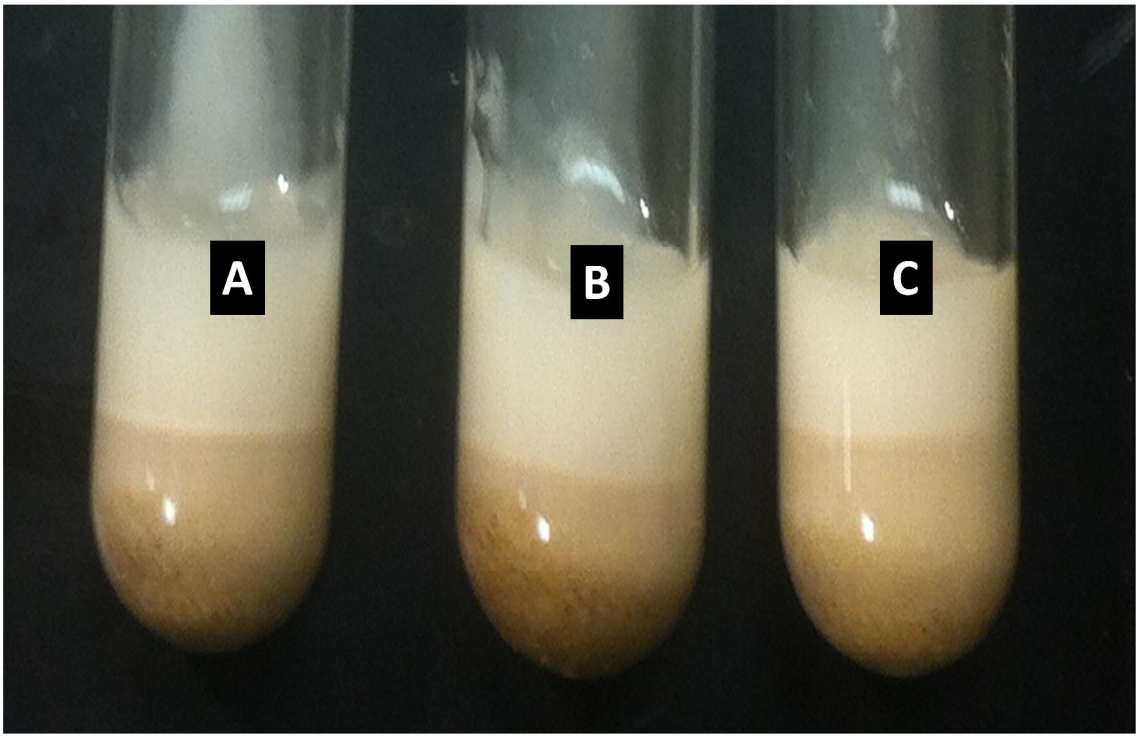
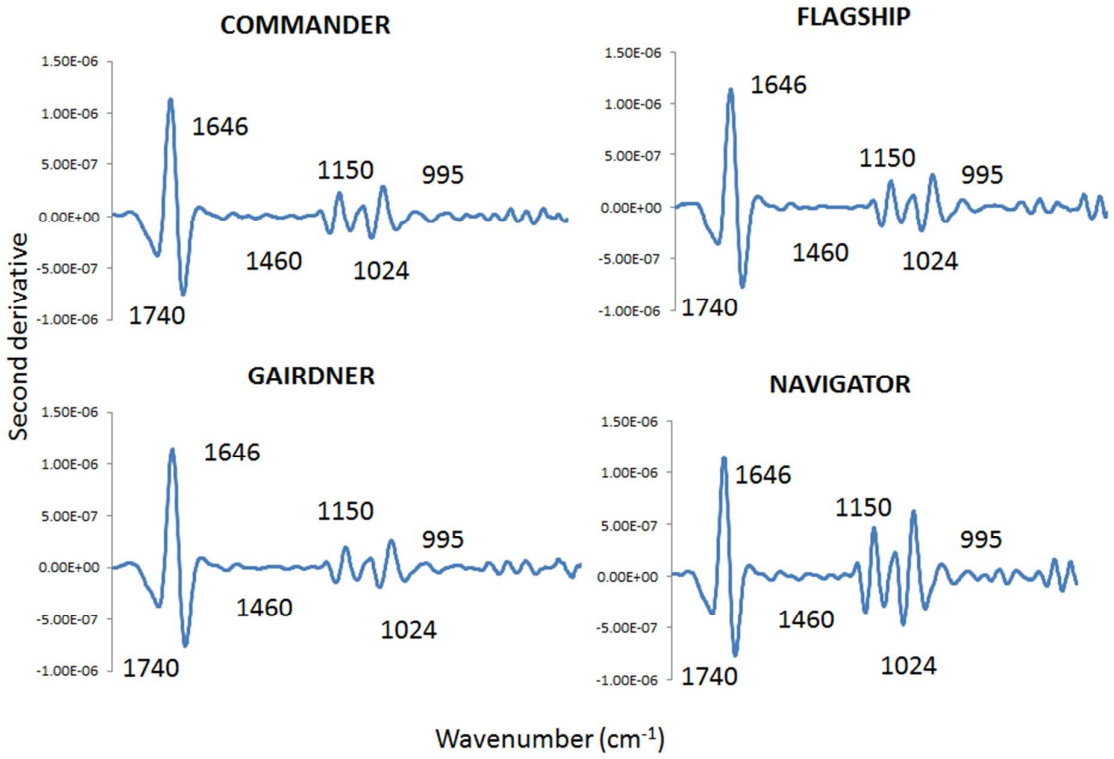
Figure 1. Creamy and grist layer obtained after hot extraction of malt samples sourced from two varieties and two localities.



