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

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Extensive research has been conducted for understanding starch structure and composition in relation to 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 lipopolysaccharides. The formation of complexes between amylose and lipids has also been highlighted, indicating that these complexes can modulate the different biochemical, chemical and physical properties of different cereal grains. 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 in food properties (*e.g.* gelatinization of 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. The results from this study indicated the existence of correlations between the area in the MIR related to 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 differences in malt composition between different barley varieties.

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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 and 4% of the total dry weight (dw) of the grain.[1,2] Extensive research has been conducted for understanding starch structure and composition in relation to 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 lipopolysaccharides.[3,4] In recent years, reports on the formation of complexes between amylose and lipids have highlighted the importance of different grain components and their role in modulating the different biochemical, chemical and physical properties of 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 granules 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, lysophospholipids and mono-acylglycerides, have been reported to significantly modify the properties and functionality of starch.[12] In particular, the presence of lipids during hydrothermal treatments can decrease the swelling capacity of 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- 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

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sphingolipids.[13–18] However, the current state of knowledge of their exact biochemical, chemical and physiological roles and their effects on the biophysical properties of grains is still incomplete.[13–18] Sucrose serves as one of the main carbon sources for the synthesis of membrane lipids, phospholipids, non-storage proteins and enzymes.[13–18] In most cereals, most of the sucrose might be converted into starch, where the conversion into lipids accounts for less than 5% of the 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 been used as emulsifiers in ice cream and bread.[19]

Malt quality is an economically important characteristic 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] Biochemical components of grain 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 on the biochemistry of barley and the chemistry of 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 oligosaccharides.[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.

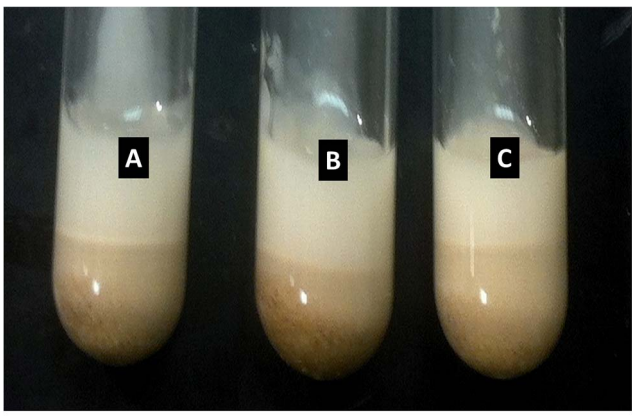
Complex biological systems such as grain and malt are very hard to analyse in isolation. Therefore, the combination of fingerprint methods such as mid-infrared (MIR) spectroscopy with multivariate data analysis has been reported as a powerful analytical tool for the qualitative and quantitative analysis 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 from 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 Fig. 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 interference 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 to esters and total carbohydrates were calculated using the infrared absorbance at specific wavenumbers between 1800 and 1600 cm^{-1} and between 1600 and 1000 cm^{-1} for esters and total



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

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

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

Fig. 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 into three distinctive phases: an upper liquid phase (the wort was not analysed in this study), a middle emulsified phase or a creamy layer, and a 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. Fig. 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), 2923 cm^{-1} (C–H, aldehydes) (not shown), 1646 cm^{-1} C–O (carbonyl group, aldehydes), and 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

