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An attenuated total reflectance mid infrared (ATR-MIR) spectroscopy study of gelatinization in barley

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ABSTRACT

The aim of this study was to evaluate the use of attenuated total reflectance and mid infrared (ATR-MIR) spectroscopy and to understand the gelatinization and retro-gradation of flour barley samples and the relationship with malting quality. Samples were sourced from two commercial barley varieties exhibiting high hot water extract (HWE) namely Navigator ($n = 8$), and Admiral ($n = 8$). Samples were analysed using the Rapid Visco Analyser (RVA) and ATR-MIR analysis. These results showed that ATR-MIR spectroscopy is capable of characterising gel samples derived from barley flour samples having different malting characteristics. Infrared spectra can effectively represent a 'fingerprint' of the sample being analysed and can be used to simplify and reduce analytical times in the routine methods currently used.

1. Introduction

Starch is found extensively in many foods and knowledge of the behaviour of its polysaccharide components is important in order to manipulate or improve the quality of starchy foods (Hu, Burton, & Yang, 2010). Amylose and amylopectin are the two main constituents of starch granules. Both polysaccharides consist of α (1-4)-linked glucose units, while amylose is linear, amylopectin is a highly branched polymer with α (1,4,6)-linked glucose units occurring every 20–25 residues (Singh, Dartois, & Kaur, 2010). These polysaccharides (amylose and amylopectin) when heated in water above a critical temperature, produce a consistent gel on cooling (Singh et al., 2010). The process of gelation and the return of these systems to an ordered state, known as "retrogradation", are important characteristics of the starch as well as one of the main factors in the processing of food systems (Batey, 2007; Singh et al., 2010; Warren, Perston, Royall, Butterworth, & Ellis, 2013). It has been reported that gelatinization is affected by starch granule size, where large-granule starches gelatinize earlier than small ones, despite their higher enthalpies (Patindol, Mendez-Montealvo, & Wang, 2012; Vasanthan & Bhatty, 1996). The gelatinization range of small granules is wider and may be attributed to the higher number of granules per unit weight of starch (Patindol et al., 2012; Vasanthan & Bhatty, 1996).

The hydrolysis of malted barley starch is not a simple, single process and is affected by multiple factors such as starch structural features, the physical access of enzyme to the starch, substrate viscosity, and the availability of water (Batey, 2007; Patindol et al., 2012). Malt enzymes that include α-amylase, β-amylase, α-glucosidase, and limit dextrinase are involved in the hydrolysis of starch during mashing (Patindol et al., 2012; You & Izydorczyk, 2002). As such, the combined activity of these enzymes has been traditionally used as an indicator of malt quality through the measurement of diastatic power (DP). However, there have been instances reported in which barley cultivars and/or lines of similar DP perform differently in the brewhouse (Patindol et al., 2012). Given that starch gelatinization and hydrolysis are key factors for efficient mashing and brewing, it is of significance to study in detail the relationships between gelatinization and malting quality parameters such as hot water extract (HWE) and fermentability. Therefore, improvements of the amylose content in barley requires a better understanding of the genetic mechanism controlling amylose metabolism, although variations in amylose content are affected by both genetic and environmental factors (Hu et al., 2010; You & Izydorczyk, 2002).

Most of the previous studies on gelation and retro-gradation of starch and its components have used differential scanning calorimetry (DSC), X-ray diffraction, and various rheological methods, while few studies related with the use of vibrational spectroscopy, either Raman or infrared have been reported (Barron & Rouau, 2008; Delval et al., 2004; Goodfellow & Wilson, 1990; Iizuka & Aishima, 1999; Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008;

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Shao, Cen, He, & Liu, 2011; Stevens & Elton, 1971; Warren et al., 2013). Vibrational spectroscopy, in particular mid infrared (MIR) spectroscopy, has been used to characterise the crystallinity and gelatinization of starch due to the characteristics that can be measured at specific vibrational frequencies (Barron & Rouau, 2008; Capron, Robert, Colonna, Brogly, & Planchot, 2007; Goodfellow & Wilson, 1990; Iizuka & Aishima, 1999; Lopez-Rubio et al., 2008; Warren et al., 2013; Wilson & Belton, 1988; Wilson, Kalichevsky, Ring, & Belton, 1987). However, most of these studies reporting the use of MIR were based on pure or extracts of starch and none of them in whole flour.

