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Exploring the potential of NIR hyperspectral imaging for automated quantification of rind amount in grated Parmigiano Reggiano cheese

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Abstract

Parmigiano Reggiano (P-R) is one of the most important Italian food products labelled with Protected Designation of Origin (PDO). The PDO denomination is applied also to grated P-R cheese products meeting the requirements regulated by the Specifications of Parmigiano Reggiano Cheese. Different quality parameters are monitored, including the percentage of rind, which is edible and should not exceed the limit of 18% (w/w). The present study aims at evaluating the possibility of using near infrared hyperspectral imaging (NIR-HSI) to quantify the rind percentage in grated Parmigiano Reggiano cheese samples in a fast and non-destructive manner. Indeed, NIR-HSI allows the simultaneous acquisition of both spatial and spectral information from a sample, which is more suitable than classical single-point spectroscopy for the analysis of heterogeneous samples like grated cheese. Hyperspectral images of grated P-R cheese samples containing increasing levels of rind were acquired in the 900-1700 nm spectral range. Each hyperspectral image was firstly converted into a one-dimensional signal, named hyperspectrogram, which codifies the relevant information contained in the image. Then, the matrix of hyperspectrograms was used to calculate a calibration model for the prediction of the rind percentage using Partial Least Squares (PLS) regression. The calibration model was validated considering two external test sets of samples, confirming the effectiveness of the proposed approach.

Keywords

Grated cheese; Rind percentage; NIR hyperspectral imaging; Multivariate calibration; Multivariate image analysis.

36 **1. Introduction**

37 Parmigiano Reggiano (P-R) is a long-ripened, cooked, hard cheese produced in Italy and registered
38 with Protected Denomination of Origin (PDO). P-R represents one of the most important typical
39 Italian food products and it is exported worldwide. This cheese is manufactured from raw and
40 unheated bovine milk, and the whole production chain must take place in a restricted area in Northern
41 Italy (Malacarne et al., 2008).

42 The PDO is extended also to grated cheese obtained from Parmigiano Reggiano cheese wheels,
43 provided that the product is grated in the specific production area and packaged immediately
44 afterwards, in order to avoid modifications of its organoleptic properties.

45 Furthermore, grated cheese products designated as Parmigiano Reggiano should meet technical and
46 technological parameters ruled by the Specifications of Parmigiano Reggiano Cheese
47 (https://www.parmigianoreggiano.com/consortium/rules_regulation_2/default.aspx), that regulates
48 all stages of P-R production, including cow feeding, cheese manufacturing and ripening process.

49 One of the different quality parameters of grated P-R cheese regulated by the Specifications is the
50 amount of rind. The rind is the external part of cheese wheels; although edible, in long-ripened
51 cheeses it has chemical and physical properties different from those of the inner part of the cheese.

52 These differences are mainly caused by exposure to environmental conditions during ripening, which
53 yields a decrease in moisture content, proteolytic activity and a higher degree of oxidation (Cattaneo
54 et al., 2008; Karoui et al., 2007; Malacarne et al., 2019). Furthermore, the different properties of rind
55 and pulp also affect size and shape of grated particles. In fact, rind particles are generally finer and
56 less circular than those derived from the pulp (Alinovi et al., 2019). Due to its peculiar chemical and
57 textural properties, an excessive amount of rind in grated cheese can be perceived by consumers,
58 negatively affecting the organoleptic properties of the product (Zannoni and Hunter, 2015). For these
59 reasons, the percentage of rind in grated P-R cheese should not exceed the 18% (w/w) threshold value.

