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1 **Use of near infrared spectroscopy coupled with chemometrics for fast detection of**
2 **irradiated dry fermented sausages**

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28 **Abstract**

29 Chemometric analysis of near infrared (NIR) spectroscopy data was applied to investigate the
30 possibility to rapidly detect dry fermented sausages treated with ionizing radiation at 0; 0,5; 1; 2 and
31 3 kGy. NIR spectra from 1000 to 2500 nm were subjected to an exploratory principal component
32 analysis (PCA) followed by orthogonal partial last square-discriminant analysis (OPLS-DA) to
33 develop classifiers able to distinguish samples according to the irradiation dose.
34 Models provided a correct classification rate of 100% for both irradiated and non-irradiated
35 specimens. As for the different irradiation doses, 100% of sausages treated at 1 and 3 kGy dose, 92%
36 of those at 2 kGy and 83% of those at 0,5 kGy were correctly classified.
37 The successful results obtained confirmed suitability of chemometric analysis applied to NIR data for
38 fast detection of dry fermented sausages subjected to irradiation treatment.

39

40

41 **Abbreviations**

42 2-alkylcyclobutanones, ACBs; cross-validation, CV; analysis of variance testing of cross-validation
43 predictive residuals, CV-ANOVA; FT-IR, Fourier transform-infrared; IR, infrared; mid-infrared,
44 MIR; near infrared, NIR; orthogonal partial last square-discriminant analysis, OPLS-DA; principal
45 component analysis, PCA; principal component, PC; root mean square error from cross-validation,
46 RMSECV; root mean square error of estimation, RMSEE; root mean square error of prediction,
47 RMSEP; second derivative, SD; Savitzky-Golay smoothing, SG; semi-intensive system, SI; standard
48 normal variate, SNV; variable influence on projection, VIP.

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51 **Keywords**

52 Irradiation; dry fermented sausages; NIR spectroscopy; chemometrics

53 **1. Introduction**

54 In 2017, Italian pork products accounted for 1177200 tons, and the export volume achieved around
55 15.2% of the global production, equivalent to about 1516 million Euros. About 80% of the exported
56 products are addressed to European countries, in particular Germany, France, United Kingdom, Spain
57 and Austria, whereas about 20% of the products reaches third countries such as USA, Japan, Canada
58 and many others. The positive and increasing trend of the export observed in the last years represents
59 the driving force of the pork processing sector characterized by the uncertainty of the internal market
60 (Assica, 2018).

61 Being a non-thermal process, food irradiation, can be used to increase food safety and to extend the
62 shelf-life of a wide range of foods. In the European Union the only category of foodstuffs that may be
63 treated with ionizing radiation at the maximum overall average absorbed dose of 10kGy is “dried
64 aromatic herbs, spices and vegetable seasonings” (Directive 1999/3/EC). Moreover, the Directive
65 1119/2/EC established the provisions for authorizing food irradiation, marketing irradiated food and
66 food ingredients, and labeling treated foodstuffs.

67 The availability of analytical methods is pivotal for the official control systems to assess the
68 compliance with regulatory requirements and prevent food fraud in the national and international
69 markets. A single analytical method to be used to control all the types of foods is not currently
70 available, and the distinction between irradiated and non-irradiated food in some cases is still an
71 unsolved analytical problem. In fact, most of the chemical compounds induced by irradiation
72 treatment are not unique radiolytic products and therefore they are not adequate markers for the
73 detection of ionizing radiation use (Zanardi, Caligiani & Novelli, 2018). Despite the analytical
74 methods available for the detection of some irradiated foods are numerous, the European Commission
75 promotes the development of new techniques and setup of new protocols aimed at simplifying or
76 improving the already existing procedures in order to ensure that detection methods exist for all food
77 products (Delincée 2002; Boniglia 2004).

78 The effects induced by irradiation treatment on cured pork products were investigated by some
79 authors in a limited number of studies. Hwang (1999) suggested some hydrocarbons (C16:2, C17:1,
80 C17:2 and C16:3) as a useful tool for determining whether bacon and ham are irradiated at 1 kGy or
81 higher. In a study involving three representative Italian cured pork products (salame Milano, coppa
82 and pancetta), a significant increase in the degree of fatty acid and cholesterol oxidation was observed
83 starting from 8 kGy irradiation dose (Zanardi, Battaglia, Ghidini, Conter, Badiani, Ianieri, 2009). In
84 the same products, 2-alkylcyclobutanones (2-ACBs), formed by radiolysis in fat-containing foods
85 exposed to irradiation treatment, were recorded occasionally at 2 kGy and constantly at higher
86 irradiation doses (5 and 8 kGy) (Zanardi, Battaglia, Ghidini, Conter, Badiani, Ianieri, 2007).