In recent years, the combination of MIR spectroscopy with sampling methods such as attenuated total reflectance (ATR) has been reported as an analytical tool in different food systems (Griffiths & De Haseth, 1986; Wilson & Tapp, 1999; Subramanian & Rodrigez-Saona, 2009). This sampling method allows the measurement of solids and paste "alike" consistency samples. Currently, in conventional and routine chemical analysis of barley, harsh chemical reagents are used to destroy the biophysical characteristics of the sample that are of importance in order to understand malting quality (Karoui, Downey, & Blecker, 2010; Subramanian & Rodrigez-Saona, 2009; Wilson & Tapp, 1999). The main advantages of using ATR-MIR spectroscopy is the availability of simple, easy to use and state of the art instrumentation that can be used as a tool for high throughput screening in breeding programs (Brewer & Wetzel, 2012; Subramanian & Rodrigez-Saona, 2009; Wilson & Tapp, 1999).

Two-dimensional correlation spectroscopy (2D-COS) was originally developed by Noda (2008) and involved the study of spectral changes associated with deformations or perturbations of samples as a function of time (Noda, 2008; Noda, Dowrey, Marcott, Story, & Ozaki, 2000; Noda & Ozaki, 2004). Recently, generalized 2D-COS theory has also been applied to a series of spectra being recorded from one or more samples either during or following perturbations (e.g. temperature, pressure, spatial orientation or changes in concentration), with the resulting spectral data matrix enabling identification of compositional differences within and between samples as a result of the perturbation (Kirwan, Clark, Barnett, Nierre, & Adams, 2008; Noda, 2008; Noda & Ozaki, 2004).

The aim of this study was to evaluate the use of ATR-MIR spectroscopy to understand the gelatinization and retro-gradation in whole barley flour samples and its relationship with malting quality.

2. Materials and methods

2.1. Samples and sample pre-processing

Samples were sourced from two commercial barley varieties Navigator ($n = 8$) and Admiral ($n = 8$) both exhibiting high hot water extract (HWE) characteristics. Samples were harvested in 2012 at one locality, Charlick, South Australia. Grain samples were milled using an UDY Cyclone Mill (Sweden) through a 0.8 mm screen, before rapid visco analysis (RVA) and ATR-MIR analysis (Cozzolino, Allder, Roumeliotis, & Eglinton, 2012; Cozzolino, Roumeliotis, & Eglinton, 2013a).

2.2. Instrumental analysis

The gel samples were prepared as follows. Ground barley flour samples (4.0 g of flour corrected using the moisture content of the sample, ± 0.01 g) were slurried with distilled water (25.0 g as function of the amount of adjusted sample, ± 0.1 g) in an aluminium can. The pasting properties of the slurries were determined with a RVA instrument (Tecmaster, Perten Instruments, NSW, Australia). Details about the RVA test profile and parameters were reported in previous studies (Cozzolino et al., 2013a; Cozzolino, Roumeliotis, & Eglinton, 2013b). After cooling at room temperature, gels from each of the samples were scanned in a MIR instrument using a platinum diamond ATR single reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany). The ATR-MIR spectra were recorded on OPUS software (version 7.0) provided by Bruker Optics. The spectrum for each sample was obtained by taking the average of 64 scans (resolution of 4 cm^{-1}, between 4000 cm^{-1} and 375 cm^{-1}) with a scanner velocity of 7.5 kHz (background of 64 scans) (Cozzolino et al., 2013b). The samples were held against the ATR crystal using the pressure applicator or sample clamp mechanism supplied by the instrument manufacturer in order to ensure that the same and constant pressure was applied for all samples. Duplicates of each sample were scanned (repacked) and the average ATR-MIR spectrum of each sample was used for further analysis. Air was used as reference background spectra. The ATR diamond surface was cleaned with ethanol (95% v/v) before each sample was scanned. Flour samples were also scanned following the same scanning protocol (Cozzolino et al., 2013b).