60 In order to ensure quality compliance of commercial products of grated P-R cheese and to avoid
61 counterfeits, it is essential to implement effective analytical methods to correctly estimate the rind
62 amount, meeting the requirements of low costs, limited sample preparation and short times of
63 analysis. To date, no official analytical methods for the quantification of rind are considered by the
64 PDO regulation (Alinovi et al., 2019). A method that can be used to measure the rind amount in grated
65 cheese is based on the determination of the different patterns of casein degradation in rind and pulp
66 by means of capillary electrophoresis (Pellegrino et al., 2003; Cattaneo et al., 2008). However, this
67 analytical procedure is expensive, time-consuming and, according to Pellegrino et al. (2003), could
68 allow the presence of rind percentage (RP) values up to 24 % (w/w), which exceed the 18% (w/w)
69 threshold value by 6 RP units.

70 Near Infrared (NIR) spectroscopy has been widely employed for fast and non-destructive analysis
71 and characterization of food products, thanks to its ability to easily provide a spectral fingerprint
72 codifying the chemical composition of the analysed sample (Curda et al., 2004; Woodcok et al., 2008;
73 Foca et al., 2013; Kraggerud et al.; 2014). In particular, previous research studies evaluated the
74 effectiveness of NIR spectroscopy in verifying the authenticity of P-R grated cheese and in
75 discriminating compliant from non-compliant samples (Cevoli et al., 2013a, Cevoli et al., 2013b).
76 However, grated cheese is an inhomogeneous food matrix composed by unevenly dispersed particles
77 derived from both cheese rind and pulp, which are characterized by different chemical properties.
78 When dealing with heterogeneous samples, classical NIR spectroscopy may lead to inaccurate results
79 since it is based on the acquisition of “average” spectra over a given sample area, thus losing the
80 information related to the compositional variability within the sample. The importance of spatial
81 information for the analysis of P-R grated cheese was recently demonstrated by Alinovi et al. (2019),
82 who found a relationship between rind percentage and parameters related to particle size and
83 distribution calculated from digital RGB images of the cheese samples. In the same paper, the Authors
84 used also NIR spectroscopy to estimate rind percentage, which led to better results than those obtained
85 using RGB image analysis.

86 The advantages of image-based methods and NIR spectroscopy can be coupled in NIR Hyperspectral
87 Imaging (NIR-HSI), an analytical technique based on the acquisition of particular types of images,
88 called hyperspectral images, where a whole NIR spectrum is registered for each image pixel (Gowen
89 et al., 2007; Amigo et al., 2013; Calvini et al.; 2018). More in detail, a hyperspectral image, also
90 called *hypercube*, is a three-dimensional data array with two spatial dimensions (x pixel rows and y
91 pixel columns) and one spectral dimension, corresponding to the λ acquired wavelengths. Therefore,
92 the hypercube can be seen as a stack of spectrally resolved images, each one acquired at a given
93 wavelength, or as a series of spatially resolved spectra, where each spectrum characterizes one pixel
94 of the image (Wu and Sun, 2013a; Amigo et al., 2015; Calvini et al.; 2016).

95 Considering that each NIR spectrum acts like a fingerprint of the chemical properties of a specific
96 pixel, thanks to hyperspectral imaging it is possible to obtain both spatial and spectral information
97 from a sample by observing variations in the chemical composition over the sample surface. These
98 aspects are particularly useful for the analysis of heterogeneous matrices like food products (Wu and
99 Sun, 2013b; Dale et al., 2013; Liu et al., 2017), generally overcoming the performances obtained with
100 single-point spectroscopy (Burger and Geladi, 2006; Shonbichler et al., 2013).

101 However, the high amount of information contained in hyperspectral images can also become a
102 drawback, since each hypercube contains up to tens of thousands of pixel spectra, resulting in data
103 handling and data storage issues. Indeed, in order to extract the relevant information from this kind

104 of data, the application of proper multivariate statistical methods is mandatory (Burger and Gowen,
105 2011). This is known as Multivariate Image Analysis (MIA), which is based on the application to
106 images of common chemometric methods, like e.g. Principal Component Analysis (PCA). The MIA
107 approach essentially consists in considering each pixel of the image as a separate object and the main
108 goal is to find similarities or differences among clusters of pixels based on their spectral signatures
109 (Prats-Montalban et al., 2011; Amigo et al., 2015).