87 Two reference methods based on analysis of secondary radiolytic products from fatty acids, namely,
88 hydrocarbons (EN 1784, 1996) and 2-ACBs (EN 1785, 2003) were validated by the European
89 Committee of Standardization (CEN) for pork, poultry, and eggs (Marchioni, 2006). However, they
90 have the disadvantage of being quite time consuming, requiring the use of considerable amounts of
91 organic solvents due to a long and complex sample preparation, and highly trained staff.

92 Near infrared (NIR) spectroscopy has been widely documented as a rapid, cost-effective, and non-
93 destructive technique able to return reliable estimates of several physico-chemical properties in
94 different food matrices (Boschetti et al., 2013; Ottavian et al, 2012a; Foca, Ferrari, Ulrici, Ielo,
95 Minelli, & Lo Fiego, 2016; Bagchi, Sharma & Chattopadhyay, 2016). The recent approach of NIR
96 spectroscopy and other spectroscopic techniques applied to food science is the exploitation of the
97 spectra as a fingerprinting to be analyzed by chemometrics. The applications of the fingerprinting
98 techniques seem to be a useful and powerful tool in view of a comprehensive food control. As a
99 matter of fact, many studies have shown the **applicability of these non-targeted strategies to**
100 **successfully monitor characteristics and overall quality of several food and foodstuffs, including food**
101 **of animal origin (Fan, Liao, & Cheng, 2018; Cheng & Sun, 2017; Barbin et al., 2015) and, in**
102 **particular, a wide range of food and beverage of plant origin (Condurso, Cincotta, Tripodi, &**
103 **Verzera, 2018; Lachenmeier, 2007; Chen et al., 2018; Cirilli et al., 2016).**

104 Alongside with the quality monitoring, one of the most promising application of NIR spectroscopy in
105 combination with multivariate data analysis is its application to the assessment of food authenticity
106 (Esslinger, Riedl, & Fauhl-Hassek, 2014). The technique provided the recognition of patterns useful
107 in assessing the authenticity of wild fish (Ottavian et al., 2012b), the adulteration of dairy products
108 (Kamal and Karoui, 2015) and minced beef (Morsy & Sun, 2013), as well as the exact origin of
109 honey (Maione, Barbosa, & Barbosa, 2019), edible oils and fats (Yang, Irudayaraj, & Paradkar, 2005)
110 and cereal products (Cozzolino, 2014).

111 In the context of food control for irradiation treatment, the vibrational spectroscopic techniques were
112 mainly applied to specific extracted components of food which are considered the most susceptible to
113 structural changes induced by the ionizing radiation, such as DNA (Hamad, Fahmy & Elshemey,
114 2019). Nevertheless, vibrational spectroscopy was also applied directly to the entire food matrix, in
115 order to study the effects induced by irradiation on the compositional/structural properties of the
116 lipidic and proteic fractions of starch (Kizil, Irudayaraj, & Seetharaman, 2002), egg products
117 (Seregély, Farkas, Tuboly, & Dalmadi, 2006), and hazelnuts (Dogan, Siyakus, & Severcan, 2007).

118 Other applications of vibrational spectroscopy led to the successful quantification of the irradiation
119 dose of irradiated milk powders (Kong, Zhang, Liu, Gong, & He, 2013), starch samples (Kizil et al.,
120 2002), and rice (Shao, He, & Wu, 2008).

121 In addition, some variations in the spectral profile of meat products treated with growing irradiation
122 doses were previously reported in literature. Absorption intensities of functional groups of lipids and
123 proteins in chicken breast samples (Liu, Barton, Lyon, & Chen, 2003), or the appearance of new
124 absorption band of 2-dodecylcyclobutanone generated by radiolysis from palmitic acid in beef
125 samples (Badr, 2012) were detected and associated to the irradiation treatment.

126 Therefore, considering the global chemical and structural modifications occurring in food treated with
127 ionizing radiations, the aim of the present study was to verify the possibility of using untargeted NIR
128 spectral fingerprinting in combination with chemometric analysis for the rapid distinction of

129 irradiated and non-irradiated dry fermented sausages and for their classification according to different
130 irradiation doses.

