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1 **Use of Raman microspectroscopy to predict malting barley husk adhesion quality**

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12

13 *Keywords:* Barley (*Hordeum vulgare*); grain skinning; husk adhesion

14 *Abbreviations:* PC, Principal component

15

16

17 **ABSTRACT**

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

24

25 **1. Introduction**

26 Raman spectroscopy has been successfully used for food and cereal quality applications,
27 including determining suitability of wheat for flour production based on protein structure
28 (Guzmán et al., 2012; Piot et al., 2002). Premium quality malting barley (*Hordeum vulgare*)
29 has a husk, which adheres to the caryopsis (barley fruit) at harvest. When adhesion quality is
30 poor, the grain quality defect “skinning” results, which is the partial or complete loss of the
31 husk at harvest or during handling. Skinning is a significant economic problem affecting the
32 wider malting industry, reducing malting productivity by affecting germination efficiency
33 (Okoro et al., 2017). Newer malting cultivars are more susceptible to skinning than older
34 cultivars (M. Brennan et al., 2017) and development of cultivars resistant to skinning, but
35 which retain desirable malting characteristics is needed. Husk-caryopsis adhesion is mediated
36 through a lipid cementing layer produced by the pericarp (fruit coat) during grain
37 development (M Brennan et al., 2017; Harlan, 1920; Hoad and Brennan, 2016; Taketa et al.,
38 2008). Changes in caryopsis surface lipid composition during cementing layer development
39 have been quantitatively linked to grain skinning (Brennan et al., 2017). Cultivars with
40 increased proportions of sterols, triterpenoids and fatty acids, and lower proportions of
41 alkanes were associated with good quality husk adhesion, and consequently reduced skinning.
42 Traditional wet-chemical analyses are time-consuming and impractical in a breeding context.
43 Here, we used Raman micro-spectroscopy on caryopsis surface-lipid extracts to determine
44 whether this technique could distinguish among cultivars with differential adhesion qualities,
45 as a potential tool for identifying skinning-resistant cultivars.

46

47 **2. Materials and methods**

48 Fifteen commercially relevant malting barley cultivars with husk adhesion qualities from
49 “good” (low skinning) to “poor” (high skinning) were grown in triplicate in a glasshouse at
50 Scotland’s Rural College, Edinburgh. Skinning was assessed as described in Brennan et al.
51 (2017), where grains with more than 20% husk loss by area are considered to be skinned.
52 Caryopses from one main shoot ear of each replicate were harvested at 15 days post-anthesis,
53 after cementing layer development. Soluble surface lipids were extracted from all caryopses
54 (~30) from each ear by dipping in dichloromethane (puriss p.a. grade for GS >99.9%, Sigma-
55 Aldrich, UK) for 20 s each. Surface lipid extracts were evaporated onto a quartz microscope
56 slide, and examined with a Raman microscope (Renishaw, UK) equipped with a Leica
57 DMLM microscope using the 100× objective, calibrated each day with a silicon wafer (520
58 cm⁻¹) at the University of Edinburgh’s School of Engineering Bioimaging Facility. Three
59 spectra were acquired from each sample (three acquisitions each) from 400 to 3200
60 wavenumbers, with exposure time 10 s at 100% laser power. For each, a background
61 spectrum of the quartz slide was acquired at the same magnification, then subtracted from the
62 corresponding sample spectrum. Spectral pre-processing was done in R (R Development Core

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

74

75 3. Results and discussion

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

100

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105

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- 160
- 161
- 162

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	δ CCC
14	-	494	
14	-	528	
14	-	682	ν CC, ring
11	-	832	
14	-	870	
11	+	890	ν CC, backbone
14	-	894	ν CC, backbone
14	-	942	ν CC, ν COC
11	+	948	ρ CH ₃ , ν CC, ν COC
14	-	982	β CH
11	+	1064	ν CC
14	+	1074	ν CC
11	+	1094	ν CC
14	-	1096	ν CC
14	-	1124	ν CC
11	+	1126	ν CC
14	+	1156	ν CC
14	-	1240	δ =CH
14	-	1260	δ =CH, ν CH <i>cis</i>
11	+	1296	τ CH ₂
14	+	1306	τ CH ₂
14	-	1416	β CH ₂
11	+	1432	α CH ₂ , α CH ₃ , δ CH ₂ , δ CH ₃
14	+	1444	α CH ₂ , α CH ₃ , δ CH ₂ , δ CH ₃ , β CH ₂
11	+	1454	β CH ₂ , β CH ₃ , δ CH ₂ , δ CH ₃
14	+	1468	β CH ₂ , β CH ₃
14	-	1488	
14	-	1504	
14	-	1554	
14	+	1604	ν C=C, aromatic
11	+	1638	ν C=C, unsaturated alkyl
14	-	1656	ν C=C, alkyl
14	+	1716	
11	-	2852	ν =CH ₂ , s
11	-	2880	ν =CH ₂ , s
11	+	2904	ν CH ₂ , ν CH ₃ , s, as
14	+	2916	ν CH ₃ , s, as
11	+	2962	ν CH ₃ , as

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

163 ^aA "+" indicates this wavenumber increased husk adhesion quality; a "-" indicates this wavenumber decreased husk adhesion
164 quality.

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

166

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

