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Use of Raman microspectroscopy to predict malting barley husk adhesion quality

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Abbreviations: PC, Principal component

ABSTRACT

Good quality husk-caryopsis adhesion is essential for malting barley, but that quality is influenced by caryopsis surface lipid composition. Raman spectroscopy was applied to lipid extracts from barley caryopses of cultivars with differential adhesion qualities. Principal component regression indicated that Raman spectroscopy can distinguish among cultivars with good and poor quality adhesion due to differences in compounds associated with adhesion quality.

1. Introduction

Raman spectroscopy has been successfully used for food and cereal quality applications, including determining suitability of wheat for flour production based on protein structure (Guzmán et al., 2012; Piot et al., 2002). Premium quality malting barley (*Hordeum vulgare*) has a husk, which adheres to the caryopsis (barley fruit) at harvest. When adhesion quality is poor, the grain quality defect "skinning" results, which is the partial or complete loss of the husk at harvest or during handling. Skinning is a significant economic problem affecting the wider malting industry, reducing malting productivity by affecting germination efficiency (Okoro et al., 2017). Newer malting cultivars are more susceptible to skinning than older cultivars (M. Brennan et al., 2017) and development of cultivars resistant to skinning, but which retain desirable malting characteristics is needed. Husk-caryopsis adhesion is mediated through a lipid cementing layer produced by the pericarp (fruit coat) during grain development (M Brennan et al., 2017; Harlan, 1920; Hoad and Brennan, 2016; Taketa et al., 2008). Changes in caryopsis surface lipid composition during cementing layer development have been quantitatively linked to grain skinning (Brennan et al., 2017). Cultivars with increased proportions of sterols, triterpenoids and fatty acids, and lower proportions of alkanes were associated with good quality husk adhesion, and consequently reduced skinning. Traditional wet-chemical analyses are time-consuming and impractical in a breeding context. Here, we used Raman micro-spectroscopy on caryopsis surface-lipid extracts to determine whether this technique could distinguish among cultivars with differential adhesion qualities, as a potential tool for identifying skinning-resistant cultivars.

2. Materials and methods

Fifteen commercially relevant malting barley cultivars with husk adhesion qualities from "good" (low skinning) to "poor" (high skinning) were grown in triplicate in a glasshouse at Scotland's Rural College, Edinburgh. Skinning was assessed as described in Brennan et al. (2017), where grains with more than 20% husk loss by area are considered to be skinned. Caryopses from one main shoot ear of each replicate were harvested at 15 days post-anthesis, after cementing layer development. Soluble surface lipids were extracted from all caryopses (~30) from each ear by dipping in dichloromethane (puriss p.a. grade for GS >99.9%, Sigma-Aldrich, UK) for 20 s each. Surface lipid extracts were evaporated onto a quartz microscope slide, and examined with a Raman microscope (Renishaw, UK) equipped with a Leica DMLM microscope using the 100× objective, calibrated each day with a silicon wafer (520 cm^{-1}) at the University of Edinburgh's School of Engineering Bioimaging Facility. Three spectra were acquired from each sample (three acquisitions each) from 400 to 3200 wavenumbers, with exposure time 10 s at 100% laser power. For each, a background spectrum of the quartz slide was acquired at the same magnification, then subtracted from the corresponding sample spectrum. Spectral pre-processing was done in R (R Development Core

Team., 2008) using the HyperSpec package (Beleites and Sergo, 2017). Spectra were re-aligned on the wavenumber axis using loess interpolation. Mean spectra were calculated for the three sample replicates, which was the standardized before further analysis. Principal component analysis of the standardised spectra values for the 15 varieties was done, and re-performed with all combinations of 14 varieties to ensure that no single variety biased the results. We identified the principal components (PCs) significantly correlated with husk adhesion quality. Then, using the PC scores for the 15 varieties, linear regression between husk adhesion quality and the key PCs was done. All analysis was carried out in R (R Development Core Team., 2008). Lipid assignments were made by comparison with the literature (Czamara et al., 2015; Edwards et al., 2011; Heredia-Guerrero et al., 2014; Littlejohn et al., 2015; Prats Mateu et al., 2016; Prinsloo et al., 2004; Wu et al., 2011).