131 **2. Materials and Methods**

132 *2.1 Samples preparation and irradiation*

133 A local manufacturer supplied Salame Milano (five batches of 2 sausages each) at the end of the
134 maturation time (60 days). Sausages were sliced, and aliquots of 2-slices each were vacuum packed
135 into oxygen-impermeable nylon/polyethylene bags (Polinyl 145 mm, O₂ trans. rate 8–10 cm³/m²/24
136 h/atm). Fifty bags (ten for each of the five batches) were randomly chosen for comparison purposes
137 (non-irradiated control samples, C, n=50) and forty bags (ten for each of four batch) were randomly
138 allotted in four groups intended for irradiation treatment at 0.5, 1, 2 and 3 kGy irradiation doses,
139 respectively (treated samples, T, n=40 each). The bags of each group were arranged in a corrugated
140 cardboard box. Irradiation was performed using a ⁶⁰Co γ -irradiator (1.17–1.33 MeV). Alanine
141 dosimeters were positioned at the top and bottom surfaces of each box and the absorbed dose was
142 within $\pm 5\%$ the targeted dose. The samples were stored for 30 days at 5 ± 1 °C prior to analysis.

143 *2.2 NIR analysis of dry fermented sausage samples*

144 The NIR spectra of samples under investigation were recorded at room temperature after having
145 transferred approximately 2–3 g of minced sample into a 34 mm round quartz holder. Reflectance (R)
146 data were recorded at 1 nm resolution step, covering the whole 1000–2500 nm region of the
147 electromagnetic spectrum, by means of a NIRFlex® N-500 (Büchi Labortechnik AG, Flawil,
148 Switzerland) instrument. To reduce physical effects deriving from the intrinsic physical properties of
149 the samples, the spectrometer was set to perform 32 scans for each spectral recording; moreover, each
150 single sample was acquired eight times in sequence. The eight individual spectra were then averaged
151 and transformed into absorbance (A) spectra, by calculating the logarithm of reciprocal of the
152 reflectance values ($A = \log 1/R$). The 1000–1249 nm wavelengths region was removed from the

153 dataset because it was characterized by the presence of noise and did not contain relevant information
154 (Fig. 1). A total of 210 samples spectra and of 1251 wavelengths were therefore included in the final
155 spectral data matrix.

156 *2.3 Statistical analysis of spectral data*

157 Multivariate data analysis was performed by using the chemometric software SIMCA-P v.14.1
158 (Umetrics, Umeå, Sweden). Pre-processing is an important step of data analysis; pre-processing
159 methods are mainly used to adjust the variability of each measured variable and their relationships to
160 comply better with the data-analysis goal (Engel et al., 2013). In the present study three different pre-
161 processing techniques were applied to raw 1250–2500 nm spectra, with the aim to flat noise and
162 upwards shifting effects, and to resolve the wide overlapping bands, namely standard normal variate
163 (SNV), second derivative (SD; quadratic polynomial order) and Savitzky-Golay smoothing (SG; 15
164 points in each sub-model) (Barnes, Dhanoa & Lister, 1989; Savitzky & Golay, 1964). Next, a
165 principal component analysis (PCA) was computed on the NIR pre-treated data, to find out
166 correlation, trends of clustering or separation, whose reliability was estimated by means of an internal
167 10-fold venetian blind cross-validation (CV). During this step, the ability of the extracted principal
168 components (PCs) to collect and to predict as much total variability of dataset as possible, was
169 assessed by evaluating the R^2X_{cum} (fitting ability) and Q^2_{cum} (prediction ability) values, respectively.
170 The possible presence of outlier samples was also uncovered through the application of the
171 Hotelling's T^2 test ($p \leq 0.5$).

172 Then, orthogonal partial last square-discriminant analysis (OPLS-DA) was applied to classify
173 samples on the basis of the irradiation dose they were subject to. As with PCA, an internal 10-fold
174 CV was performed to evaluate the robustness of the models, but, with a view to avoid getting
175 overoptimistic results, only 70% of the original spectra were used to compute the models (Westad &
176 Marini, 2015). In particular, $n = 35$ non-irradiated control samples, $n = 28$ samples irradiated at 0.5
177 kGy, $n = 28$ samples irradiated at 1 kGy, $n = 28$ samples irradiated at 2 kGy, and $n = 28$ samples

178 irradiated at 3 kGy were shifted into a new sub-dataset (training set, $n = 147$), while the remaining $n =$
179 15 control samples, $n = 12$ samples irradiated at 0.5 kGy, $n = 12$ samples irradiated at 1 kGy, $n = 12$
180 samples irradiated at 2 kGy, and $n = 12$ samples irradiated at 3 kGy were included into the test set (n
181 $= 63$, 30% of the original spectra), which was used to externally validate the classification results
182 obtained through the training set.