2.3. Data analysis

Multivariate data analysis was carried out using *The Unscrambler* X software (version 10.1; CAMO ASA, Norway). Principal component analysis (PCA) was performed to determine relevant and interpretable structure in the ATR-MIR data (Brereton, 2000; Naes, Isaksson, Fearn, & Davies, 2002). The ATR-MIR spectral data was processed using the second derivative Savitzky–Golay (2nd derivative, 40 smoothing points and 2nd polynomial order) in order to remove and correct for baseline effects (Savitzky & Golay, 1964; Duckworth, 2004). The second derivative is a measure of the change in the slope of the curve ignoring the offset and is very effective in removing both baseline offset and slope from a spectrum (Duckworth, 2004). The MIR region related with non-structural (e.g. starch, amylose and amylopectin) carbohydrates (between 1200 cm^{-1} and 990 cm^{-1}) peak height intensities and their ratios were also used for analysis. These chemical function groups were identified according to published reports (Griffiths & De Haseth, 1986). The ratios were calculated using the infrared absorbance intensity at one characteristic peak divided by another one for the same variety or line. The ratios were analysed statistically (Student *t*-test) using GenStat (14th Ed., VSN International, UK, 2011) ($p < 0.05$).

In addition, the ATR-MIR profiles from both barley flour and gel samples were analysed using two dimensional correlation spectroscopy (2D-COS). In this way, 2D-COS has greatly aided structural analysis, where spectral peaks were not readily distinguishable in one-dimension (raw or processed). Two dimensional correlation analysis not only enhances spectral resolution, but also provides insight into variation in composition and structure (Noda, 2008; Noda & Ozaki, 2004). Changes in data are visualized according to 2D-COS maps where the synchronous matrix represents correlations between measured variables and the asynchronous matrix represents sequential variations occurring in the data (Kirwan et al., 2008). Both synchronous and asynchronous matrices are displayed as contour maps. Peaks located on the diagonal (called autopeaks) show regions of the MIR spectrum which are changing with respect to the average MIR spectrum of the series, while peaks off the diagonal (called cross peaks) show the correlation intensity (positive or negative) of different bands (Noda, 2008). The synchronous and asynchronous 2D-COS correlation processing was performed using

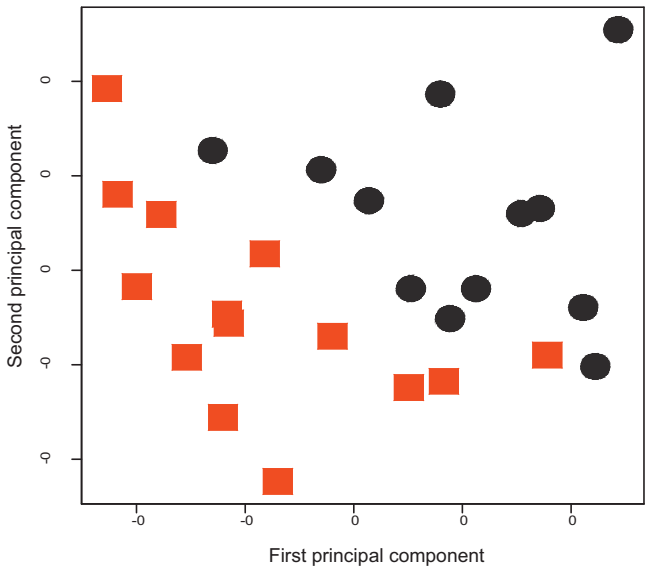

Fig. 1. Principal component score plot of the gel of barley flour samples analysed using ATR-MIR spectroscopy. Navigator ●, Admiral ■.

