

1 **Title**

2 Estimation of Apple Mealiness by means of Laser Scattering Measurement

3

4 **Author information**

5 Daiki IIDA<sup>1</sup>, Mito KOKAWA<sup>2\*</sup>, Yutaka KITAMURA<sup>2</sup>

6 <sup>1</sup> Graduate School of Science and Technology, University of Tsukuba, 1-1-1 Tennodai,  
7 Tsukuba, Ibaraki 305-8572, Japan

8 <sup>2</sup> Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai,  
9 Tsukuba, Ibaraki 305-8572, Japan

10 \* Corresponding author: [kokawa.mito.ke@u.tsukuba.ac.jp](mailto:kokawa.mito.ke@u.tsukuba.ac.jp)

11

12 **Abstract**

13 Mealiness is a phenomenon in which intercellular adhesions in apples loosen during storage, causing a soft  
14 and floury texture at the time of eating, and leading to lower consumer preference. Although apples can be  
15 stored and commercially sold throughout the year, the occurrence of mealiness is not monitored during  
16 storage. Therefore, the objective of this research was to non-destructively estimate the mealiness of apple  
17 fruit by means of laser scattering measurement. This method is based on laser light backscattering imaging  
18 but can quantify a wider range of backscattered light than the conventional method by utilizing high  
19 dynamic range (HDR) rendering techniques. Lasers with wavelengths of 633 nm and 850 nm were used as  
20 a light source, and after acquiring backscattered images, profiles and images were obtained. Profile features  
21 such as curve fitting coefficients and profile slopes and image features such as statistical image features  
22 and texture features were extracted from the profiles and images, respectively. PLS, SVM and ANN models  
23 were used for the estimation of mealiness. The results of the estimation based on these features showed that  
24 the ANN model combining both wavelengths had a higher performance (R=0.634, RMSE=7.621) than the  
25 models constructed from features of single wavelength measurements. In order to further improve the

26 performance of the model, we applied various ensemble learning methods to combine different estimation  
27 models. As a result, the ensemble model showed the highest performance ( $R=0.682$ ,  $RMSE=7.281$ ). These  
28 results suggest that laser scattering measurement is a promising method for estimating apple fruit mealiness.

29

### 30 **Keywords**

31 Apple Mealiness, Laser Scattering Measurement, Backscattering imaging, Ensemble Learning, Image  
32 Analysis, Non-destructive technology

33

### 34 **Statements and Declarations**

35 The authors declare that they have no conflict of interest.

36

## 37 **Introduction**

38 Apples (*Malus × domestica*) are one of the most widely cultivated fruits in the world and are sold all  
39 year due to the widespread use of controlled atmosphere (CA) storage. The palatability of apples is derived  
40 not only from their chemical properties but also from their physical properties. In fact, firmness, crispiness,  
41 and ease of swallowing are more important factors in the palatability of apples than sweetness and acidity  
42 (Chen Jie Yu et al. 2011; Hayakawa et al. 2012). However, the current market value of apples is assessed  
43 by shape, degree of damage, and Brix of fruit juice, not by indicators related to texture.

44 Therefore, the development of technologies to estimate apple texture indicators is important. However,  
45 the change in apple texture during storage varies widely among apple cultivars, and various deterioration  
46 phenomena can be observed including a decrease in flesh turgor pressure, increase in the degree of  
47 mealiness, and cell fracture (Cárdenas-Pérez et al. 2017; Gwanpua et al. 2016; Iwanami et al. 2005;  
48 Iwanami, Moriya, Kotoda, and Abe 2008; Iwanami, Moriya, Kotoda, Takahashi, et al. 2008). For instance,  
49 the decrease in turgor pressure and increase in mealiness are less likely to occur in “Kanzi” and “Fuji”  
50 apples, which are known to soften due to the fracture of the microstructure. On the other hand, “Jonagold”  
51 apples are known to become mealy and soften.

52 Mealiness is an internal damage phenomenon in which the adhesion between cell tissues loosens,  
53 causing cells to separate. Loosening of the adhesion between cell tissues is caused by the solubilization of  
54 pectin in the fruit. It has been reported that non-mealy apples are juicy because the cells are strongly adhered  
55 to each other, and the cells are easily crushed. However mealy apples are broken down into several cell  
56 clusters in the mouth during eating, resulting in less crushed cells and a less juicy mouthfeel (Harker and  
57 Hallett 1992). Barreiro et al. (1998) reported that the increase in mealiness causes loss of crispiness,  
58 firmness, and juiciness, and increases a floury mouthfeel.

59 Two methods have been developed to evaluate mealiness. The confined compression method has been  
60 more widely used; the method determines whether or not the apple is mealy by assessing the hardness and

61 juiciness obtained from a compression test based on a certain threshold value (Barreiro et al. 1999). On the  
62 other hand, the fruit disc shaking method measures the degree of mealiness quantitatively. In this method,  
63 apple discs are shaken in a sucrose solution, and the degree of mealiness (degree of disc collapse) is  
64 calculated from the weight ratio before and after the shaking (Iwanami et al. 2005; Iwanami, Moriya,  
65 Kotoda, and Abe 2008; Moriya et al. 2017; Motomura et al. 2000).

66 Although the aforementioned methods of measuring mealiness provide objective information, they both  
67 involve destructive operations and have little practical application. Since the texture of agricultural products  
68 varies greatly between individuals and also changes significantly during storage (Liu et al. 2019; C. Ma et  
69 al. 2020; Saei et al. 2011), there is a need for non-destructive technologies that can inspect all products that  
70 are consumed.

71 Non-destructive methods for evaluating apple mealiness include hyperspectral backscattering imaging  
72 analysis (Huang et al. 2012; Huang and Lu 2010), biospeckle imaging (Arefi et al. 2016), laser light  
73 backscattering imaging (LLBI)(Mollazade and Arefi 2017), nuclear magnetic resonance imaging (Barreiro  
74 et al. 1999, 2000), fluorescence spectroscopy (Moshou et al. 2003), near infrared spectroscopy (Mehinagic  
75 et al. 2003), ultrasound methods (Bechar et al. 2005), and acoustic methods (M Lashgari and Imanmehr  
76 2019; Majid Lashgari et al. 2020). With the exception of LLBI, non-destructive techniques have several  
77 problems in practical application, such as the high price of equipment (e.g., hyperspectral cameras), long  
78 measurement times, and the need for contact between the fruit and the device.

79 LLBI is a technique for quantifying the spatial distribution of backscattered light by capturing the  
80 backscattering of an object with a monochrome camera. LLBI simultaneously acquires information related  
81 to both the absorption coefficient and the reduced scattering coefficient of the measured object, which are  
82 related to its chemical and physical properties, respectively. However, it is possible to focus on the physical  
83 properties of the object by selecting illumination wavelengths in which the effect of absorption is small.  
84 LLBI is a non-contact, non-destructive technology and can be operated at low cost since its basic

85 configuration is based on a monochrome camera and several single-wavelength laser sources.