3. Results and discussion

The PCs which had the highest correlation with husk adhesion quality (skinning) were PC11 and PC14. In PC11, negative scores dominated, associated with CH_2 twisting (1296) and C-C stretching (1126 and 1064). In PC14, a negative score associated with CH2 and CH3 scissoring and deformations, and CH2 bending, was observed (1444), and a positive score associated with C=C alkyl stretches (1656). The proportion of skinned grains had a positive relationship with both PCs, and using both as predictor variables, the relationship with skinning was significant as shown in Fig. 1A ($R^2 = 0.45$, $p < 0.02$). The loadings for each wavenumber in PCs 11 and 14 are shown in Fig. 1B and C. Wavenumbers with highest and lowest loadings are shown with their vibrational assignment in Table 1. A positive loading in both PCs indicates that wavenumber contributed to poor husk adhesion (high skinning). That alkyl backbone C-C stretches contributed both positively and negatively to husk adhesion is consistent with low alkanes and higher proportions of fatty acids being associated with good quality adhesion (Brennan et al., 2017). For both PCs, CH_2 twisting, and CH_2 and CH_3 stretches and deformations contributed only positively to good husk adhesion however, indicating that the presence of fatty acids may be more important in the determination of adhesion quality. The C=C aromatic ring stretches contributed positively to husk adhesion quality in PC14, consistent with higher proportions of sterols and triterpenes being associated with low skinning (Brennan et al., 2017). Our results show that Raman spectroscopy could be useful for predicting husk adhesion quality based on differences in caryopsis surface lipids among cultivars. Previously, total internal reflectance Raman was used to directly examine barley leaf surface waxes (Greene and Bain, 2005), the limited penetration depth has the advantage of less interference from cell wall autofluorescence which made surface lipid extraction necessary in our study. Such Raman technology could allow direct on-caryopsis measurements to be made and therefore be more efficacious for breeding applications.

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References

Beleites, C., Sergo, V., 2017. hyperSpec: a package to handle hyperspectral data sets in R.

Brennan, M., Shepherd, T., Mitchell, S., Topp, C.F.E., Hoad, S.P., 2017. Husk to caryopsis adhesion in barley is influenced by pre- and post-anthesis temperatures through changes in a cuticular cementing layer on the caryopsis. BMC Plant Biol. 17, 169. https://doi.org/10.1186/s12870-017-1113-4

Brennan, M., Topp, C.F.E., Hoad, S.P., 2017. Variation in grain skinning among spring barley varieties induced by a controlled environment misting screen. J. Agric. Sci. 155, 317–325. https://doi.org/http://dx.doi.org/10.1017/S0021859616000411

Czamara, K., Majzner, K., Pacia, M.Z., Kochan, K., Kaczor, A., Baranska, M., 2015. Raman spectroscopy of lipids: A review. J. Raman Spectrosc. 46, 4–20. https://doi.org/10.1002/jrs.4607

Edwards, H.G.M., Herschy, B., Page, K., Munshi, T., Scowen, I.J., 2011. Raman spectra of biomarkers of relevance to analytical astrobiological exploration: hopanoids, sterols and steranes. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 78, 191–195.

Greene, P.R., Bain, C.D., 2005. Total internal reflection Raman spectroscopy of barley leaf epicuticular waxes in vivo. Colloids Surfaces B Biointerfaces 45, 174–180. https://doi.org/10.1016/j.colsurfb.2005.08.010

Guzmán, E., Baeten, V., Pierna, J.A.F., García-Mesa, J.A., 2012. A portable Raman sensor for the rapid discrimination of olives according to fruit quality. Talanta 93, 94–98. https://doi.org/10.1016/j.talanta.2012.01.053

Harlan, H. V., 1920. Daily development of kernels of Hannchen barley from flowering to maturity, at Aberdeen, Idaho. J. Agric. Res. 19, 393–429.

Heredia-Guerrero, A., Bayer, I.S., Cingolani, R., Athanassiou, A., BenÃtez, J.J., Heredia-Guerrero, J.A., DomÃnguez, E., 2014. Infrared and Raman spectroscopic features of plant cuticles: a review. Front. Plant Sci. 5, 1–14. https://doi.org/10.3389/fpls.2014.00305

Hoad, S.P., Brennan, M., 2016. Variety choice: key performers and what to look out for in 2016, in: SRUC, AHDB (Eds.), Agronomy 2016. Carfraemill, Perth, Inverurie and Inverness.

