



Short communication

Use of Raman microspectroscopy to predict malting barley husk adhesion quality

Maree Brennan^{a,b,*}, Alison McDonald^c, Cairistiona F.E. Topp^a^a Scotland's Rural College, King's Buildings, West Mains Road, EH9 3JG, Edinburgh, United Kingdom^b LERMAB, Faculté des Sciences et Technologies, Université de Lorraine, Nancy, France^c University of Edinburgh, King's Buildings, Edinburgh, United Kingdom

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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 (Brennan et al., 2017a,b) 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 (Brennan et al., 2017a,b; 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., 2017a,b). 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 microspectroscopy 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. (2017a,b), 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 Sergio, 2017). Spectra were re-aligned

Abbreviations: PC, Principal component

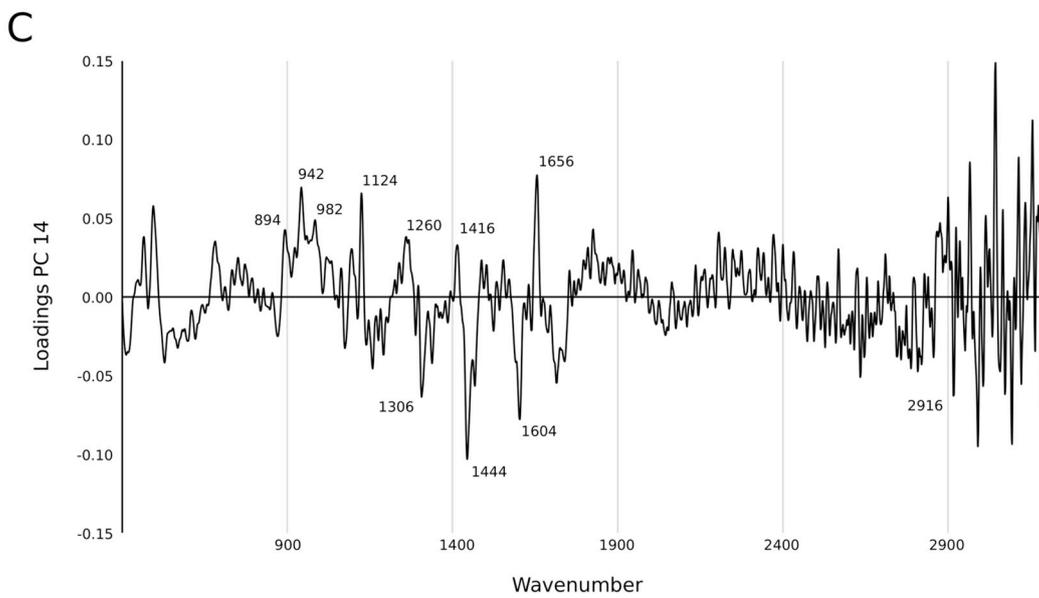
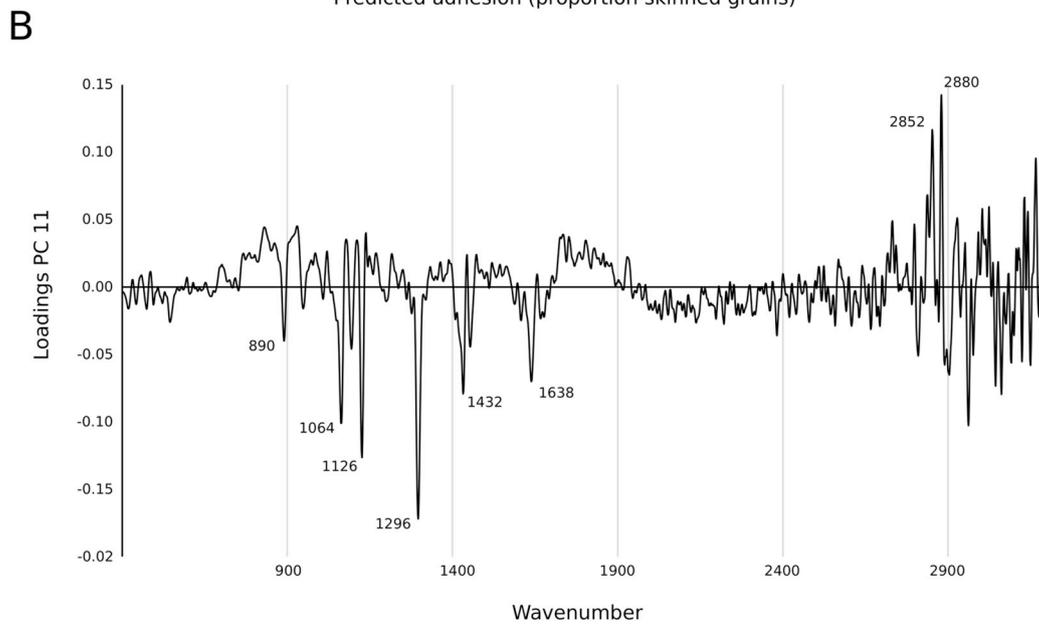
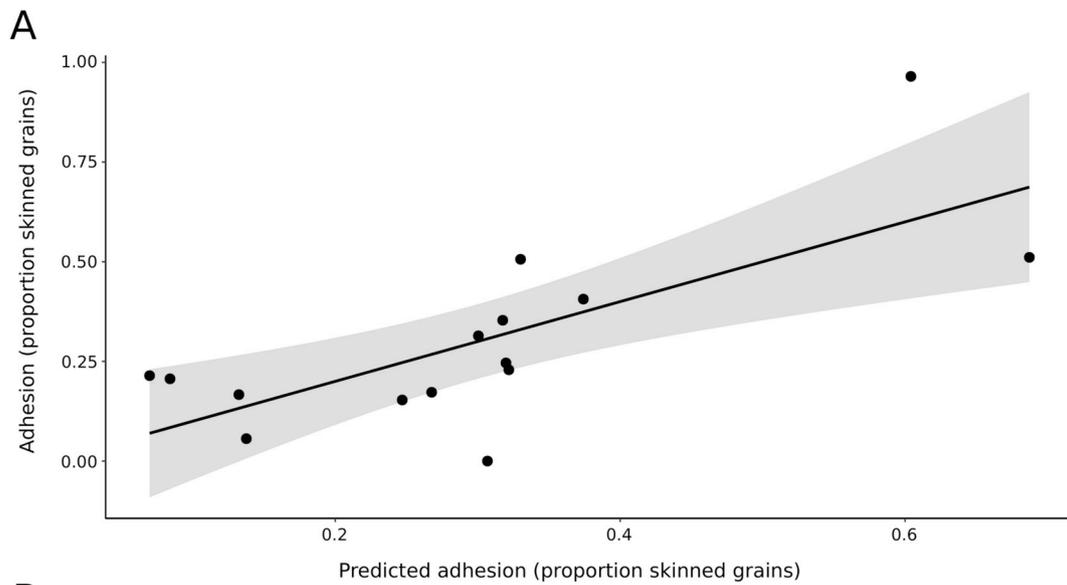
* Corresponding author. LERMAB, Faculté des Sciences et Technologies, Université de Lorraine, Nancy, France

E-mail address: Maree.Brennan@univ-lorraine.fr (M. Brennan).<https://doi.org/10.1016/j.plaphy.2019.04.024>

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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.

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 <i>cis</i>
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	νC = C, aromatic
11	+	1638	νC = C, unsaturated alkyl
14	-	1656	νC = C, alkyl
14	+	1716	
11	-	2852	ν = CH ₂ , s
11	-	2880	ν = CH ₂ , s
11	+	2904	νCH ₂ , νCH ₃ , s, as
14	+	2916	νCH ₃ , s, as
11	+	2962	νCH 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; ν, stretching; s, symmetric; as, asymmetric.

on the wavenumber axis using loess interpolation. Mean spectra were calculated for the three sample replicates, which was the standardised 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 CH₂ 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., 2017a,b). For both PCs, CH₂ twisting, and CH₂ and CH₃ 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., 2017a,b). 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.

Declarations of interest

None.

Contributions

MB conceived the study. MB grew the plants, did the phenotyping and extractions. MB and AM did the spectroscopy. CT and MB did the data analysis. MB drafted the paper and all authors contributed to revisions. All authors approved the final version for submission.

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