A: Admiral, CHA; B: Navigator, CHA; C: Navigator, RAC

Figure 2. Second derivative of the mid infrared spectra of creamy layer in each of the barley samples analysed. **Panel A**: fingerprint range; **Panel B**: Carbonyl esters region (1700-1760 cm^{-1}).

Panel A: fingerprint range (700-1800 cm^{-1}).



Panel B: Carbonyl esters region (1700-1760 cm^{-1})

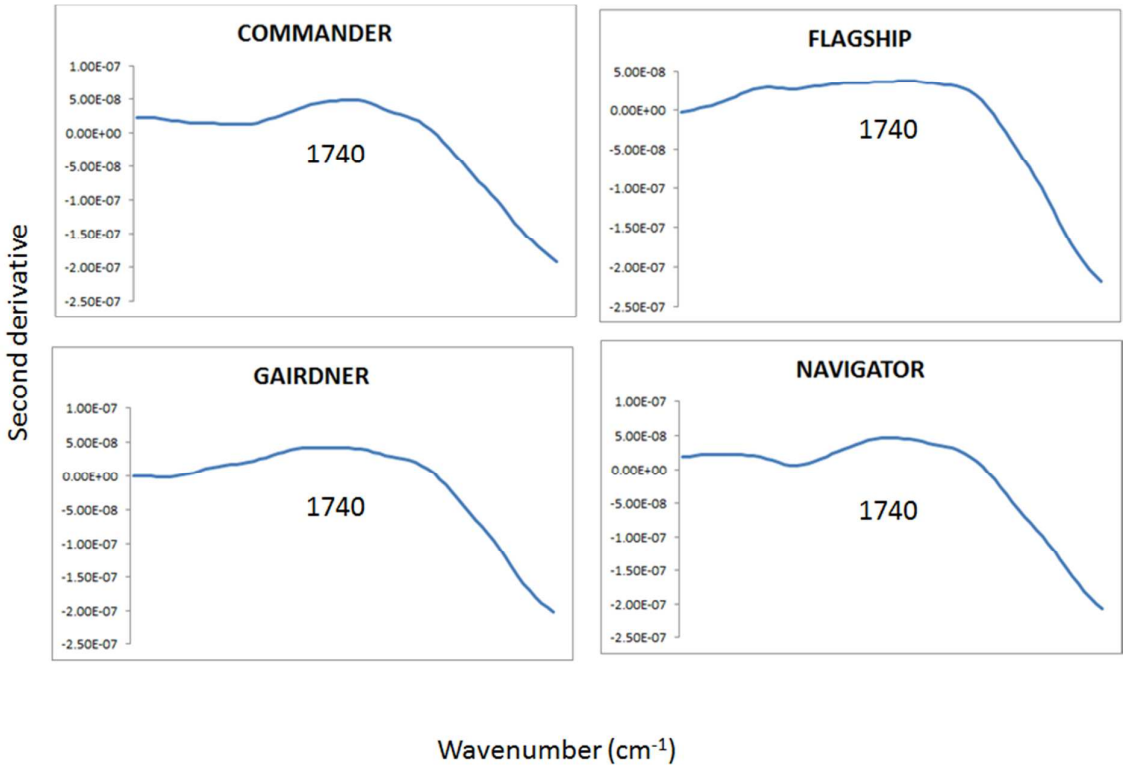


Figure 3. Area from the MIR corresponding to carbohydrates, esters and ratio between carbohydrates and esters in four barley malt varieties. NAV: Navigator, ADM: Admiral; SCH: Schooner, FLAG: Flagship.

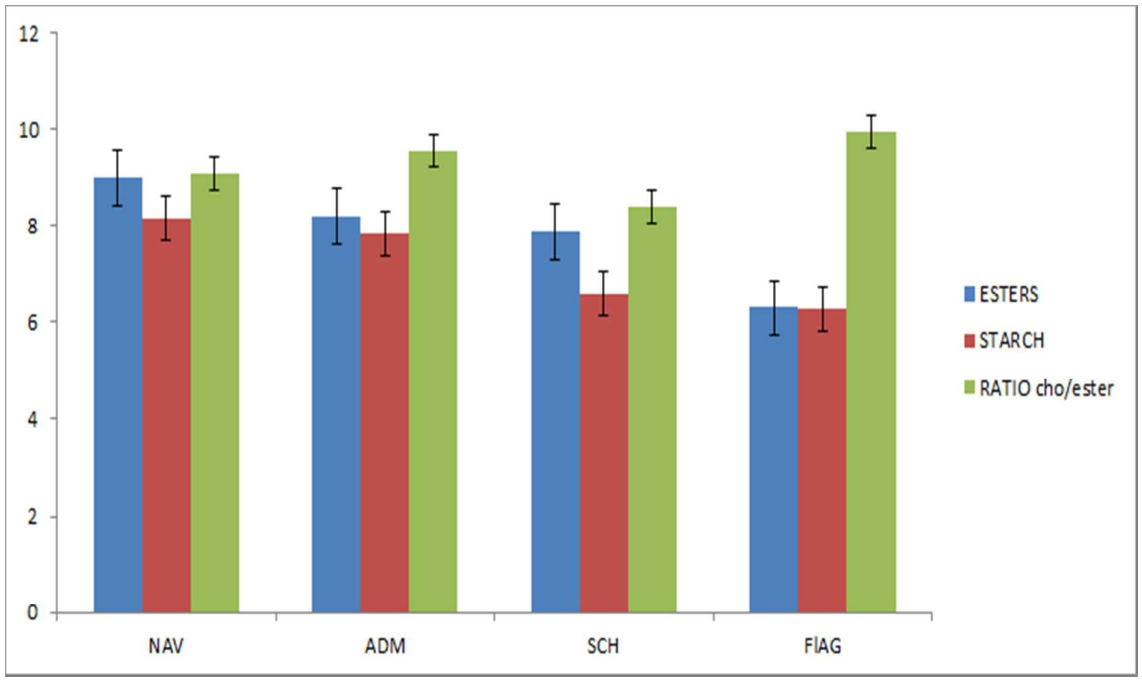


Figure 4. Linear correlations between hot water extract and the area in the mid infrared associated with esters.