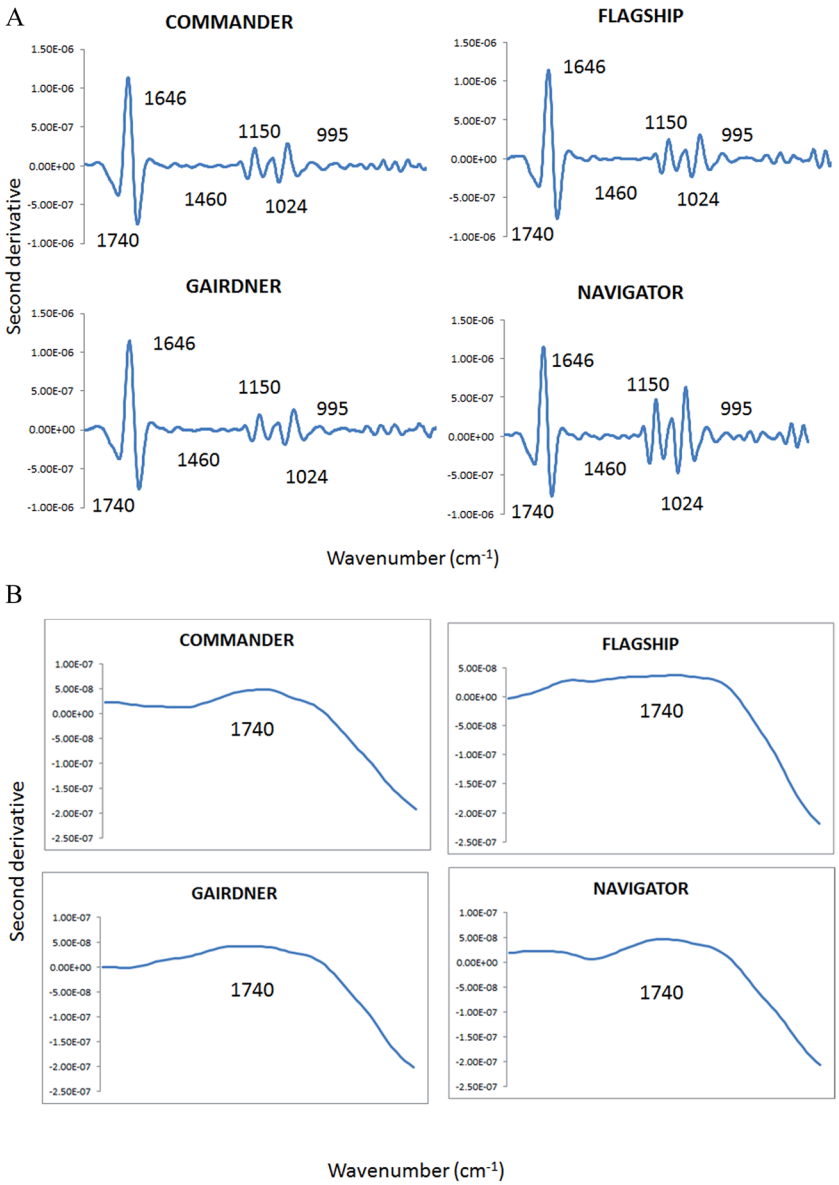


Fig. 2 Second derivative of the mid-infrared spectra of the creamy layer in each of the barley samples analysed. Panel A: fingerprint range (700–1800 cm^{-1}); Panel B: carbonyl ester regions (1700–1760 cm^{-1}).

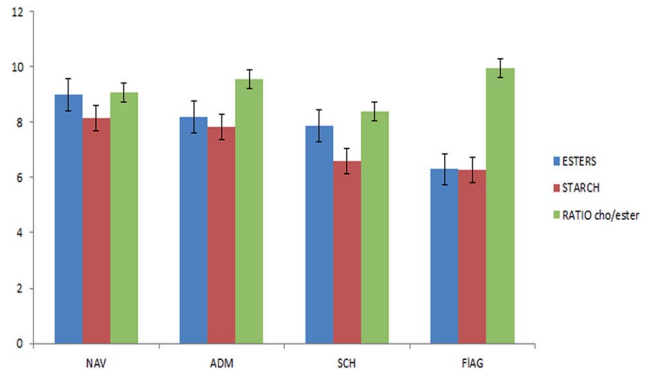


Fig. 3 Area from the MIR corresponding to carbohydrates, esters and ratio between carbohydrates and esters in four barley malt varieties. NAV: Navigator, ADM: Admiral; SCH: Schooner, FLAG: Flagship.

absorptions associated with sucrose were found around the region between 1130 and 1025 cm^{-1} (specific peaks at 926, 1005, 1048, and 1124 cm^{-1}).[35–40] Sucrose is characterized by 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, and 1460–1470 cm^{-1} (C–H stretch in CH_3 and/or CH_2).[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 the 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 and 1750 cm^{-1}) have been reported by other authors to be associated with sucrose esters that exhibit a maximum carbonyl band intensity (Panel B).[40–42]