110 When dealing with a large number of hyperspectral images that should be analysed altogether,
111 classical pixel-level MIA can become unfeasible due to the intensive computational loads, since it
112 would imply the simultaneous analysis of numerous images, each one with tens of thousands of pixel
113 spectra. In these situations, a possible solution is to move from a pixel-level approach to an image-
114 level approach, which consists in performing the analysis considering the image of each sample as a
115 single object and extracting a feature vector characterizing the whole image, and thus the
116 corresponding sample. In this manner, it is possible to analyse data matrices containing these feature
117 vectors, in order to gain a general overview of the image dataset, to identify images sharing similar
118 features or to quantify whole sample properties (Ulrici et al., 2012; Kucheryavskiy, 2013; Giraud et
119 al., 2018; Orlandi et al., 2018a; Orlandi et al., 2018b; Oliveri et al., 2019; Calvini et al., 2020).

120 To this aim, a data dimensionality reduction method has been proposed, which consists in converting
121 each hyperspectral image of the dataset into a one-dimensional signal, named hyperspectrogram,
122 obtained by merging in sequence the frequency distribution curves of quantities derived from a PCA
123 model calculated on the images (Ferrari et al., 2013; Ferrari et al., 2015; Xu et al., 2016; Calvini et
124 al., 2016; Corti et al., 2017). In this manner, each hyperspectrogram summarizes the relevant
125 information contained in the corresponding hyperspectral image and a large dataset of hyperspectral
126 images is converted into a matrix of signals, which in turn can be analysed by means of common
127 chemometric methods.

128 In this context, the main goal of the present study consisted in evaluating the possibility of exploiting
129 the advantages of NIR-HSI coupled to data dimensionality reduction, in order to monitor the rind
130 percentage of grated P-R cheese products. In particular, hyperspectral images of grated P-R cheese
131 samples were analysed by means of the hyperspectrograms approach, and the resulting
132 hyperspectrograms were then used to predict the rind percentage by means of Partial Least Squares
133 (PLS) regression.

134

135 **2. Materials and Methods**

136 **2.1 Grated cheese samples**

137 Samples of grated Parmigiano Reggiano cheese containing varying rind percentages (RP) were
138 provided by Parmigiano Reggiano cheese Consortium. The grated cheese samples were prepared
139 considering the following 15 percentages (w/w) of rind in pulp: 0%, 5%, 10%, 12%, 14%, 16%, 18%,
140 20%, 22%, 24%, 26%, 28%, 30%, 35% and 40%.

141 In order to minimize possible effects of unwanted variations, the mixtures were prepared starting
142 from the same matrices of cheese pulp and rind, obtained by grating pulp and rind pieces derived
143 from different cheese wheels matured for a period of 12 months using a knife mill (Grindomix GM
144 200, Retsch). Cheese pulp was grated for 15 seconds with a speed equal to 4500 rpm, while cheese
145 rind was grated for 30 seconds at a speed equal to 8500 rpm. In order to ensure homogeneity of
146 particle size and distribution, the mixtures were further grated for 15 seconds at a speed of 4500 rpm.
147 The mixtures were replicated twice (deliveries A and B) as reported in Table 1, each time following
148 a different random order. Firstly, the matrices of grated pulp and rind were prepared, and a part of
149 them was then used to obtain the first set of 15 mixtures. The remaining part of the grated pulp and
150 rind matrices was stored in the dark at 4 °C, and after one week it was used to prepare the second set
151 of 15 mixtures. For both the replicate sets, the samples were stored in the dark at 4°C and the day
152 after their preparation they were delivered to the laboratory, where they were immediately analysed.
153 Furthermore, 15 additional samples with unknown rind percentage were provided by Parmigiano
154 Reggiano cheese Consortium (X1-X15). These samples were prepared, delivered and stored
155 considering the same procedure followed for the samples with known RP values.