183 Three different OPLS-DA elaborations were performed. The first one was aimed at discriminating
184 irradiated from non-irradiated salami, the second one at discriminating among the different irradiation
185 doses samples were subjected to (0, 0.5, 1, 2, and 3 kGy), and, finally, the third one at specifically
186 discriminating the control samples (0 kGy, non-irradiated) from the lowest-dose irradiated samples
187 (0.5 kGy).

188 The R^2X_{cum} and Q^2_{cum} , together with RY^2_{cum} (cumulative explained variation related to classes),
189 RMSECV (Root Mean Square Error from cross-validation), RMSEE (Root Mean Square Error of
190 Estimation), and RMSEP (Root Mean Square Error of Prediction from external validation) values
191 were considered as useful performance indicators for evaluating the global quality of models.

192 Moreover, analysis of variance of the cross-validated residuals (CV-ANOVA, p -value ≤ 0.05) was
193 performed to further assess the statistical significance of the results and a permutation test with 200
194 random interactions was also designed to support the absence of overfitting and overprediction of the
195 models. Values of R^2 and Q^2 y-intercepts ≤ 0.4 and ≤ 0.05 , respectively, were considered satisfactory
196 (Van der Voet, 1994).

197 Finally, the Variable Importance in Projection (VIP) scores for predictive OPLS-DA components
198 were designed to find the strongest influence exerted by single absorption bands over samples
199 classification: as a rule, VIP values ≥ 1 were used to identify the most relevant wavelengths
200 (Andersen & Bro, 2010; Ottavian et al., 2015).

201 **3. Results and discussion**

202 *3.1 NIR spectra characteristics and early steps of chemometric analysis*

203 The NIR absorbance spectra of the 210 salami samples collected in the range of 1000 to 2500 nm are
204 represented in Fig. 1. Original spectra (Fig. 1A) showed deceptive baseline shifts and intense but
205 undefined absorption peaks at three main wavelength ranges, which were strongly improved by SNV,
206 SD and SG mathematical transformations (Fig. 1B). As briefly mentioned above, the 1000-1249 nm
207 NIR region was found to be noisy and useless and, for this reason, it was excluded from the set of
208 variables used for multivariate data analysis.

209 According to the Fig. 1B, sharp absorption peaks for pre-processed NIR spectra emerged from the
210 1250–1350 nm region, which, just as peaks falling within the 1660–1780 nm and the 2200-2500 nm
211 regions, are related to the first or the second overtones of C–H bonds of lipid and fatty acids (Prieto,
212 Andrés, Giráldez, Mantecón, & Lavín, 2006; Ding & Xu, 2000; Fan, et al., 2018). Absorption peaks
213 around 1360–1400 nm correspond to N–N bonds vibration of peptides and proteins, while those found
214 at 1800–2000 nm are the result of both N–H combination bands of protein and O–H stretching and
215 deformation vibrations: the major peak around 1940 nm is typical of water absorption and it is partly
216 responsible for masking protein absorption signal (Brøndum et al., 2000; Sinelli, Limbo, Torri, Di
217 Egidio, & Casiraghi, 2010).

218 PCA was initially used as a preliminary tool to examine the structure and characteristics of samples
219 under investigation. PCA model was constructed using the SNV-SD-SG pre-processed NIR spectra
220 (over the range 1250–2500 nm) of the overall 210 salami dataset studied. Internal CV allowed to
221 optimally extract a total of 8 PCs, which explained 98.6% of the cumulative variance of the spectra
222 ($R^2X_{cum} = 0.986$), the first two PCs accounting for the major variability present in the dataset (90.2%).
223 As for the percentage of variability predicted by PCA model on the basis of the variability of the
224 original spectra, it was found to be around 81.5% ($Q^2_{cum} = 0.815$).

225 The two-dimensional score scatter plots for PC1 and PC2 (68.5% and 21.7% of the total variance,
226 respectively) reported in Fig. 2 was designed to show the interrelations among samples according to
227 irradiation dose they were exposed. As it can be observed, all samples distributed confusingly both as
228 positive and negative score on PC1 and PC2. All the analysed groups strongly overlapped each other

229 and no clusters among the different classes was obtained, probably as consequence of the very low
230 degree of modification of the physical and chemical characteristics of samples induced by the
231 irradiation treatments.