86 LLBI has been used to predict the pre- and post-harvest quality for a variety of agricultural products  
87 such as apples (Baranyai et al. 2009), bananas (Zulkifli et al. 2019), pears (Adebayo et al. 2017), sweet  
88 potatoes (Sanchez, Hashim, Shamsudin, and Nor 2020), apricots (Mozaffari et al. 2022), plums (Rezaei  
89 Kalaj et al. 2016), potatoes (Babazadeh et al. 2016), and cocoa beans (Lockman et al. 2019). However, one  
90 of the problems in LLBI is that it relies on the analysis of a limited image area. Imaging devices can  
91 typically capture the whole object, but the scattered light is saturated near the incident point and cannot be  
92 analyzed. In contrast, areas that are far from the incident point are too dark to acquire relevant signals, since  
93 the intensity of scattered light in turbid materials decreases rapidly with increasing distance from the  
94 incident point. Typically, a circular region with a diameter of 15 mm at most from the incident point can be  
95 analyzed (Abildgaard et al. 2015; Cen et al. 2013; Højager Attermann et al. 2011). In other words,  
96 conventional LLBI has used local image information to estimate the overall quality.

97 This problem led us to modify the LLBI method by developing a system that can capture multiple  
98 scattering images obtained at different exposure times. These multiple images can then be combined into a  
99 high dynamic range (HDR) composite to quantify a wider range of surface area, including areas near and  
100 far from the incident point. This modified method, which we termed as the laser scattering method, has  
101 been used to estimate the firmness of apples (Iida et al. 2022), and has been shown to increase the analysis  
102 area fourfold. This study attempted to estimate apple mealiness using the laser scattering method. As a  
103 method for quantifying mealiness, we used the fruit disc shaking method, which provides more detailed  
104 information on the degree of mealiness, rather than the confined compression method that has been widely  
105 used in previous studies but only distinguishes whether or not the apple is mealy (Mollazade and Arefi  
106 2017). In addition, the relationship between light scattering and mealiness degree was investigated by  
107 measuring the 3-dimensional microstructure using X-ray CT.

108

## 109 **Materials and Methods**

### 110 **Materials**

111 One hundred fresh and externally undamaged 'Jonagold' apples which had been harvested in 2021 were  
112 purchased from Aomori Prefecture, Japan. The apples were stored in an incubator (LTE510, Tokyo  
113 Rikakikai Co., Ltd., Japan) to accelerate mealiness. The apples were divided into 2 groups; the first group  
114 was immediately stored at 20°C, while the other group was first stored at 4°C for 1 month and were then  
115 transferred to the 20°C incubator. Ten apples were measured each week, including the day of purchase (0  
116 week), and the maximum storage period at 20°C was four weeks.

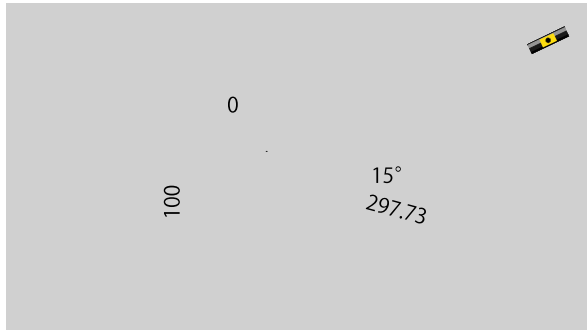
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### 118 **Laser Scattering Measurement**

#### 119 **Laser scattering system**

120 The laser scattering measurement system was constructed as shown in Fig. 1, following the basic  
121 structure explained in previous studies (Babazadeh et al. 2016; Iida et al. 2022; Mollazade and Arefi 2017;  
122 Sanchez, Hashim, Shamsudin, and Mohd Nor 2020). A 12-bit monochrome CMOS camera (ORCA-spark,  
123 Hamamatsu Photonics Co, Tokyo, Japan) was used for image capture. The camera was mounted on a  
124 camera stand (EMVA-SL, Misumi Group Inc., Tokyo, Japan) at a height of 210 mm from the optical surface  
125 plate, and images of the samples were captured in a horizontal direction. In order to limit the effect of light  
126 absorption by the apple peel, lasers with wavelengths of 633 nm (Self-Contained He-Ne Laser, 0.8 mW,  
127 Thorlabs, Inc.) and 850 nm (Alignment laser diode 5 mW, Edmund Optics Japan Co.) were used for  
128 illumination, and both lasers were fixed at a height of 185 mm. He-Ne lasers with a typical wavelength of  
129 633 nm have been used in previous studies for backscattering imaging (Askoura et al. 2016). On the other  
130 hand, the 850 nm laser was selected based on the report that the near-infrared region between 800 nm and  
131 1200 nm shows reduced light absorption in apples (van Beers et al. 2017). Due to limited space around the  
132 camera, the 633 nm laser was placed behind the sample and was reflected by three mirrors before being  
133 irradiated on the sample at an angle of 15.0 degrees. The laser light was focused on the sample surface with  
134 an achromatic lens (MgF<sub>2</sub> coated achromatic lens, Edmund Optics Japan Co.). The 850 nm laser was

135 directly irradiated onto the sample surface at an angle of 25.5 degrees. These measurements were performed  
136 in a dark room.