156

157 **2.2 Image acquisition**

158 For each sample, three randomly sampled aliquots containing about 13 g of grated cheese were
159 collected and placed inside a plastic Petri dish of 5.5 cm diameter. Each hyperspectral image included
160 the three aliquots of two different samples. The samples were positioned according to a 3 × 2
161 chessboard scheme, as reported in Figure 1.

162 The hyperspectral images were acquired using a line scanning system (NIR Spectral Scanner, DV
163 Optic) equipped with a Specim Inspector N17E imaging spectrometer coupled to a Xenics Xeva-1.7-
164 320 camera (320 × 256 pixels) embedding Specim Oles 31 f/2.0 optical lens. The hyperspectral
165 system covers the 900-1700 nm spectral range with a spectral resolution equal to 5 nm. Due to the
166 low signal-to-noise ratio at the edges of the spectral range, only 143 spectral channels between 960
167 and 1670 nm were considered for further analysis.

168 The hyperspectral images were acquired using a black silicon carbide sandpaper sheet as background
169 and including in the image scene also a white ceramic tile with a 99 % reflectance standard reference
170 and two ceramic tiles with intermediate reflectance values corresponding to 89 % and 46 %,
171 respectively. The raw data were converted into reflectance values using the instrumental calibration
172 based on the measure of the white high reflectance standard reference and of the dark current (Ulrici
173 et al., 2013).

174

175 **2.3 Image elaboration**

176 The first step of image elaboration consisted in the application of an additional internal calibration
177 procedure in order to reduce possible variations over time. This correction procedure is based on the
178 comparison of the average reflectance values of the white standard reference, of the two ceramic tiles
179 and of the black silicon carbide sandpaper between an image chosen as reference and all the remainder
180 images of the dataset. Further details about the image correction algorithm can be found in Ulrici et
181 al. (2013).

182 Subsequently, the corrected images were cropped in order to obtain a single hyperspectral image for
183 each aliquot of grated cheese sample. At the end of the cropping procedure, a total of 135
184 hyperspectral images were obtained (= 45 grated cheese samples \times 3 aliquots), as reported in the last
185 column of Table 1.

186 After cropping, the pixels related to the black sandpaper background were removed using a
187 thresholding procedure carried out considering a wavelength equal to 1100 nm. Indeed, at 1100 nm
188 the pixels with reflectance value lower than 0.5 were ascribable to the background or to the plastic
189 Petri dish, thus they were not considered in the subsequent steps.

190 Finally, an additional morphological erosion procedure was performed using a disk-shaped
191 structuring element with radius equal to 8 pixels (Van Den Boomgaard and Van Balen, 1992).
192 Morphological erosion allowed to remove the pixels placed at the edges of the region of interest
193 obtained after background removal, since these pixels were mainly influenced by scattering
194 phenomena and specular reflections of the plastic Petri dish.

195 The image elaboration steps, which are summarized in Step 1 and Step 2 of Figure 1, were performed
196 with routines written *ad hoc* in MATLAB language (ver. 9.3, The Mathworks Inc., USA).

197

198 **2.4 Data analysis**

199 **2.4.1. Exploratory analysis**

200 As a preliminary assessment, Principal Component Analysis (PCA) was performed at the pixel level
201 on some representative images (Prats-Montalbán et al., 2011). More in detail, three images

202 corresponding to RP values equal to 0%, 20% and 40% were merged together and analysed by means
203 of PCA after applying standard normal variate (SNV) and mean center as spectral preprocessing
204 methods. This preliminary analysis was carried out in order to obtain a qualitative evaluation of the
205 differences between samples containing an increasing amount of rind.