232 To conclude, PCA of salami spectral data was considered inadequate for a good sample distinction
233 based on irradiation treatment. However, further efforts of classification were made by applying
234 supervised classification techniques.

235 *3.2 OPLS classification results*

236 Since the spectra of the samples covered by the present study were probably characterised by too
237 much useless and disturbing information due to the high between-class variability, OPLS-DA was
238 thought to be the most appropriate chemometric technique to elaborate spectral data of salami
239 samples. OPLS-DA offers several advantages over other supervised classification techniques,
240 deriving, first of all, from its ability to identify the non-related (orthogonal) variability present in the
241 spectra, which, therefore, can be separated from the informative variability related to class
242 membership of samples.

243 OPLS-DA algorithm was applied to the 147 SNV-SD-SG NIR spectra of the training subset by
244 following a multi-step methodology. An initial OPLS model was intended to discriminate non-
245 irradiated (control, C) from irradiated (treated, T) samples (Fig. 3); after that, a much more specific
246 approach was followed, aimed at discriminating salami sample in five different groups corresponding
247 to the delivered irradiation dose of 0, 0.5, 1, 2 and 3 kGy (Fig. 4). Since the separation between non-
248 irradiated samples (0 kGy) and the samples treated by the lowest dose of irradiation (0.5 kGy) failed,
249 one last discriminant analysis was performed only on these two groups of samples (Fig. 5).

250 *3.2.1 OPLS-DA for non-irradiated and irradiated samples*

251 Classification models able to distinguish between C and T salami samples were built on pre-
252 processed training spectra, using 1 predictive component ($t[1]$) and 4 orthogonal components ($t[o]$),
253 which, in CV, collected more than 89% of the total variability in the dataset ($R^2X_{cum} = 0.891$) and

254 showed a predictive ability of approximately 49% ($Q^2_{cum}=0.487$) (Table 1). To ascertain that the good
255 statistical outcomes on the training set were not just the product of data overfitting during CV, the
256 OPLS model reliability was further checked by means of a permutation test, whose results confirmed
257 the validity of the model in sample discrimination (R^2 y-intercept ≤ 0.4 and Q^2 y-intercept ≤ 0.05).
258 As it can be observed from score scatter plot reported in Fig. 3, the two groups of samples were
259 perfectly separated in the bi-dimensional space, since C samples distributed along the positive t[1]
260 component and the T samples along the negative one (with a statistical significance assessed at 95%
261 confidence interval, $p_{CV-ANOVA} \leq 0.05$). Nevertheless, a mild degree of heterogeneity was observed
262 within samples of the same group which were not closely grouped along the vertical direction.
263 This intra-class non-predictive variability, of which 19.9% was collected by the first t[0] component,
264 was expected to be present, especially among T samples at different irradiation doses, but it did not
265 hinder the correct discrimination of C from T samples.

266 The check of the reliability and robustness of the model was carried out by way of the test set-based
267 external validation. The classification performances of the model previously computed on the training
268 subset were thus assessed as percentages of correctly classified and misclassified samples in the
269 confusion matrix (Table 2). As a result, classification rates of 100% for both C and T samples were
270 achieved, which, together with a low mean value of RMSEP (0.211), confirmed the accuracy of the
271 model in prediction of the class the samples belonged to.

272 3.2.2 OPLS-DA for the different irradiation doses

273 The second purpose of the present work was to investigate whether OPLS-DA of NIR spectral data
274 was useful for the discrimination of irradiated salami on the basis of the extent of irradiation they
275 were exposed. Classification ability of the model was at first evaluated on the basis of the results
276 obtained from CV of the training set: the values of R^2X_{cum} and Q^2_{cum} obtained were higher than the
277 model discriminating control from treated samples, while the value of R^2Y_{cum} was lower but
278 sufficiently good for the performances to be considered more than satisfactory (see Table 1). The
279 predictive variation extracted, in fact, explained about 88 % of the information related to the specific

280 class membership of the samples, allowing to obtain a perfect separation among samples of different
281 groups and a high degree of clusterisation among samples of the same group. Nevertheless, as it can
282 be observed from the score plot reported in Fig. 4, no distinct clusters were observed among the
283 control (0 kGy) and the lowest-irradiated (0.5 kGy) samples.

284 At the same time, model's overall classification performance of 74.6 % in external validation was
285 satisfying (Table 2). In this context, the samples exposed to 1 and 3 kGy irradiation doses were 100%
286 correctly allocated in their class, while the weakest statistical performances were shown by samples
287 exposed to both 0 and 0.5 kGy irradiation doses and, in particular, the highest error of classification
288 was recorded for 0.5 kGy samples, since only 41.7% was assigned to the proper class.