Fig. 3 shows the carbohydrate to ester 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 ester area) in relation to carbohydrates (starch content) was observed. It is important to note that the currently available laboratory methods used to measure malt extract determine the specific gravity of the wort, relating the strength of sucrose solutions to 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 to sugars in order to explain the observed differences in malt between barley varieties.[29]

In order to further establish that sucrose fatty esters are present in the malt samples and they might be associated with malting quality characteristics, correlations between HWE and the area in the MIR range related to esters (1800–1600 cm^{-1}) were reported (see Fig. 4). A 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

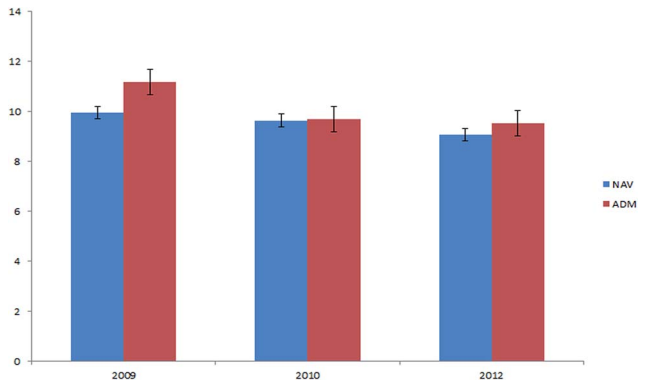


Fig. 5 Mid-infrared ratio between carbohydrates and esters in two barley varieties analysed sourced from three consecutive harvests.

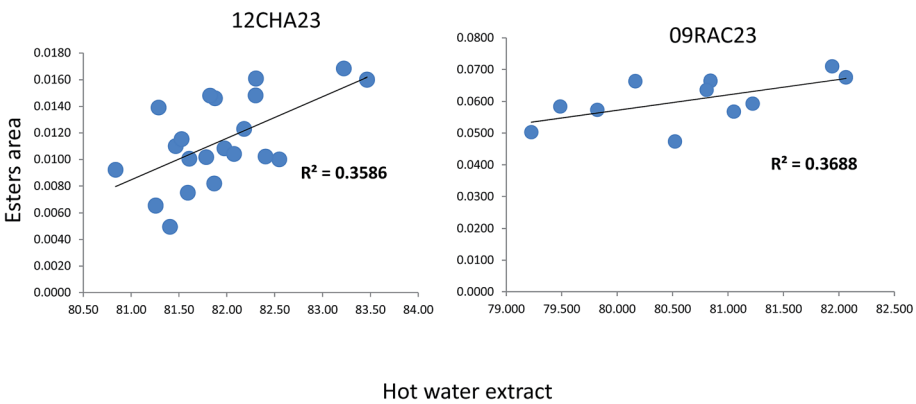


Fig. 4 Linear correlations between hot water extract and the area in the mid-infrared associated with esters.

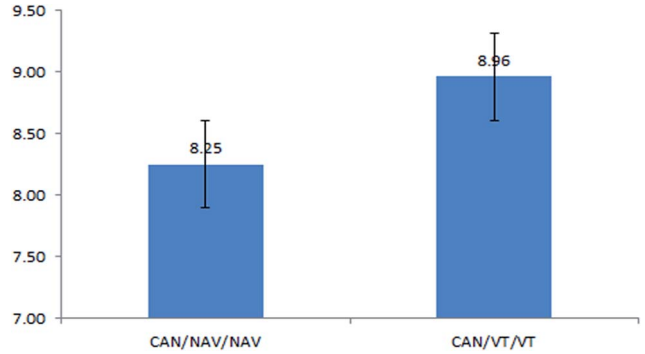


Fig. 6 Carbohydrate and ester ratio in a cross derived from Admiral and Navigator with Canada.

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 ester groups.

The ratio between carbohydrates and esters was calculated in the malt samples sourced from different harvests, localities and crosses (Figs. 5 and 6). Fig. 5 shows the calculated ratio for two malt varieties sourced from two localities and three consecutive harvests. The trend in the relationship between carbohydrates and esters was consistent in each of the varieties independent of the year of harvest. Fig. 6 shows the results of the ratio between carbohydrates and esters in a validation set using a cross derived from Navigator and Admiral with Canada (see Fig. 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 of 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 of 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 to 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.

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