206

207 **2.4.2. Calibration models**

208 *Data organization*

209 Before calculating the calibration model to predict the rind percentage, the hyperspectral images of
210 the grated cheese samples were split into training images, for model computation, and test images for
211 external validation, based on the RP values. The training images included the hyperspectral images
212 of grated cheese samples with RP values equal to 0%, 10%, 14%, 18%, 22%, 26%, 30% and 40%,
213 for a total of 48 images (= 8 RP values \times 2 deliveries \times 3 aliquots). The remainder images were
214 separated into two different test sets:

- 215 - TS_{known} : including the images acquired on cheese samples with RP values equal to 5%, 12%,
216 16%, 20%, 24%, 28% and 35%, for a total of 42 images (= 7 RP values \times 2 deliveries \times 3
217 aliquots);
- 218 - TS_{unknown} : including the images acquired on the cheese samples of unknown composition, for
219 a total of 45 images (= 15 unknown samples \times 3 aliquots).

220

221 *Conversion into Common Space Hyperspectrograms*

222 The hyperspectral images were then converted into one-dimensional signals, named Common Space
223 Hyperspectrograms (CSH) (Calvini et al., 2016). The basic idea behind the hyperspectrograms
224 approach is to convert each hyperspectral image of the dataset into a one-dimensional signal, which
225 acts like a feature vector retaining the useful spectral/spatial information of the corresponding image
226 (Ferrari et al., 2013; Ferrari et al., 2015). In the case of CSH, the signals are obtained by merging in
227 sequence the frequency distribution curves of quantities derived from a common PCA model, i.e.
228 from a PCA model calculated considering all the images of the training set.

229 The first step in the computation of the CSH consisted in unfolding all the three-dimensional
230 hyperspectral images into two-dimensional matrices with as many rows as the pixels retained after
231 background removal and erosion, and as many columns as the number of spectral channels. Then, the
232 unfolded hypercubes were row-preprocessed using SNV and scaled according to the global mean
233 spectrum, obtained by averaging all the retained pixel spectra of the training images. After unfolding
234 and spectral preprocessing, for each training image the corresponding variance-covariance matrix
235 was calculated. Then, all the resulting variance-covariance matrices were summed in order to obtain

236 the kernel variance-covariance matrix of the whole training set (Geladi and Grahn, 1996). The kernel-
237 variance covariance matrix was then decomposed by singular value decomposition (SVD) to obtain
238 the loading vectors of the common PC space. In this case, the common PC space was calculated
239 considering 3 principal components, based on the results of the previous exploratory data analysis
240 described in Section 2.4.1.

241 Once calculated the PC space common to the training images, all the hyperspectral images belonging
242 to both training and test sets were projected onto the PC space to obtain the corresponding scores, Q
243 residuals and Hotelling's T^2 vectors. Finally, for each image, the corresponding CSH signal was
244 obtained by merging in sequence the frequency distribution curves of the three score vectors, of the
245 Q residuals vector and of the Hotelling's T^2 vector. Including in the signals the frequency distribution
246 curves of the Q residuals allows to retain also the information not considered by the principal
247 components, while the inclusion of the frequency distribution curves of the of Hotelling T^2 values
248 allows to better describe the features of interest that are characterized by the simultaneous
249 contribution of more PCs.

250 The frequency distribution curves were calculated considering a number of bins equal to 150. Their
251 range was defined separately for each PC, and it was calculated considering the minimum and the
252 maximum score values of all the training images. Similarly, the range of the frequency distribution
253 curves of the Q residuals was defined between 0 and the maximum value calculated over all the
254 training images, and the same procedure was also applied to the frequency distribution curves of the
255 Hotelling T^2 values.

256 Since each image of the dataset has a different number of pixels, the corresponding CSH signal was
257 normalized by dividing each frequency distribution curve by the number of pixels retained after image
258 elaboration, as described in Section 2.3. Therefore, in this case each hyperspectrogram was a 750
259 points long vector, resulting from 150 bins of each frequency distribution curve \times 5 quantities
260 derived from PCA (3 PCs + Q residuals + Hotelling's T^2). Further details about the algorithm used to
261 calculate the CSH can be found in Calvini et al. (2016). The conversion of the hyperspectral images
262 into CSH signals is schematically depicted in Step 3 of Figure 1.