137  
138 **Fig. 1** Schematic diagram of laser scattering measurement device

139 M: Mirror, L: Achromatic lens

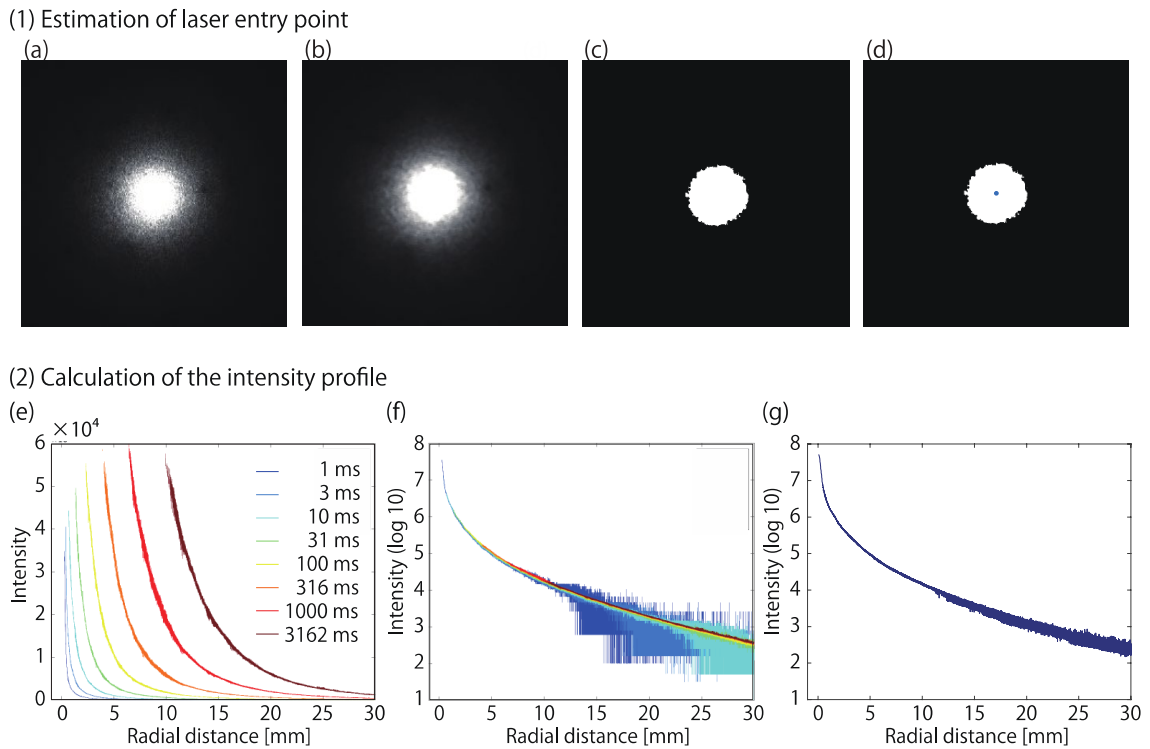
140

141 Backscattering images were captured at two opposing points on the equatorial line of the apple. After the  
142 apples were fixed using a ring-shaped spherical container, the height of the apple was adjusted so that the  
143 incident point was just on the equatorial plane.

144 To obtain high-dynamic-range images, the images were captured with eight different exposure times,  
145 ranging from  $10^0$  ms to  $10^{3.5}$  ms at  $10^{0.5}$  ms intervals. For all images, the offset and gain were both set to 0,  
146 and the binning was set to  $1 \times 1$ . LabVIEW 2018 (National Instruments Corp., USA) was used to control  
147 the camera, and the acquired images were saved in the TIFF format.

### 148 **Obtaining the intensity profile (Analysis of backscattered images)**

149 Fig. 2 shows the image analysis flow after capturing images with the eight different exposure times  
150 explained above. First, the laser incident point was estimated from the image. Generally, the incident point  
151 can be detected as the point in the image with the highest intensity. However, since the strong laser light  
152 causes saturation near the incident point even in the image taken with a minimum exposure time of 1 ms,  
153 the incident point could not be detected with this method. Therefore, the image taken with an exposure time  
154 of 100 ms was binarized using the Otsu method (Otsu 1979) after smoothing with a  $5 \times 5$  median filter and  
155 the incident point was calculated as the point of gravity of the scattering area.



157

158 **Fig. 2** Flow from image analysis to acquisition of the high-dynamic-range intensity profile

159 (1) The laser incident point is estimated by using the image acquired with 100 ms exposure time: (a) Raw

160 image, (b) Image processed with a median filter to reduce noise, (c) Binarization by Otsu method, (d)

161 Calculation of the center of gravity (estimated incident point)

162 (2) The intensity profile is acquired: (e) Radial averaging is performed for images acquired with the eight

163 exposure times, (f) After dark correction, each profile is multiplied by the inverse of the exposure time

164 and transformed logarithmically, (g) Finally, the average of the eight intensity profiles are calculated.

165

166 Next, a circular region with a diameter of 70 mm from the incident point was set as the region of interest

167 (ROI). The distance from the incident point was calculated for all pixels in the ROI, and the average

168 intensity value was calculated for the points with equal radial distances. The intensity of the dark frame

169 acquired with the cap on the camera was subtracted from all intensities (dark correction), resulting in eight



170 profiles shown in Fig. 2(e). The intensities were normalized by multiplying each of them with the reciprocal  
171 of their exposure time (Fig. 2(f)). Furthermore, the normalized intensity values for the eight exposure times  
172 were averaged after logarithmic transformation. The distribution of intensity of scattered light along the  
173 radial distance from the incident point is called the intensity profile. Since the intensity was saturated within  
174 1 mm of radial distance from the incident point, the intensity data between 0 and 1 mm radial distance were  
175 removed from the obtained profile.

176 Finally, the intensity profiles were corrected for the effect of fruit curvature. This is important because  
177 the backscattering images are captured as images taken from the plane surface of a semi-infinite object and  
178 fruit curvature affects both the intensity and radial distance of the measured scattered light. In this study, a  
179 correction method based on the Lambert cosine law was used to correct the intensity of scattered light  
180 (Yankun Peng and Lu 2006; Qing et al. 2007). This method assumes that backscattered light is strongest in  
181 the direction normal to the sample surface and that backscattered light in other directions are proportional  
182 to the cosine of the angle between the normal. Since the backscattered light captured with the camera is at  
183 an angle with the normal due to the fruit curvature, it is converted back to the maximum intensity using the  
184 diameter of the fruit. Similarly, the radial distance was corrected following the method developed by the  
185 same authors (Yankun Peng and Lu 2006). These correction methods have been shown to increase  
186 estimation performance by 2.0–3.5% in estimating apple firmness and soluble solids content (Yankun Peng  
187 and Lu 2006; Qing et al. 2007).

188 Various libraries in MATLAB2021b (Mathworks) and Python (Version 3.8.13) were used for these  
189 analyses.

### 190 **Fruit disc shaking method**

191 Referring to previous studies (Iwanami et al. 2005; Iwanami, Moriya, Kotoda, and Abe 2008; Moriya et  
192 al. 2017), the degree of mealiness was quantified using the modified fruit disc shaking method. First, for  
193 each incident point, discs (10 mm in diameter, 5 mm thick) were taken just below the skin by a cork borer.

194 The discs were soaked in 12% sucrose solution for 45 min under vacuum condition. After soaking, excess  
195 water was wiped off with gauze, and the discs were weighed ( $W_i$ ). The discs were then transferred to a 30  
196 mm diameter test tube containing 10 mL of 12% sucrose solution, shaken in a continuous shaker (PLUS  
197 SHAKER EP-1, TAITEC, Tokyo) for 7 h, and reweighed ( $W_s$ ). Finally, the degree of mealiness ( $(W_i -$   
198  $W_s)/W_i \times 100\%$ ) was calculated.

## 199 **Observation of Microstructure using Micro-CT**

### 200 **Micro-CT**

201 Three apples stored at 20°C for 0, 2, and 4 weeks were prepared as samples for the observation of  
202 microstructure. Five cylindrical samples of 12 mm diameter and 15 mm height were cut out from each  
203 apple with their skin on. The samples were wrapped in plastic wrap to prevent drying until observation.  
204 Samples were scanned using a high-resolution X-ray  $\mu$ -CT system (inspXio SMX-100CT, Shimadzu Corp.,  
205 Japan). The X-ray CT conditions were as follows: tube voltage 60 kV, tube current 100  $\mu$ A, no metal filter,  
206 600 views, and 12 $\times$ 1 averaging. The measurement time was 4 min per sample. The measured projections  
207 were digitized by an ultra-high-speed computing system (HPC inspXio, Shimadzu Corp., Japan) as 512  $\times$   
208 512 size, 16-bit images, with a voxel size of 10  $\mu$ m.