Littlejohn, G.R., Mansfield, J.C., Parker, D., Lind, R., Perfect, S., Seymour, M., Smirnoff, N., Love, J., Moger, J., 2015. In vivo chemical and structural analysis of plant cuticular waxes using stimulated raman scattering microscopy. Plant Physiol. 168, 18–28. https://doi.org/10.1104/pp.15.00119

Okoro, P., Brennan, M., Bryce, J., Smith, P., Kelly, H., Hoad, S., 2017. Effects of grain skinning on the malting performance of barley, in: Worldwide Distilled Spirits Conference. Glasgow, UK.

Piot, O., Autran, J.C., Manfait, M., 2002. Assessment of cereal quality by micro-Raman analysis of the grain molecular composition. Appl. Spectrosc. 56, 1132–1138.

Prats Mateu, B., Hauser, M.T., Heredia, A., Gierlinger, N., 2016. Waterproofing in Arabidopsis: following phenolics and lipids *in situ* by confocal Raman microscopy. Front. Chem. 4, 1–13. https://doi.org/10.3389/fchem.2016.00010

Prinsloo, L.C., Du Plooy, W., Van Der Merwe, C., 2004. Raman spectroscopic study of the epicuticular wax layer of mature mango (*Mangifera indica*) fruit. J. Raman Spectrosc. 35, 561–567. https://doi.org/10.1002/jrs.1185

151 R Development Core Team., 2008. R: A language and environment for statistical computing.

152 Taketa, S., Amano, S., Tsujino, Y., Sato, T., Saisho, D., Kakeda, K., Nomura, M., Suzuki, T.,
153 Matsumoto, T., Sato, K., Kanamori, H., Kawasaki, S., Takeda, K., 2008. Barley grain
154 with adhering hulls is controlled by an ERF family transcription factor gene regulating a
155 lipid biosynthesis pathway. Proc. Natl. Acad. Sci. 105, 4062–4067.
156 https://doi.org/10.1073/pnas.0711034105

157 Wu, H., Volponi, J. V., Oliver, A.E., Parikh, A.N., Simmons, B.A., Singh, S., 2011. In vivo
158 lipidomics using single-cell Raman spectroscopy. Proc. Natl. Acad. Sci. 108, 3809–3814.
159 https://doi.org/10.1073/pnas.1009043108

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Table 1 Wavenumbers that had the highest and lowest loadings for PCs 11 and 14, assignments and their contribution to husk adhesion quality

PC	Contribution[a]	Wavenumber	Assignment of vibrational mode[b]
14	-	412	
14	-	466	δCCC
14	-	494	
14	-	528	
14	-	682	νCC, ring
11	-	832	
14	-	870	
11	+	890	νCC, backbone
14	-	894	νCC, backbone
14	-	942	νCC, νCOC
11	+	948	ρCH_3, νCC, νCOC
14	-	982	βCH
11	+	1064	νCC
14	+	1074	νCC
11	+	1094	νCC
14	-	1096	νCC
14	-	1124	νCC
11	+	1126	νCC
14	+	1156	νCC
14	-	1240	δ=CH
14	-	1260	δ=CH, νCH *cis*
11	+	1296	τCH_2
14	+	1306	τCH_2
14	-	1416	βCH_2
11	+	1432	αCH_2, αCH_3, δCH_2, δCH_3
14	+	1444	αCH_2, αCH_3, δCH_2, δCH_3, βCH_2
11	+	1454	βCH_2, βCH_3, δCH_2, δCH_3
14	+	1468	βCH_2, βCH_3
14	-	1488	
14	-	1504	
14	-	1554	
14	+	1604	υC=C, aromatic
11	+	1638	υC=C, unsaturated alkyl
14	-	1656	υC=C, alkyl
14	+	1716	
11	-	2852	υ=CH_2, s
11	-	2880	υ=CH_2, s
11	+	2904	υCH_2, υCH_3, s, as
14	+	2916	υCH_3, s, as
11	+	2962	υCH_3, as

14	+	2990
14	-	3044
14	+	3094
14	-	3156
14	+	3186

[a]A "+" indicates this wavenumber increased husk adhesion quality; a "-" indicates this wavenumber decreased husk adhesion quality.

[b]α, scissoring; β, bending; δ, deformation; ρ, rocking; τ, twisting; υ, stretching; s, symmetric; as, asymmetric.

Fig. 1. A, Adhesion quality predicted by cultivar scores of PCs 11 and 14 is plotted against measured adhesion quality. The fitted model is shown, with a 95% confidence interval in grey. Loadings for B, PC11 and C, PC14 are plotted for each wavenumber. Wavenumbers with the greatest influence and for which vibrational assignments could be made are indicated.