289 *3.2.3 OPLS-DA for non-irradiated and the lowest irradiated samples*

290 The model designed to classify samples by growing irradiation dose failed in discriminating the
291 control samples from the lowest irradiated samples and, for this reason, an additional OPLS-DA was
292 performed on a training set composed by total of 63 samples, of which n = 35 samples were not
293 treated and n = 28 samples were treated at 0.5 kGy). This approach was meant on the ground that
294 lower irradiation doses are still effective in improving safety and health quality of food, and, at the
295 same time, led to minor undesirable radiolysis effects affecting the global sensory and
296 physicochemical characteristics of meats (Feng et al, 2019).

297 This time, higher predictive ability ($Q^2_{cum} = 0.531$) and higher rate of variation directly linked to the
298 explanation of the two groups examined ($R^2Y_{cum} = 0.954$), were achieved, with a consequent
299 beneficial impact on the discrimination of samples (Table 1). Non-irradiated samples neatly located
300 as positive scores on the t[1], while 0.5 kGy irradiated samples as scores on the negative axis of the
301 same component, to such a point that no evident overlays between them were produced (Fig. 5). As in
302 the case of OPLS model discriminating C from T samples, a certain degree of intra-class variability
303 was present, both among non-irradiated and 0.5 kGy irradiated samples, which resulted in the
304 samples of the same group to be distributed far enough away from each other. Once again, the intra-
305 class variability modelled in the vertical direction by the first t[0], despite showing values of 20.9%,

306 did not negatively interfere with the correct discrimination of samples achieved along the horizontal
307 t[1] direction.

308 Taking a closer look to the results obtained by internal CV of the classification model, it is important
309 to emphasise also the very low values of RMSECV and RMSEP obtained, meaning that the model
310 presented the highest achievable accuracy so as to enable reasonable assurance of the prediction of
311 the class of new samples (Table 1). As showed in Table 2, this characteristic was confirmed by the
312 application of the external test set validation (n = 15 non-irradiated samples and n = 12 samples
313 irradiated at 0.5 kGy): correct class predictions for 100% and 83% of non-irradiated and irradiated
314 samples were respectively obtained, since only two samples of the 0.5 kGy group were misclassified.

315 *3.3 Analysis of the discriminating NIR wavelengths*

316 Ionizing radiations are responsible for the generation of highly reactive free radicals which can
317 induce several chemical and structural modification when interacting with the food matrix. These
318 modifications affect both lipidic and proteic fractions of foods, which can easily undergo
319 peroxidation and hydrolysis induced by the activity of radicals (Severcan, & Bozkurt, 2010).

320 Therefore, in the present work, the VIP analysis was applied in order to identify the NIR spectral
321 regions which mostly contributed to the successful separation of the salami samples achieved by
322 OPLS-DA and which can be considered markers of modifications induced by irradiation.

323 As for the most influential variables to discrimination of C from T samples, few specific NIR
324 absorption bands were found to be characterised by VIP values ≥ 1 . These NIR regions mainly
325 corresponded to the absorption of secondary radiolytic products from fatty acids, namely
326 hydrocarbons at 1670–1750 nm and 2150–2200 nm (Fig. 6A); the highest VIP value for predictive
327 components around 2420 nm was just the result of the absorption of C–H groups during the second
328 overtone. (Manley, Downey, & Baeten, 2008; Ribeiro, Ferreira, & Salva, 2011). In addition, other
329 wavelengths associated to lipid absorption contributed mostly to the discrimination of samples,
330 among which those located at 1720 nm (first overtone of the C–H stretching vibrations) and around
331 2160–2170 nm (C–H stretching vibrations and C–H deformation combination, typical of the double

332 bonds HC=CH). With regard to the significant VIP values for the 2050 nm region, they were
333 specifically associated to the vibration of N–H moiety of CONH and CONH₂ groups (amides II and
334 III) (Shenk et al., 2008).