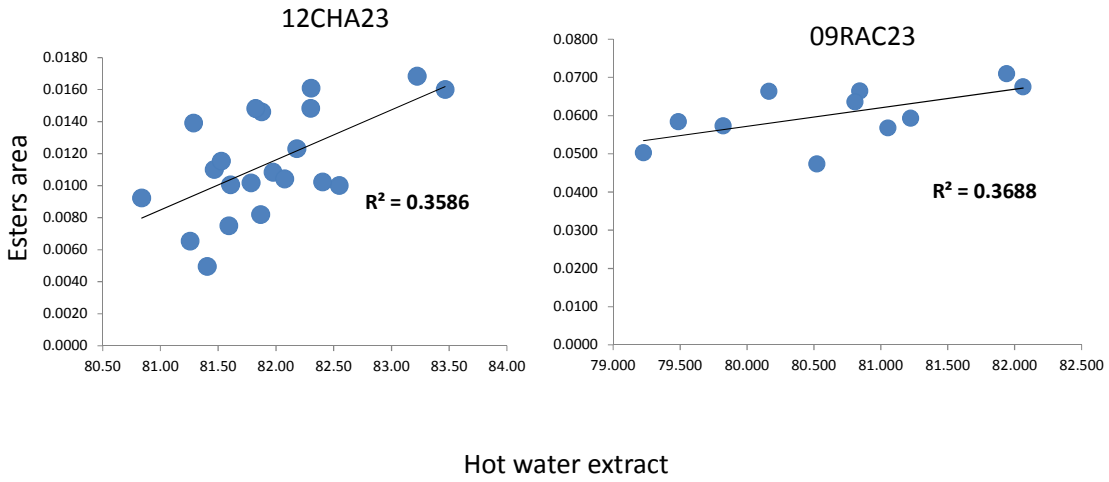


Figure 5. Mid infrared ratio between carbohydrates and esters in two barley varieties analysed sourced from three consecutive harvests.

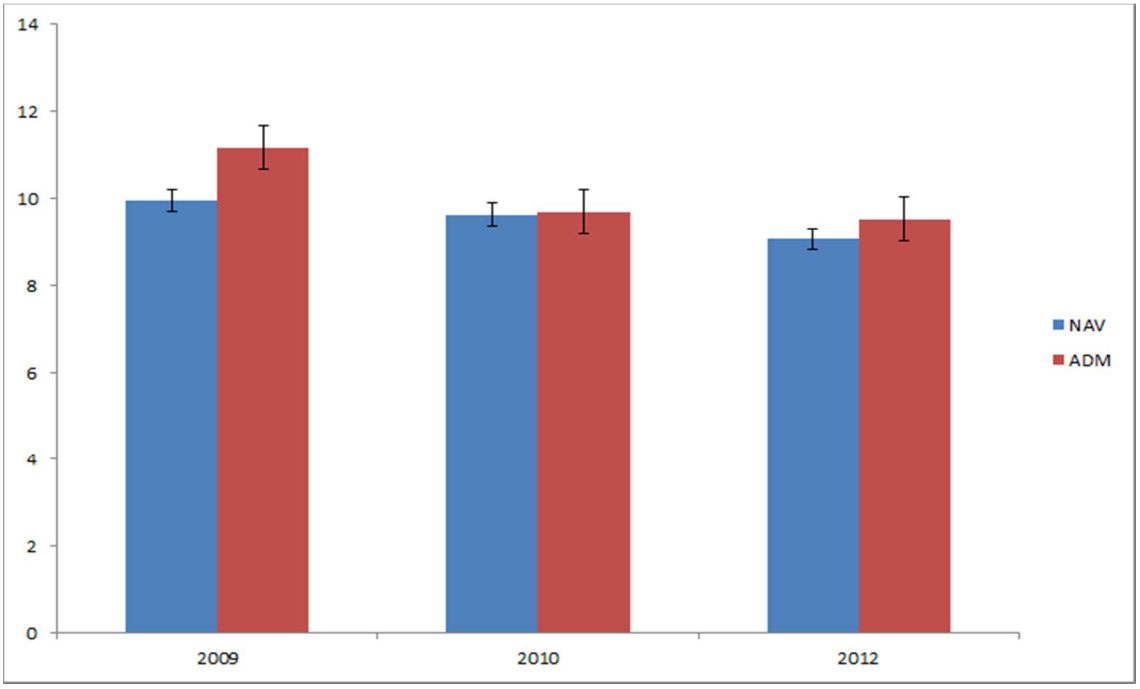


Figure 6. Carbohydrate and ester ratio in a cross derived from Admiral and Navigator with Canada.