### 209 **Image analysis**

210 A square ROI of 300  $\times$  300 pixels was cropped from the center of the 512  $\times$  512 pixel image and was  
211 used for further analysis. Gaussian smoothing (standard deviation: 5) was applied to all images to reduce  
212 the noise before applying binarization using the Otsu method (Otsu 1979) to segment the image into cells  
213 (white) and pores (black). The volume distribution of individual pores was then calculated for each sample.  
214 Image preprocessing was performed using Pydicom (ver. 2.3.0) and OpenCV (ver. 4.6.0) of Python (ver.  
215 3.8.13), and pore volume distribution was calculated using BoneJ plugin (Richard et al. 2021). BoneJ has  
216 been used in previous studies to quantify microstructural differences between different apple varieties with  
217 comparable porosity (Ting et al. 2013).

## 218 **Estimation of mealiness**

### 219 **Feature engineering from profiles and backscattered images**

220 In order to construct an estimation model, it is necessary to extract features from the backscattered images.

221 Features obtained from backscattered images can be classified into two types: profile features and image  
222 features (Mollazade et al. 2013; Mollazade and Arefi 2017; Romano et al. 2008).

223 The profile features were calculated from the intensity profiles and consisted of two types of features:  
224 the fitting coefficients obtained by approximating the profile with mathematical functions, and the gradients  
225 of the profile. The former was calculated by fitting 11 mathematical functions to the profiles: nine types of  
226 semi-Gaussian functions (Mollazade et al. 2012), a Gaussian-Lorentzian function (Mollazade and Arefi  
227 2017), and Farrell's simplified function (Thomas J. Farrell et al. 1992). Since these 11 functions were  
228 developed to fit the intensity profile before logarithmic transformation, the intensity profile was converted  
229 back by exponential multiplication. In addition, the intensity profiles were scaled to a maximum value of 1  
230 for Farrell's simplified function. The latter type of profile feature was calculated as the slope of the profile  
231 obtained at 1 mm intervals (Iida et al. 2022). A total of 62 profile features were obtained for each laser  
232 wavelength.

233 The image features were obtained from the original laser scattering images. Before any meaningful  
234 features could be extracted, it was necessary to segment the ROI from the backscattered image. The ROI  
235 segmentation step was performed by first binarizing the backscattered image using the Otsu method to  
236 separate the background from the light scattering areas. Subsequently, saturated areas near the incident  
237 point were removed, resulting in a donut-shaped ROI.

238 Two types of image features were obtained by analyzing the ROI, namely, statistical and texture features.  
239 Statistical features are parameters which could be calculated from the image using standard statistical  
240 calculations. Eighteen statistical features such as mean intensity and area of light scattering were calculated.  
241 On the other hand, texture features are characterized by the spatial arrangement of the brightness values of  
242 the pixels in a region (Zheng et al. 2006). In addition to the texture features used in LLBI analysis

243 (Mollazade et al. 2013; Mozaffari et al. 2022): Gray-Level Co-Occurrence Matrix (GLCM) features, Gray  
244 Level Run Length Matrix (GLRLM) features, and Local binary patterns (LBP) features, the following  
245 texture features were added to obtain a comprehensive understanding of the data: Neighborhood Gray Tone  
246 Difference Matrix (NGTDM) feature, Statistical Feature Matrix (SFM) feature, Law's Texture Energy  
247 Measures (LTE) features, Fractal Dimension Texture Analysis (FDTA) features, and Fourier Power  
248 Spectrum (FPS) features (Christodoulou et al. 2003; Kaplan 1999; Wu et al. 1992; Wu and Chen 1992).  
249 These added up to 70 image features for each wavelength. In order to extract these features from the images,  
250 pyfeats (ver. 1.0.0), a related library in Python was used.

251 In total, 264 features were calculated with 132 features for each wavelength. The complete list of  
252 calculated features can be found in Supplementary data (SI1).

### 253 **Construction and evaluation of estimation model**

254 To construct an estimation model that retains generalizability, the data were divided into training (80%)  
255 and test (20%) sets. Data were divided so that multiple measurements acquired from the same apple would  
256 not be separated into the training and test datasets. Next, the training data were standardized to have mean  
257 0 and variance 1, and the test data were standardized with the same mean and variance. In order to eliminate  
258 redundant features, the filter method was applied to the features. Specifically, correlation coefficients were  
259 calculated between the mealiness and features in the training data, and features with correlation coefficients  
260 lower than an absolute value of 0.1 were not used in model construction and estimation.

261 PLS, SVM and ANN were used as estimation models to evaluate the possibility of mealiness estimation.  
262 Hyperparameters were optimized by Grid Search using 10-fold cross-validation, and the hyperparameters  
263 which obtained the lowest average Root Mean Squared Error (RMSE) were chosen. The hyperparameter in  
264 the PLS model was the number of latent variables, and those in the SVM model were the regularization  
265 term and the gamma coefficient of the Radial Basis Function (RBF) which was used as the kernel function.  
266 Finally, the ANN model was structured with 3 hidden layers, and a linear function and Rectified Linear

267 Unit (ReLU) function were used for the output layer and hidden layer, respectively. Adaptive moment was  
 268 adopted for the learning algorithm. The hyperparameters for the ANN model were the node size for each  
 269 layer, batch size, and the L2-regularization term. The estimation model set up with the obtained  
 270 hyperparameters was trained again on the training data, and the test data were estimated with the constructed  
 271 estimation model to obtain predicted values.

272 The multiple correlation coefficient (R) and the root mean square error (RMSE) were used as metrics to  
 273 evaluate the performance of the models. Moreover, the ratio of prediction to deviation (RPD), the range  
 274 error ratio (RER), and the evaluation index (EI) were used as assessment guidelines for model performance.  
 275 RPD, RER, and EI were calculated by the following formulas, respectively.

$$276 \quad R = \frac{R^2}{R^2 + RMSE^2} \quad (1)$$

$$277 \quad RPD = \frac{R}{RMSE} \quad (2)$$

$$278 \quad RER(\%) = 100 \times 2 \times \frac{SD}{RANGE} \quad (3)$$

279 where SD indicates the standard deviation of the target and RANGE is the difference between the maximum  
 280 and minimum values of the target.

281 RPD values below 1.5 indicate that the model performance is not usable, values between 1.5 and 2.0  
 282 suggest a possibility to distinguish between high and low values, and values over 2.0 reveal a possibility of  
 283 quantitative prediction (Saeys et al. 2005). The RER is related to the range of the objective variable, where  
 284 values over 4.0 indicate that the model is acceptable for sample screening and values over 10.0 reveal a  
 285 quality control level (Gohain et al. 2021). The EI is also a metric that takes into account the distribution  
 286 range of the objective variable. The EI can be assigned to the following five ranks: very high (EI<12.4%),  
 287 high (12.5–24.9%), slightly high (25.0–37.4%), low (37.5–49.9%), and very low (EI>50%). Models with  
 288 EI below 37.4% are described as "practical" (Mizuno et al. 1988; Suzuki et al. 2008).