335 VIP analysis for samples discrimination according to the irradiation dose highlighted the strongest
336 contribution exerted by a remarkably high number of spectral regions: in particular, VIP values for
337 predictive components > 1.5 were achieved around 1310, 1670, 1750, , 2170, and 2420 nm (Fig. 6B),
338 corresponding to absorption of the C–H bonds of aliphatic chains (Ribeira, Ferreira, & Salva, 2011),
339 and at 1850 nm and 2290 nm, corresponding to the absorption of the N–H bonds of proteins (Shenk,
340 Workman, & Westerhaus, 2008). The two peaks at 1790 and 1940 were instead attributed to
341 combination and bending vibrations of O–H group, mainly related to variations of the water content
342 and/or distribution (Shenk et al., 2008). Similar results were previously reported in chicken breast
343 samples by Liu and collaborators according to whom some variation of the C–H, O–H, and N–H
344 absorption intensity ad different wavelengths can be observed after the irradiation treatment (Liu et
345 al., 2003).

346 Similarly, the most influent variables to the separation of 0 from 0.5 kGy irradiated samples (VIP
347 value ≥ 2) were found to be before the 1240 nm region and at 1705 and 1780 nm (first and second
348 overtone of C–H stretching vibrations), but also around 1840 (absorptions of proteic molecules) and
349 at 1790 nm (O–H combination vibrations of water) (see Fig. 6C) (Williams & Norris, 1987).

350 The results achieved in the present work may be explained by the ability of irradiation to accelerate
351 the lipid and protein oxidation of meat during storage (Feng, Jo, Nam, Ahn, 2019). However, to the
352 best of our knowledge, only Jensen, Christensen, & Engelsen (2004) reported the application of NIR
353 spectroscopy to predict the oxidation status in pork. In particular, these authors observed changes in
354 the absorbance levels for the oxidized pork scratchings with respect the fresh samples in the spectral
355 region 1400–2250 nm; moreover, first and second overtone of C–H and the combination bands above
356 1800 nm, i.e. changes in the fat fraction, played the main contribution in discriminating pork
357 scratchings at different hexanal content. Therefore, the variations of lipids- and proteins-associated

358 absorption bands are the key for the interpretation of the results obtained. The C-H stretching regions
359 was already reported to be as the most important for discrimination of irradiated and non-irradiated
360 food and of foodstuff according to irradiation dose, both in the MIR (Kizil et al., 2002; Reid,
361 Woodcock, O'Donnell, Kelly, & Downey, 2005) and in the NIR regions (Reid et al., 2005). In
362 particular, a lower intensity of the lipid bands associated to stretching vibrations of the C-H groups,
363 as well as decreases and shifts of the protein absorption bands were found in the MIR spectra of
364 hazelnut samples after high-dose irradiation treatments, and these variations were associated to
365 oxidation of lipids on one hand and structural modifications of proteins on the other (Dogan et al.,
366 2007). At the same time, the stretching vibration of the C=O group of fats was reported to be
367 increased after irradiation as a consequence of the generation of the new radiolytic product 2-
368 dodecylcyclobutanone (Badr, 2012) or the polymerization of radiolytic products from lipids (Dogan
369 et al., 2007). However, in the present work wavelengths associated to the C=O groups were not found
370 to be significant for the discrimination of irradiated and non-irradiated salami samples, probably
371 because they were masked by high spectral complexity of the salami samples investigated.

372 **4. Conclusions**

373 A comprehensive analysis that allows the detection of violations of the provisions with regard to a
374 prohibited use of technological processes such as irradiation is of urgent need in food control.
375 The present study demonstrates the feasibility of a simple and rapid detection of dry fermented
376 sausages treated at 0,5 - 3 kGy irradiation doses by using NIR spectroscopy combined with
377 chemometric analysis. Supervised and unsupervised classification models were built. Although a
378 preliminary classification performed by PCA analysis was not satisfying enough, results of OPLS-DA
379 showed a clear discrimination of 100% sample by irradiation treatment. To interpret discriminant
380 information, the VIP index was additionally used. Spectral bands associated with secondary radiolytic
381 products from fatty acids absorption were found to be significant towards a sample discrimination.
382 Therefore, the excellent ability of the models to discriminate between irradiated and non-irradiated
383 samples provides high potential with regard to the identity verification of food in view of

384 comprehensive food control and, compared to the traditional methodologies of food analysis, offers
385 the advantages of rapidity and the ease of use in routine operations on a large-scale.
386 Nevertheless, applications based on the fingerprinting approaches present still some limitations to be
387 overcome prior to the potential implementation of these protocols in food control analyses, of which
388 the development of standardized operating procedures, the establishment of joint comprehensive
389 databases, and the establishment of quality assurance measures represents the principal ones.

390 **Declaration of interest**

391 None.

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398 treatment.