263 At the end of the conversion procedure three different matrices of signals were obtained: the training
264 set (TR), the test set derived from the TS_{known} images and the test set of unknown samples derived
265 from the TS_{unknown} images.

266 Figure 2 shows a plot of the CSH signals belonging to the training set and coloured according to the
267 rind percentage of the corresponding sample.

268

269 *Calibration model*

270 The training set matrix containing the CSH signals calculated from the training images was used to
271 calculate the calibration model to predict the RP value, using Partial Least Squares (PLS) algorithm
272 (Geladi and Kowalski, 1986). The signals were preprocessed using mean center and the optimal
273 number of latent variables (LVs) was chosen by minimizing the cross-validation error. In particular,
274 a custom cross-validation scheme was followed, considering 2 deletion groups, each one containing
275 the signals derived from samples belonging to the same delivery day.

276 The performances of the calibration models were evaluated both in terms of Root-Mean-Square Error
277 (RMSE) and of coefficient of determination (R^2). These parameters were calculated in calibration
278 (RMSEC and R^2 Cal), cross-validation (RMSECV and R^2 CV) and prediction of the external test set
279 (RMSEP and R^2 Pred).

280 The conversion of the hyperspectral images in CSH signals was done using a specific Graphical User
281 Interface (GUI), that was previously developed by some of the authors of the present work. The GUI,
282 which works under the MATLAB environment (ver. 9.3, The Mathworks, USA) and is named
283 *Hyperspectrograms GUI*, is freely downloadable from www.chimslab.unimore.it/downloads. PCA
284 and PLS models were calculated using PLS Toolbox (ver. 8.5, Eigenvector Research Inc., USA) and
285 MIA Toolbox (ver. 3.0.4, Eigenvector Research Inc., USA).

286

287 **3. Results and discussion**

288 **3.1. PCA at the pixel-level**

289 For a first evaluation of the differences between grated cheese samples containing different amounts
290 of rind, three hyperspectral images corresponding to samples with RP values equal to 0%, 20% and
291 40% were merged together to obtain a unique hyperspectral image, which was analysed at the pixel-
292 level by PCA. Figure 3.a reports the resulting PC1-PC2 score plot, where the first two principal
293 components account for 72.83 % and 16.58 % of total variance, respectively. In the score plot each
294 object represents a single pixel and is coloured according to pixel density, i.e. a yellowish colour
295 represents a region of the PC1-PC2 score space with a high density of pixels, while blue corresponds
296 to low pixel density. From this score plot it is possible to observe the presence of three clusters of
297 pixels, corresponding to the imaged samples with different RP values. The separation between
298 samples with different rind levels is particularly evident along PC2. Indeed, the sample containing
299 only cheese pulp is characterized by higher PC2 score values, while samples with increasing
300 percentages of rind have decreasing PC2 score values, as shown in the PC2 score image reported in
301 Figure 3.b.

302 In order to investigate the spectral features involved in the definition of the PC space, the
303 corresponding PC1-PC2 loading vectors are reported in Figure 3.c. The highest absolute values of the
304 PC2 loading vector can be found in the 1195-1225 nm wavelength range, corresponding to the C-H
305 bond second overtone ascribable to lipids (Burns and Ciurzak, 2008; Karoui et al., 2006), in the 1330-
306 1340 nm spectral range corresponding to asymmetric stretching vibration of water (Ozaky, 2002),
307 and in the region centred at 1400 nm ascribable to the O-H bond first overtone of free water (Burns
308 and Ciurzak, 2008).