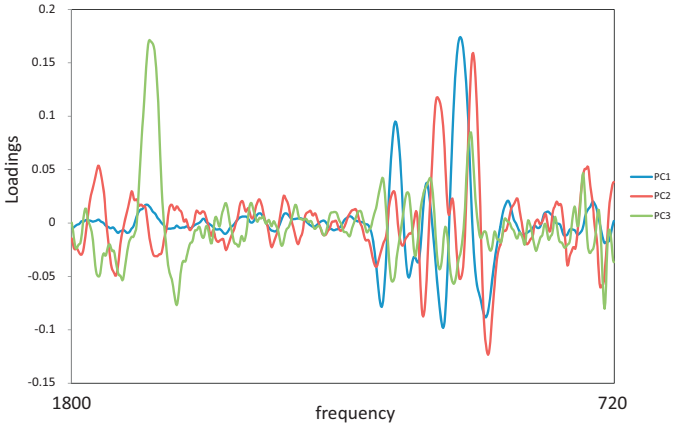

Fig. 2. Loadings of the first three principal components of gel samples derived from the barley gel samples analysed using ATR-MIR spectroscopy.

2Dshige© 1.3 software (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005), according to the theory of generalized 2D-COS correlation analysis developed by Noda and Ozaki (Noda, 2008; Noda & Ozaki, 2004).

3. Results and discussion

Principal component scores of the first two principal components that explained 90% of the variability in the set of gel samples analysed is shown in Fig. 1. Separation was observed between the gel samples analysed sourced from the two varieties. The highest loadings (Fig. 2) were observed in the MIR spectral range associated with amylose and amylopectin (1100–900 cm^{-1}), amide I (1600–1690 cm^{-1}) and lipids (1750 cm^{-1}) (Bock & Damodaran, 2013; Bock, Connelly, & Damodaran, 2013; Brewer & Wetzel, 2012; Goodfellow & Wilson, 1990; Van Soest, Tournois, De Wit, & Vliegenthart, 1995; Warren et al., 2013; Zhang & Yu, 2012). The MIR band region between 1100 cm^{-1} and 900 cm^{-1} is associated with amylopectin and amylose, where bands observed around 1022 cm^{-1}, at 1045 cm^{-1} and approximately around 995 cm^{-1} have also been assigned to the crystalline structure of the starch by other authors (Brewer & Wetzel, 2012; Goodfellow & Wilson, 1990; Li, Dobraszczyk, Dias, & Gil, 2006; Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2013; van de Weert, Haris, Hennink, & Crommelin, 2001; Van Soest et al., 1995; Warren et al., 2013; Zhang & Yu, 2012). Recently, Capron and co-workers (2007) reported that the ratio between MIR absorbance values at 1000 cm^{-1} and 1022 cm^{-1} correlated with the crystallinity index determined by X-ray powder diffraction for linearized, granular, and extruded starches at various water contents (Delval et al., 2004; Capron et al., 2007; Warren et al., 2013). In addition, regions in the MIR range between 1150 cm^{-1} and 1024 cm^{-1} were reported to be associated with glycolipids, and around 1740 cm^{-1} and 1469 cm^{-1} with phosphordiesters (Brewer & Wetzel, 2012; Goodfellow & Wilson, 1990; Griffiths & De Haseth, 1986; Van Soest et al., 1995; Warren et al., 2013; Zhang & Yu, 2012). These wavenumbers can be associated with amylose-lipid complexes present in the grain. Absorption bands related with proteins were observed in the MIR range between 1600 cm^{-1} and 1500 cm^{-1}, where specific peaks associated with amide II (N—H bend, C—N stretch and C—C stretch) at 1641 cm^{-1} and with amide I (C—O stretch, C—N stretch), at 1587 cm^{-1}, 1537 cm^{-1} and 1510 cm^{-1} (Brewer & Wetzel, 2012; Liu & Yu, 2010; Li et al., 2006; van de Weert et al., 2001; Warren et al., 2013). Structural carbohydrates such as cellulosic compounds were related to the absorption band around 1240 cm^{-1} (Stuart, 1996; Brewer & Wetzel, 2012).