289

290 **Results and Discussion**

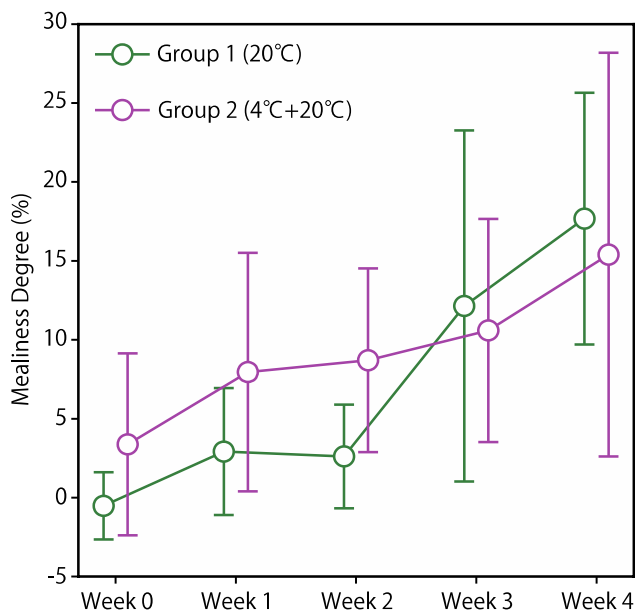
291 **Changes in apple quality during storage**

292 **Changes in mealiness**

293 The “Jonagold” apples measured in this study are known to become mealy during storage. Fig. 3 shows  
294 the change in mealiness as the average of 20 measurements from 10 apples each week. Mealiness increased  
295 with the increase in storage period, as reported in previous studies (Iwanami et al. 2005; Iwanami, Moriya,  
296 Kotoda, and Abe 2008). Moreover, the increase was especially rapid in the latter half of the storage period.

297 Two-way ANOVA was performed to determine if mealiness changed by storage group and storage period.  
298 As a result, both the storage group and storage period were shown to significantly affect mealiness ( $p<0.05$ ).  
299 The reason for the significant difference between storage groups is likely to be due to the effect of one  
300 month of refrigerated storage. These results of two-way ANOVA can be found in Supplementary data (SI2).

301



302

303 **Fig. 3** Change in mealiness during storage

304 The horizontal axis indicates the storage period at 20°C, and the vertical axis shows the mealiness degree.

305 The error bars show the standard deviation for each storage period.

306

307 The degree of mealiness sometimes showed negative values, meaning that the apple discs weighed  
308 heavier after the 7-h shaking than their initial weight. In these cases, previous studies (Iwanami et al. 2005)  
309 replaced the negative values with zero. However, since the final goal of this study was to estimate the degree  
310 of mealiness and the adjustment of the objective variable was expected to affect the performance of the  
311 estimation model, the negative values were used as they were in this study.

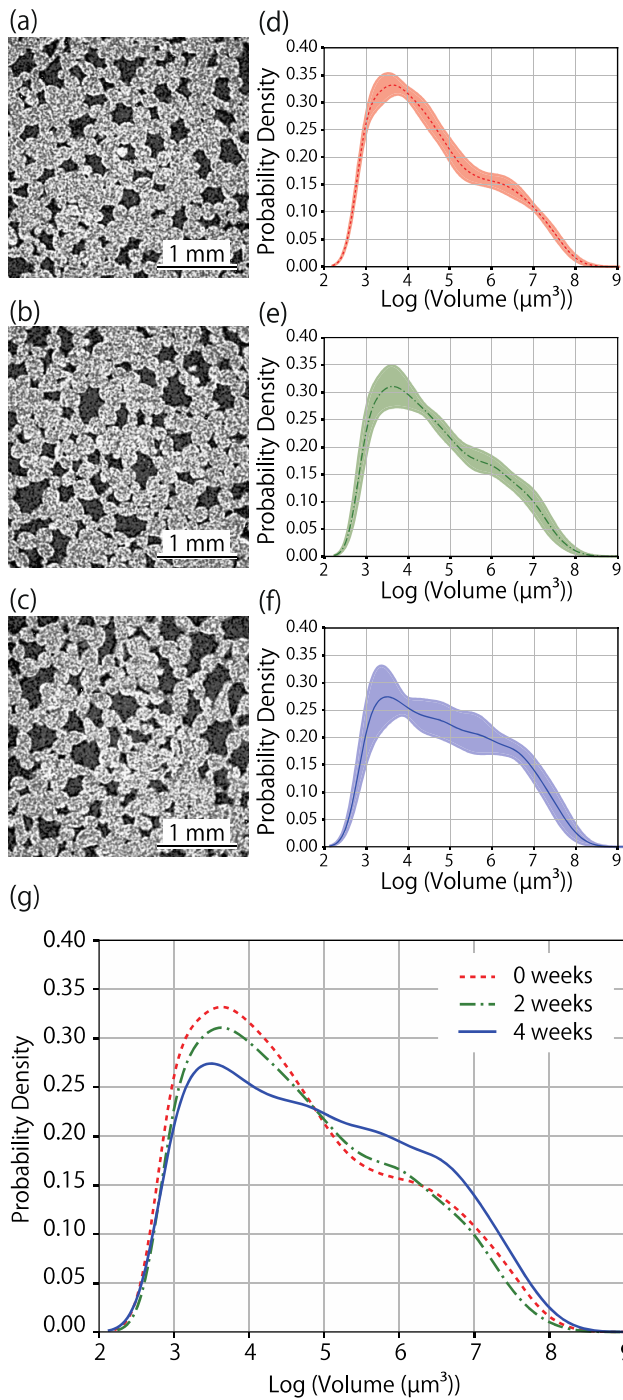
312 As can be seen from the large error bar in Fig. 3, mealiness varied largely among apples stored for the  
313 same storage period, with standard deviations ranging from 2.13 to 12.78. Furthermore, the mealiness of  
314 the two samples obtained from one apple varied greatly, with differences ranging from 0.16% to 27.76%,  
315 indicating that the degree of mealiness was not uniform even within the same apple. This variation may be  
316 attributed to the heterogeneous microstructure of the apple, which has been reported for many other  
317 vegetables and fruits (Chaïb et al. 2007; Ella Missang et al. 2011; Iida et al. 2022; Khan and Vincent 1990;  
318 T. Ma et al. 2021). It should also be noted that the degree of mealiness obtained by the fruit shaking method  
319 may be influenced by the amount of liquid adhering to the apple disks when weighed, leading to a margin  
320 of error.

321 The high variability in mealiness among apples stored in similar conditions and even within each apple  
322 indicate that mealiness cannot be estimated from storage conditions alone. Therefore, technologies that  
323 allow apple mealiness to be estimated non-destructively and on multiple points would be valuable.

### 324 **Change in microstructure during storage and its relationship to mealiness**

325 Fig. 4(a), (b), and (c) are representative X-ray CT images of apple samples after 0, 2, and 4 weeks of  
326 storage. All images are acquired at the same depth from the skin. These images indicate the increase in large  
327 pores and the decrease in adhesion between cell tissues during storage. This tendency was analyzed  
328 quantitatively.