309 Therefore, the amount of rind of the cheese samples can be somehow described by the distribution of
310 the corresponding pixel spectra along the principal components. A convenient way to summarize this
311 pixel distribution consists in using the frequency distribution curves of the score vectors of each
312 sample, as reported in Figure 3.a that shows the frequency distribution curves of both PC1 and PC2
313 score vectors for each image. From this figure it is possible to observe the presence of a clear shift of
314 the frequency distribution curves of PC2, which is related to the rind percentage of the corresponding
315 samples. Although less marked, a variation with RP can be observed also for the frequency
316 distribution curves of PC1 scores, which tend to become sharper and with a maximum located at
317 lower PC1 values with increasing values of rind percentage.

318 Since the hyperspectrograms approach is based on the use of frequency distribution curves of score
319 vectors calculated from a PCA model in order to summarize the relevant information contained in the
320 images, this preliminary analysis suggests the effectiveness of this approach for the determination of
321 the rind amount in hyperspectral images of grated cheese samples.

322

323 **3.2 PLS calibration model with the CSH approach**

324 The training set of CSH signals reported in Figure 2 was then used to calculate a PLS regression
325 model for the quantification of rind percentage in the samples of grated Parmigiano Reggiano cheese,
326 leading to the results reported in Table 2.

327 The optimal model dimensionality was found to be equal to 8 LVs, leading to a RMSECV value equal
328 to 1.70 RP units, corresponding to a R^2 CV value of 0.979.

329 The calibration model was then used to predict the RP of the samples belonging to the TS_{known} test
330 set. In this case, the prediction results were calculated considering both the whole range of rind levels
331 (0% - 40%) and only the interval of rind percentages ranging from 10% to 30%, which better reflects
332 RP values that generally may occur in real situations.

333 The prediction results confirm the effectiveness of the calibration model in quantifying the rind
334 percentage, leading to RMSEP values equal to 1.91 and 1.85 RP units in the 0-40% and 10-30%
335 ranges, respectively.

336 Figure 4 shows the plot of the rind percentage values predicted for the TS_{known} test set versus the
337 corresponding experimental values, where the samples are coloured according to the delivery day.
338 Generally, all the objects are close to the bisector, indicating the good prediction performances of the
339 model. In addition, from Figure 4 it is also possible to observe that there is no evidence of systematic
340 variations in the prediction results caused by the different delivery days, which further confirms the
341 robustness of the calibration model toward replicated measurements.

342 Considering that compliant Parmigiano Reggiano grated cheese samples should have an RP value
343 less than or equal to 18%, all the test set samples with a rind percentage falling outside this limit are
344 correctly identified by the model.

345 The calibration model was also used to predict the samples with unknown composition belonging to
346 the TS_{unknown} test set. The predicted rind percentages of the unknown samples were communicated to
347 the Parmigiano Reggiano Cheese Consortium, who then revealed the corresponding experimental
348 values. In this manner, it was possible to perform a further external validation of the calibration
349 model.

350 It has to be considered that, for each unknown sample, three different aliquots were imaged and,
351 therefore, three RP values were obtained in prediction by the model. In order to have a single estimate
352 of the RP value for each unknown sample, the three RP predicted values corresponding to the three
353 aliquots were averaged for each sample. The results are reported in Table 3, together with the
354 corresponding experimental values, further confirming the good prediction ability of the calibration
355 model. More in detail, an RMSEP value equal to 2.50 RP units was obtained, corresponding to an R^2
356 value equal to 0.955. The highest difference between predicted and experimental rind percentage,
357 equal to 5 RP units, was observed for sample X15, while samples X2 and X4 were exactly predicted.

358 Compared to a recent study of Alinovi et al. (2019), who reported the use of NIR spectroscopy to
359 predict rind percentage, the calibration model reported in the present work led to a lower error both
360 in cross-validation and in prediction, suggesting the advantage of accounting also for spatial
361 information brought by NIR-HSI. Indeed, using single point NIR spectroscopy Alinovi et al. obtained
362 an RMSECV value equal to 2.86 RP units and an RMSEP value equal to 3.44 RP units.