The comparison of the MIR spectra (range between 1200 cm^{-1} and 800 cm^{-1}) of flour samples having high and low HWE values sourced from the two varieties is showed in Fig. 3. Samples having low malt extract showed differences in the MIR range at wavenumbers around 1823 cm^{-1}, 1881 cm^{-1}, 1799 cm^{-1}, 1517 cm^{-1} and 1213 cm^{-1}. On the other hand samples having the highest malt extract values showed differences at 2929 cm^{-1}, 2851 cm^{-1}, 1754 cm^{-1}, 1210 cm^{-1} and 801 cm^{-1}. Earlier work identified that the MIR region between 1300 cm^{-1} and 800 cm^{-1} might be related to starch, where a number of absorbance bands related to C—C, C—O stretching and C—O—H bending modes can be found (Liu & Yu, 2010; Li et al., 2006; van de Weert et al., 2001; Warren et al., 2013). Although these bands are yet to be fully assigned and the analysis is complicated by spectral overlap, it has been known that several bands in this region are sensitive to the crystallinity of the starch (Goodfellow & Wilson, 1990; Li et al., 2006; Sivam et al., 2013; van de Weert et al., 2001; Van Soest et al., 1995; Zhang & Yu, 2012). Some of these bands have been assigned to either the amorphous or the crystalline form, and band ratios have been used to provide a measure of crystallinity (Goodfellow & Wilson, 1990; Li et al., 2006; Sivam et al., 2013; van de Weert et al., 2001; Van Soest et al., 1995; Zhang & Yu, 2012). The principal band associated with the amorphous form was observed at 1022 cm^{-1}, while shoulders at 1045 cm^{-1} and approximately at 995 cm^{-1} have been assigned to crystalline starch by other authors.

The spectrum of a starch suspension in water is dominated by the presence of major OH absorbance bands arising from OH bond stretching (3700–3000 cm^{-1}, not shown) and bending (1640 cm^{-1}) modes of water. The bending mode at 1640 cm^{-1} shows only limited temperature sensitivity, while the stretching band at 3700–3000 cm^{-1} undergoes dramatic changes during heating due to its sensitivity to changes in hydrogen bonded structures (Warren et al., 2013). The structure of the OH stretching band is highly sensitive to changes in hydrogen bonding structure within an aqueous sample, and it has previously been suggested that changes in its structure may be used to probe hydrogen bonding interactions between water and polymers in aqueous polymer systems. Gelatinization results in a significant decrease at wavenumbers around 995 cm^{-1} and an increase at 1022 cm^{-1}. The peak at wavenumbers around 1080 cm^{-1}, also previously reported to depend on the crystallinity, appears to grow