329



330

331 **Fig. 4** Change in microstructure due to storage

332 (a)–(c) Representative X-ray CT images for each storage period, (d)–(f) The pore volume distribution for

333 each storage period. The lines show the mean of the probability density functions estimated by kernel

334 density estimation, and the filled areas show the standard deviations of the probability density function



335 among samples stored for the same period. (g) Comparison of the mean pore volume distribution between  
336 different storage periods.

337

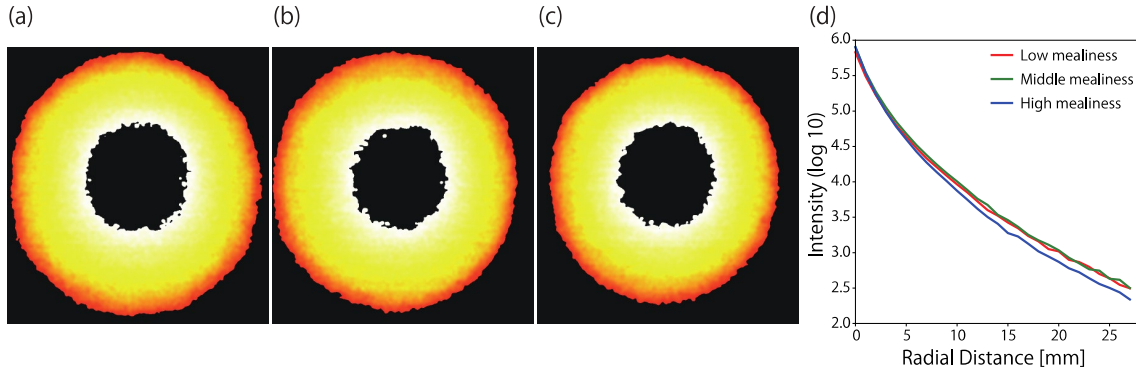
338 X-ray CT images for five samples per storage period (0, 2, and 4 weeks) were 3-dimensionally-analyzed  
339 and the individual pore volumes were calculated (Fig. 4(d)–(f)). Fig. 4(g) is given to compare of the  
340 common logarithmically transformed average pore volume distribution for each storage period. The  
341 average pore volume was approximately  $0.002 \text{ mm}^3$  and increased with prolonged storage. The proportion  
342 of small pores was relatively high in all samples, and the proportion of large pores varied among different  
343 storage periods, increasing at 4 weeks of storage. The occurrence of large pores is consistent with the  
344 increase in the degree of mealiness, and this relationship between the increase in large pores and cell  
345 detachment due to cell separation and disintegration has been reported in previous studies (Li et al. 2020;  
346 Muziri et al. 2016).

### 347 **Change in the laser scattering properties during storage and their relationship to** 348 **mealiness**

349 Changes in cell microstructure during storage are known to affect their interactions with light as well as  
350 the sensory characteristics of the apple such as mealiness. The former was quantified as the intensity profiles  
351 and backscattered image features obtained from laser scattering measurement, and their relationship to the  
352 degree of mealiness was clarified. Fig. 5 shows average backscattered images and average profiles  
353 calculated for three groups: the first group consisting of five samples with the highest degree of mealiness,  
354 the second group of five samples closest to the average degree of mealiness, and the third group of five  
355 samples with the lowest degree of mealiness. The degrees of mealiness for the high, average, and low  
356 groups were  $41.12 \pm 5.37\%$ ,  $7.88 \pm 0.27\%$ , and  $-3.55 \pm 0.61\%$ , respectively. The profiles showed little  
357 difference between the low and middle groups, but the overall intensity of the profiles in the high mealiness  
358 group decreased. In addition, the backscattered image showed a decrease in the scattered region with the  
359 increase in mealiness. These observations were quantitatively confirmed by calculating the correlation

360 between the features obtained from the laser scattering measurement and the degree of mealiness.

361



362

363 **Fig. 5** The changes in backscattered images and intensity profiles with the increase in mealiness

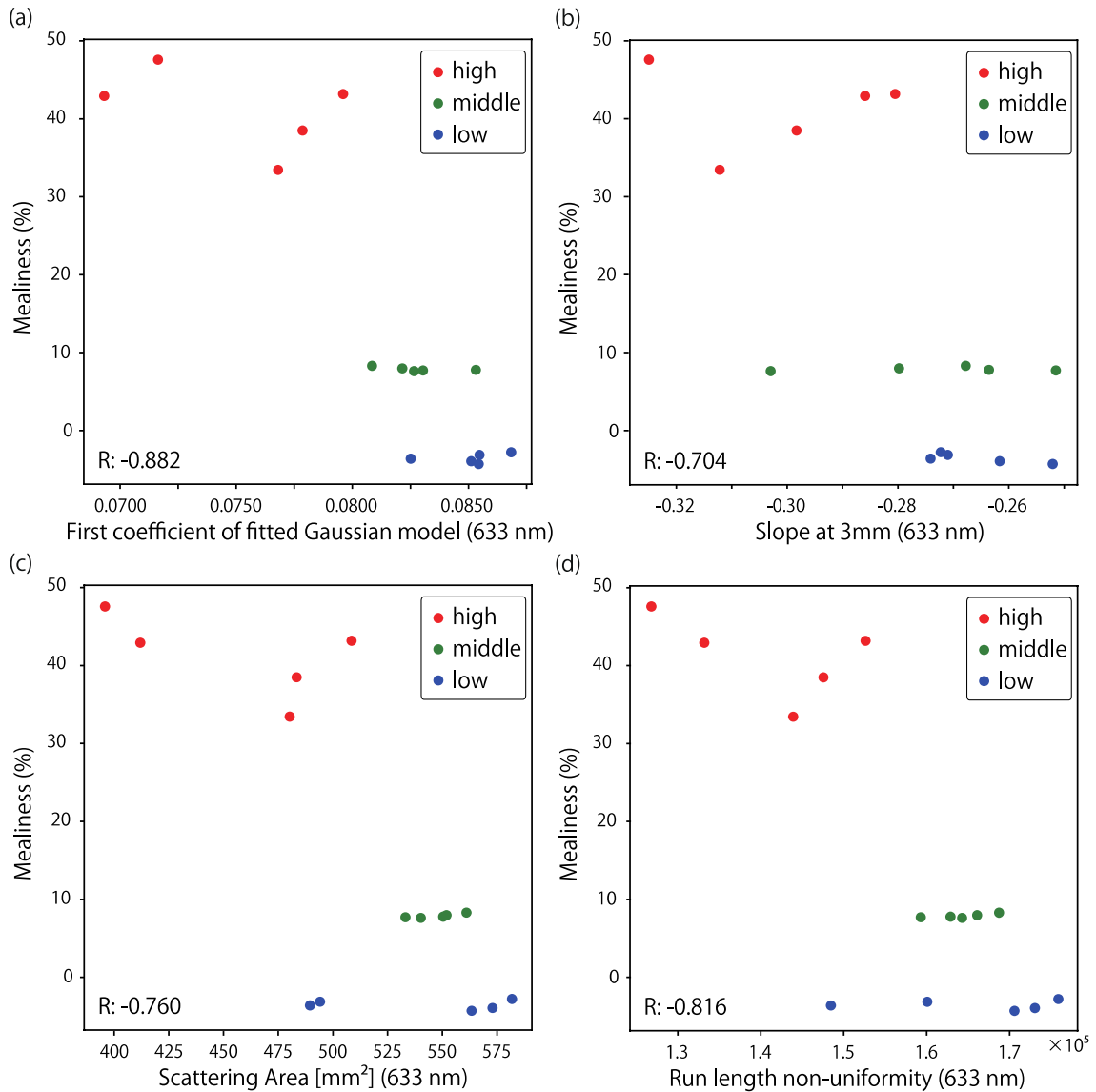
364 (a)–(c) Average backscattered image for (a) low, (b) middle, and (c) high mealiness groups