363 In order to have a comprehensive evaluation of the hyperspectrogram regions most relevant to the
364 calibration model, Figure 5.a reports the Variable Importance in Projection (VIP) scores: variables
365 with VIP score values higher than one are considered significant for the model. This figure shows
366 that all the frequency distribution curves of the PCA quantities included in the CSH signals have
367 regions with significant variables. In particular, among the frequency distribution curves of the three
368 score vectors included in the signals, the regions related to the frequency distribution curve of PC2
369 reach the highest VIP score values, together with the signal regions related to the frequency

370 distribution curves of the Hotelling T^2 values. Therefore, among the different PCA quantities
371 considered in the hyperspectrograms, PC2 and Hotelling T^2 have the major relevance in accounting
372 for the rind percentage of the grated cheese samples. In order to further verify these findings, Figure
373 5.b reports the PC2 score images of some representative test set samples with increasing percentage
374 of rind. Similarly to what was previously reported in Section 3.1, images of samples with a lower RP
375 value are characterized by higher PC2 score values, and the increase of the RP value in the grated
376 cheese samples causes a shift of the corresponding pixels toward lower PC2 score values. Actually,
377 this is due to the fact that the PC2 loading vector of the common PCA model used to calculate the
378 CSH signals is very similar to the PC2 loading vector calculated on the three sample hyperspectral
379 images, reported in Figure 3c.

380

381 **Conclusions**

382 The present study demonstrated the possibility of using NIR-HSI as a tool for the quantification of
383 the amount of rind in grated Parmigiano Reggiano cheese samples. The combined use of a data
384 dimensionality reduction approach, namely the Common Space Hyperspectrograms approach, with
385 PLS regression allowed to obtain good prediction performances. The calibration model was validated
386 using two different test sets: the first test set consisted of cheese samples with known RP values,
387 while for the second test set the experimental RP values of the analysed samples were revealed by
388 the operators of Parmigiano Reggiano Cheese Consortium only after providing them with the RP
389 values predicted by the model. The RMSEP values obtained for both test sets, corresponding to 1.91
390 RP units and 2.50 RP units, respectively, confirm the advantages of coupling spatial and spectral
391 information of a sample brought by NIR-HSI in the analysis of heterogeneous products, like grated
392 cheese.

393 However, it has to be considered that commercial samples of grated Parmigiano Reggiano cheese
394 may be affected by different variability factors, such as months of ripening, fat content and rind
395 processing methods, among others. Therefore, in order to obtain a more robust calibration model the
396 influence of these factors should be properly evaluated and included in the model. As a consequence,
397 the increasing complexity of the model should be faced by including further steps in the data analysis
398 workflow, like e.g. spectral and/or spatial feature selection.

399

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403

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526

527 **Captions to Tables and Figures**

528 **Table 1.** Summary information about the grated cheese samples considered in the present study

529 **Table 2.** Results of PLS regression for the determination of rind percentage.

530 **Table 3.** Prediction results of the unknown test samples and corresponding experimental RP values.

531

532 **Figure 1.** Key steps involved in image elaboration and analysis

533 **Figure 2.** Hyperspectrograms of the training set images; the signals are coloured according to the
534 rind percentage of the corresponding grated cheese sample

535 **Figure 3.** In (a) PC1-PC2 score plot of the image containing grated cheese sample with 0%, 20% and
536 40% percentages of rind, and corresponding frequency distribution curves of PC1 and PC2 score
537 vectors calculated separately for each sample. In (b) PC2 score image. In (c) PC1 and PC2 loading
538 vectors.

539 **Figure 4.** Results of the PLS model: TSkknown test set predicted rind percentage (Y Predicted) vs
540 experimental rind percentage (Y measured).

541 **Figure 5.** VIP scores of the PLS model (a) and PC2 score images of samples with increasing rind
542 percentage values(b).

543