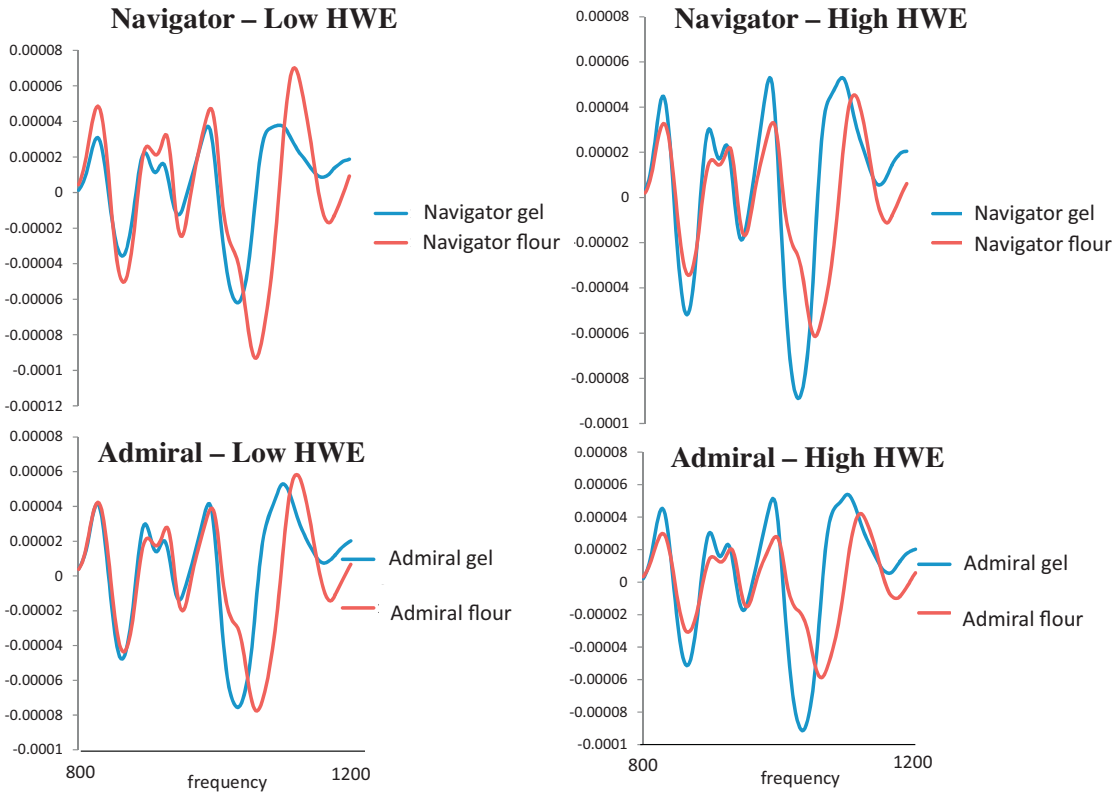

Fig. 3. Second derivative of gel and flour samples analysed using ATR-MIR spectroscopy comparing low and high hot water extract (HWE).

broader and weaker and shift to higher wavenumbers. A similar result was observed for the 1045/1022 cm^{-1} ratio. Van Soest et al. (1995) reported that the band at 1053 cm^{-1} is related with the conformational ordering involving the possible formation of double helices of the amylose or in the amylopectin branching and crystallization. This band is associated with the amylopectin aggregation and starch crystallization. For the barley flour samples analysed in this study and having low HWE the absorbance at this wavenumber (1053 cm^{-1}) did not change, however a shift was observed in this region for the samples having the highest HWE values, indicating that this band might be sensitive to polymer conformation changes during gelatinization. The absorption bands observed at 1155 cm^{-1}, 1080 cm^{-1} and 1017 cm^{-1} showed also a tendency to decrease in bandwidth during gelation while the band at 1047 cm^{-1} shows a broadening in bandwidth and might be associated with the disordering of the polymers in the crystalline phase (Van Soest et al., 1995; Warren et al., 2013). It has been reported that during gelation, the crystalline order is lost while the narrowing of the bands at 1155 cm^{-1}, 1080 cm^{-1} and 1017 cm^{-1} can be interpreted as a progression to a more uniform state of the amorphous phase (Van Soest et al., 1995; Warren et al., 2013).

Linear regressions for the ratios between 1053 cm^{-1} and 1022 cm^{-1} wavenumbers and HWE values for some of the samples sourced from the two varieties analysed were calculated and are shown in Fig. 4. High correlations but opposite in direction were observed. This can indicate that the relationship between amylose and amylopectin with HWE is not the same for both varieties. In addition, amylose-lipid complexes also play a role in determining the characteristics of the gels. Note that the correlations were intended as a tool to show the relationships between gelatinization and MIR spectra and not as predictive modelling.

The use of 2D-COS has greatly aided structural analysis, where spectral peaks were not readily distinguishable in one-dimension. Two dimensional correlation analysis not only enhances signal resolution, but also provides insight into variation in composition and structure (Noda, 2008; Noda & Ozaki, 2004). Changes in data can be visualized according to 2D-COS spectral maps where