365 (d) Average profiles for the three groups

366

367 Fig. 6 shows scatter plots of the fifteen samples analyzed, where the degree of mealiness is plotted  
368 against four feature types: coefficients of fitted functions, gradients of the intensity profile, statistical image  
369 features, and texture features. For each feature type, the feature with the highest correlation with mealiness  
370 is shown: the first coefficient of the fitted Gaussian function (Fig.6(a)), the gradient between 3–4 mm in  
371 the profile (Fig.6(b)), the area of the scattering region ( $\text{mm}^2$ ) (Fig.6(c)), and the Run Length Non-uniformity  
372 (RLN) in GLRLM (Fig.6(d)). Although all features were obtained from images acquired with both 633-nm  
373 and 850-nm lasers, features with the highest correlations with mealiness were obtained with the 633-nm  
374 laser. The correlation coefficients between these features and mealiness were -0.882, -0.704, -0.760 and -  
375 0.816, respectively.

376



377

378 **Fig. 6** Relationship between four laser scattering features and the degree of mealiness

379 (a) Fitting coefficient (the first coefficient of the fitted Gaussian model), (b) Gradient feature (gradient  
 380 between 3–4 mm), (c) Statistical image feature (the area of scattering region ( $\text{mm}^2$ )), (d) Texture feature  
 381 (Run Length Non-uniformity). Colors indicate the high-, middle- and low-mealiness groups.

382

383 The first coefficient of the fitted Gaussian function (Fig. 6(a)) is the asymptotic value of scattered light  
 384 intensity at large radial distances from the incident point (Y Peng and Lu 2005). On the other hand, the  
 385 gradient at 3–4 mm from the incident point (Fig. 6(b)) is obtained relatively close to the incident point.

386 These results show that mealiness is related to the whole profile, ranging from the initial attenuation of the  
387 profile to the intensity of scattered light at large distances from the incident point.

388 As observed from the images in Fig. 5 and shown in Fig. 6(c), the area of scattered light was reduced as  
389 mealiness increased. The area values were calculated by counting the number of pixels in the scattering  
390 area and converting pixels to mm. Mealiness has been related to an increase in intercellular space and  
391 porosity within the microstructure as well as cell separation and rupture (Li et al. 2020; Ting et al. 2013).  
392 Considering these reports, the decrease in the area of scattered light may be influenced by the decrease in  
393 scattering frequency due to the collapse of the dense microstructure of the apple fruit.

394 The texture feature Run Length Nonuniformity (RLN) quantifies the non-uniformity of the Run Length  
395 Matrix (Chu et al. 1990; Tang 1998). Run Length calculates the number of consecutive pixels with the same  
396 intensity value, and the Run Length Matrix stores this information as a single intensity value and count (run  
397 length). A lower RLN indicates that the Run Length Matrix is more uniform, meaning that the image  
398 contains a variety of run lengths (Galloway 1975) and therefore higher variability in intensity between  
399 connected pixels. Based on Fig. 6(d), the negative correlation between mealiness and RLN indicates that  
400 as mealiness increased, neighboring pixels in the laser scattering image became more varied. This may be  
401 caused by the collapse of cells leading to a more complex microstructure which causes non-uniform light  
402 scattering patterns.

### 403 **Results of estimation models**

404 To estimate apple mealiness from laser scattering measurement, a total of 132 features were calculated  
405 from the laser scattering images acquired with either the 633-nm or 850-nm laser light. Forty three of these  
406 features were obtained by fitting 11 functions to the intensity profile. All functions were fitted with a  
407 coefficient of determination ( $R^2$ ) over 0.91, which is a good fit to the measured data. The fitting performance  
408 of all the functions is shown in the supplementary data (SI3).

409 After removing redundant features as explained in the construction of the estimation model, 78 features

410 were selected from the features calculated from the 633-nm laser image, and 74 features were selected from  
411 the features calculated from the 850-nm laser image. For models using the data obtained from both lasers,  
412 a total of 152 features were used.

413 The sample size was reduced to 198 because 2 samples collapsed during the shaking process of the fruit  
414 disc shaking method and the degree of mealiness could not be calculated from these samples. The 198  
415 samples were split into 158 and 40 for the training and test data, respectively.

416 Table 1 shows the results for all estimation models. For the single wavelength model, the ANN and SVM  
417 were able to accurately estimate the test data. As discussed in previous studies (Babazadeh et al. 2016; M  
418 Lashgari and Imanmehr 2019; Mollazade and Arefi 2017; Mozaffari et al. 2022), there is a latent nonlinear  
419 relationship between scattering data and mealiness. Therefore, nonlinear models such as ANN and SVM  
420 may adapt well to laser backscattering measurements.

421 When comparing the estimation performance between models built using data acquired with 633-nm and  
422 850-nm lasers, the 633-nm models were superior for all algorithms. Furthermore, when data acquired with  
423 the two lasers were combined, estimation performance improved, with the ANN showing the lowest RMSE  
424 for the test data ( $R = 0.634$ ,  $RMSE = 7.621$ ).

425

426 Table 1 Performance of estimation models

427 Single wavelength: models constructed using features acquired from a single laser (633 nm or 850 nm),

428 Single model: models constructed with a single algorithm (PLS, SVM, or ANN), Two wavelengths:

429 models constructed using features acquired from both lasers, Ensemble model: models constructed by the

430 ensemble of predicted values from two or more single models

Model		R		RMSE		RPD		RER		EI	
		train	test	train	test	train	test	train	test	train	Test
Single wavelength / Single model											
Wavelength	Model										
633	ANN	0.57	0.61	7.76	8.05	1.18	1.24	6.69	5.06	29.90	39.50
	PLS	0.60	0.55	7.28	8.22	1.25	1.21	7.13	4.95	28.10	40.40
	SVM	0.52	0.65	8.22	8.65	1.11	1.15	6.31	4.71	31.70	42.50
850	ANN	0.50	0.59	8.12	8.45	1.12	1.17	6.39	4.82	31.30	41.50
	PLS	0.64	0.36	6.96	12.73	1.31	0.78	7.46	3.20	26.80	62.50
	SVM	0.52	0.59	8.14	8.63	1.12	1.15	6.37	4.72	31.40	42.40
Two wavelengths / Single model											
ANN		0.67	0.63	6.96	7.62	1.31	1.31	7.46	5.34	26.8	37.4
PLS		0.72	0.59	6.3	8.78	1.45	1.13	8.23	4.64	24.3	43.1
SVM		0.61	0.66	7.78	8.26	1.17	1.2	6.67	4.93	30.0	40.6
Two wavelengths / Ensemble model											
Simple averaging		0.72	0.66	6.69	7.45	1.36	1.34	7.76	5.47	25.8	36.6
Weighted averaging		0.72	0.66	6.66	7.46	1.37	1.33	7.79	5.46	25.7	36.6
Stacking		0.75	0.68	6.25	7.28	1.46	1.37	8.30	5.60	24.1	35.8