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408 **Table 1**

409 Summary of cross-validation statistics computed for each OPLS-DA models

OPLS-DA Model	CV statistics						$p_{CV-ANOVA}^*$	Permutation test**	
	NC	R^2X_{cum}	R^2Y_{cum}	Q^2_{cum}	RMSECV	RMSEE		$R^2 y$ -Intercept	$Q^2 y$ -Intercept
C vs. T	1 + 4	0.647	0.891	0.487	0.305	0.151	1.62e-07	0.30	-0.41
0 vs. 0.5 vs. 1 vs. 2 vs. 3	4 + 8	0.708	0.883	0.516	0.227	0.106	1.85e-12	0.35	-0.51
0 vs. 0.5	1 + 5	0.662	0.954	0.531	0.198	0.079	1.24e-22	0.22	-0.58

410 NC = total number of predictive + orthogonal OPLS components. R^2X_{cum} = cumulative modelled variability. R^2Y_{cum} =
411 cumulative explained variability related to class information. Q^2_{cum} = predictive variability. RMSECV = root mean square
412 error of cross-validation. RMSEE = root mean square error of estimation. $p_{CV-ANOVA}^*$ = p -value from analysis of variance
413 of the cross-validated residuals at a 95% level of significance. Permutation test** = R^2 and Q^2 y -intercepts values
414 calculated by performing 200 interactions and considered significant when lower than 0.4 and 0.05, respectively.

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427 **Table 2**

428 Classification results obtained from the external validation.

OPLS DA									
Model	C vs. T		0 vs. 0.5 vs. 1 vs. 2 vs. 3					0 vs. 0.5	
Group	C	T	0	0.5	1	2	3	0	0.5
Classification rate	100.0% (15/15)	100.0% (12/12)	46.7.0% (7/15)	41.7% (5/12)	100.0% (12/12)	91.7% (11/12)	100.0% (12/12)	100.0% (15/15)	83.3% (10/12)
RMSEP	0.211		0.364					0.263	
Total classification rate	100.0%		74.6%					92.6%	
<i>p</i> -value *	6.9e-05		5.5e-10					8.4e-09	

429 C = control samples. T = treated (irradiated) samples. RMSEP = root mean square error of prediction. 0, 0.5, 1, 2, 3 =
430 different dosed of irradiation (kGy) delivered to samples. *p*-value* = assessed by Fisher's exact test at 95% level of
431 significance.

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626 **Figure Captions**

627 **Fig. 1.** Raw (A) and SNV-SD-SG pre-processed (B) NIR spectra of n=210 non-irradiated and
628 irradiated dry fermented sausage samples.

629 **Fig. 2** Bi-dimensional score plot of PCA analysis performed on the whole dataset, where distribution
630 of non-irradiated samples (0 KGy) and samples irradiated at different doses (0.5, 1, 2, and 3 KGy) is
631 highlighted. The ellipse corresponds to the 95% confidence level for Hotelling's T^2 ; 0 KGy samples
632 (5-point stars); 0.5 KGy (reverse triangles); 1 KGy samples (triangles); 2 KGy samples (squares); 3
633 KGy samples (circles).

634 **Fig. 3.** OPLS-DA score scatter plot discriminating control (C) from treated (T) sample. The ellipse
635 corresponds to the 95% confidence level for Hotelling's T^2 . C samples (5-point stars); T samples
636 (pentagons).

637 **Fig. 4.** OPLS-DA score scatter plot related to samples discrimination according to the growing tested
638 irradiation doses. The ellipse corresponds to the 95% confidence level for Hotelling's T^2 . 0 kGy
639 samples (5-point stars) 0.5 kGy (reverse triangles), 1 kGy samples (triangles), 2 kGy samples
640 (squares); 3 kGy samples (circles).

641 **Fig. 5.** OPLS-DA score scatter plot for the discrimination between control (0 kGy) and lowest-dose
642 irradiated (0.5 kGy) samples. The ellipse corresponds to the 95% confidence level for Hotelling's T^2 .
643 0 kGy samples (5-point stars); 0.5 kGy samples (reverse triangles).

644 **Fig. 6.** VIP plots for predictive components of the OLPS-DA models, showing the most influential
645 variables to the discrimination among: control (C) and treated (T) samples (**Fig. 6A**); samples at
646 growing irradiation doses of 0 kGy, 0.5 kGy, 1 kGy, 2 kGy, and 3 kGy (**Fig. 6B**); control (0 kGy) and
647 lowest-dose irradiated (0.5 kGy) samples (**Fig. 6C**). VIP values ≥ 1 were considered significant.