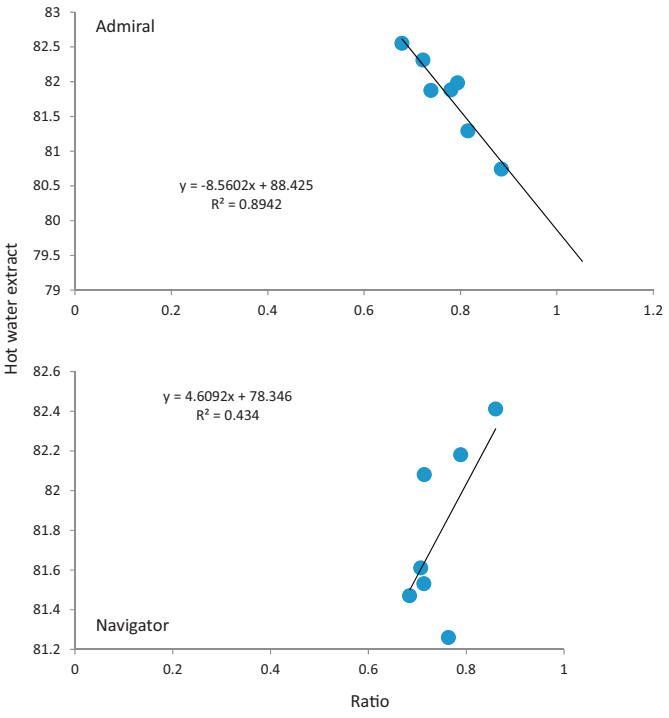

Fig. 4. Linear regression between hot water extract and the frequency ratio calculated at 1053:1020 cm^{-1} in the barley malting varieties Admiral and Navigator analysed using ATR-MIR spectroscopy.

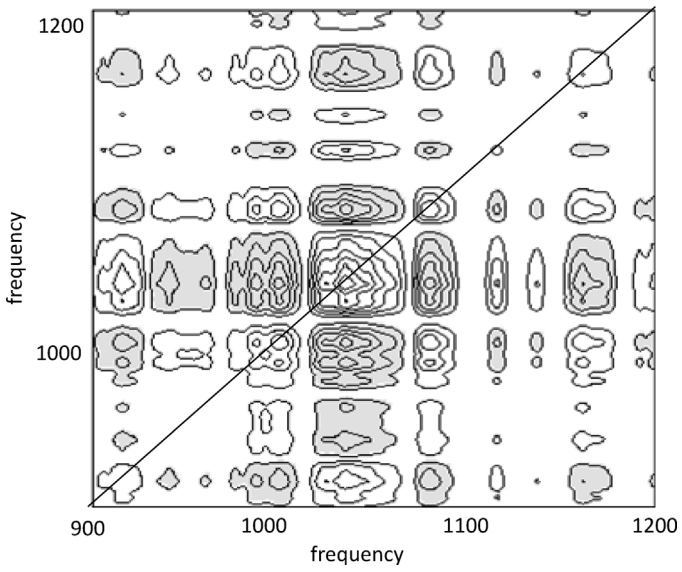

Fig. 5. Two dimensional synchronous spectroscopy of gel samples derived from the two malting barley varieties analysed using ATR-MIR.

the synchronous matrix represents correlations between measured variables and the asynchronous matrix represents sequential variations occurring in the data (Noda, 2008; Noda & Ozaki, 2004). Synchronous and asynchronous matrices are displayed as contour maps; peaks or regions located on the diagonal (e.g. autopeaks) showing regions of the data which are changing with respect to the average data of the series, while peaks of the diagonal (cross peaks) show the correlation intensity (positive or negative) of different bands (Noda, 2008; Noda et al., 2000; Noda & Ozaki, 2004). Fig. 5 shows the synchronous 2D-COS auto peaks derived from the MIR spectra of gel samples having similar and high hot water extract (HWE) content, and collected at different soaking times. The use of 2D provides a better level of information. The bands in the infrared spectra of the gel samples associated with water were not included for analytical purposes. Only the MIR region between 1200 cm^{-1} and 900 cm^{-1} was used. Strong auto peaks were observed around 1002 cm^{-1}, 1040 cm^{-1}, 1080 cm^{-1} and 1163 cm^{-1} wavenumbers. Inverse correlations were observed between 1035 cm^{-1} and 1162 cm^{-1} (Brewer & Wetzel, 2012; Goodfellow & Wilson, 1990; Katayama, Kondo, & Miyazama, 2010; Stuart, 1996; Van Soest et al., 1995; Zhang & Yu, 2012). Overall these results have shown that changes in solubility of hydrocolloids are influenced by the formation and polymerization of starch as well as by the interactions between oligosaccharides and polymers with other compounds such as lipids. These changes determine shifts at specific wavenumbers that might define the different malting characteristics between varieties.

4. Conclusions

These results showed that ATR-MIR spectroscopy is capable of characterising gel samples derived from barley flour samples having different malting characteristics. Infrared spectra can effectively represent a 'fingerprint' of the sample being analysed and can be used to simplify and reduce analytical times in the routine methods currently used. Furthermore, the technology offers the exciting prospect of potentially providing for the development of new applications of IR spectroscopy.

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