431

432

433 In order to improve the estimation model performance, ensemble learning methods were adopted.

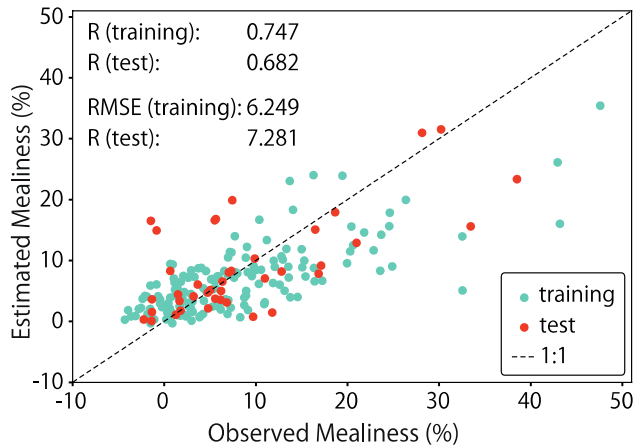
434 Ensemble learning is a method where initial predicted values are calculated from multiple independent

435 weak learning algorithms (base models) and these initial predicted values are used as inputs for further

436 modeling to output the final predicted values. In the field of food quality management, ensemble learning  
437 methods have been used as an algorithm for food safety risk prediction (Wu and Weng 2021), estimation  
438 of chicken meat authenticity (Parastar et al. 2020), and milk adulteration detection (Neto et al. 2019). In  
439 this study, the simple averaging method, the weighted averaging method, and the stacking method (Mendes-  
440 Moreira et al. 2012; Zhou 2021) were adopted as ensemble learning methods. In the simple averaging  
441 method, the predicted values from multiple base models are averaged, giving the final predicted values. In  
442 the weighted averaging method, the weighted average is used to average the predicted values from multiple  
443 base models. Finally, the stacking method builds a meta-model that uses the predicted values from multiple  
444 base models as its features (Anderson et al. 2021; Shen et al. 2020). In this study, we adopted ensemble  
445 learning with PLS, SVM, and ANN models as the base models and SVM was used as the meta-model in  
446 the stacking method.

447 The performance of all ensemble models improved over the single model, suggesting that ensemble  
448 learning can enhance the strengths of the individual base models. Among the three types of ensemble  
449 models, the stacking model showed the best performance. Fig. 7 shows the relationship between the  
450 observed and estimated degree of mealiness obtained from the stacking model. Although some of the data  
451 with high degrees of mealiness were not successfully estimated, the evaluation metrics of the stacking  
452 model were 0.682 and 7.281% for R and RMSE, respectively, and the overall tendency of the degree of  
453 mealiness could be estimated. Moreover, RPD, RER, and EI were 1.37, 5.60, and 35.8, respectively. The  
454 RPD value falls in the range of "not usable," while the ranges for RER and EI evaluated the models as  
455 "acceptable for sample screening" and "slightly high accuracy". Overall, these results indicated that the  
456 performance of the estimation model is satisfactory for practical use.

457



458

459 **Fig. 7** Observed-Estimated plot of the best ensemble model

460 Light blue plots show the training data, orange plots show the test data, and the black dashed line is the  
 461 ideal line ( $y = x$ ). The evaluation metrics of training and test data are shown in the upper left corner.

462

463 In this study, the samples were stored for one month to induce mealiness. However, there were still only  
 464 a few samples with high degrees of mealiness, leading to an imbalanced distribution of the objective  
 465 variable. For further improvement of the estimation performance, it may be necessary to increase the  
 466 number of samples with higher degrees of mealiness. Moreover, further research is required to determine  
 467 whether similar or improved performance can be obtained for estimating mealiness in other cultivars or  
 468 apples with green skin.

469

## 470 **Conclusions**

471 The objective of this study was to estimate apple mealiness by means of laser scattering measurement.  
 472 Laser scattering measurement is based on conventional backscattering imaging but is improved in terms  
 473 of exposure time and by adapting high dynamic image rendering. These leads to the advantage of being  
 474 able to quantify scattered light far from the incident point. In this study, lasers with wavelengths of 633  
 475 nm and 850 nm were used based on previous research reports. To estimate apple mealiness from the  
 476 image data acquired by the laser scattering measurement, comprehensive feature extraction was



477 conducted. The profile features that characterize the intensity profile, and image features that characterize  
478 the scattering image itself were calculated, leading to a total of 132 features for each wavelength.

479 The objective variable, mealiness, was quantified by the fruit disc shaking method, which evaluates the  
480 degree of mealiness as a continuous value and provides more detailed information than the conventional  
481 binary method. Although mealiness increased gradually due to storage, there was large variability among  
482 different apples stored for the same period, indicating the need to evaluate mealiness for each apple. On  
483 the other hand, the degree of mealiness was found to correlate with a variety of laser scattering features,  
484 such as the coefficient and slope of the fitted curve, statistical image features, and texture features. In  
485 addition, microstructure analysis focusing on pore volume using X-ray CT showed that the number of  
486 large pores increased with the storage period and suggested that differences in microstructure affected  
487 light scattering.

488 Finally, several models were calculated to estimate the degree of mealiness from laser scattering  
489 features. When comparing the estimation performance between models built using data acquired with  
490 633-nm and 850-nm lasers, the 633-nm models were superior for all algorithms. Optimization results  
491 showed that the use of feature values acquired at both wavelengths combined with a nonlinear model  
492 resulted in a good performance. Furthermore, the ensemble learning method showed improved  
493 performance ( $R=0.682$ ,  $RMSE=7.281$ ) compared to models built with a single algorithm. Overall, these  
494 results indicated that the laser scattering method can non-destructively estimate the degree of mealiness  
495 and has a potential to be applied for practical use.

496

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500

#### 501 **Author's contribution**

502 Daiki Iida designed and conducted the experiments and wrote the main manuscript. Mito Kokawa designed  
503 the research project, constructed the measurement device, edited the manuscript, and prepared the figures.

504 Yutaka Kitamura oversaw the research project and was in charge of overall supervision. All authors  
505 reviewed the manuscript.

506

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509

### 510 **Conflict of Interest Statement**

511 The authors declare that they have no known competing financial interests or personal relationships that  
512 could have appeared to influence the work reported in this paper.

513

### 514 **Data availability Statement**

515 The datasets generated during and analyzed during the current study are available from the corresponding  
516 author on reasonable request.

517

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