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Additional Information

1 **Non-destructive determination of fat content in green hams using ultrasound and**
2 **X-Rays**

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13

14 **ABSTRACT**

15 This work addresses the use of ultrasound (US) and **medical dual energy X-ray**
16 **absorptiometry methods** to predict the fat content in green pork hams. Ultrasonic
17 velocity (v) and X-Ray absorption were measured in 78 green hams. An increase in the
18 fat content involved an increase in v and a decrease in the X-Ray attenuation
19 measured **at 2 °C**. Models developed to predict the fat content from the ultrasonic
20 velocity or X-Ray parameters provided errors of 2.97% and 4.65%, respectively. The
21 combination of both US and X-Ray technologies did not improve prediction accuracy.
22 These models allowed green hams to be classification into three levels of fatness, with
23 88.5% and 65.4% of the hams correctly classified when using models based on
24 ultrasonic and X-Ray parameters, respectively. Therefore, US and X-Rays emerge as
25 useful quality control technologies with which to estimate the fat content in green pork
26 hams.

27

28 **Keywords:** Non-destructive analysis; Green ham; Meat products; Ultrasound; X-Rays

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34 1. INTRODUCTION

35 The total fat content of green hams is a key issue, since it affects the processing of
36 both cooked and dry-cured hams. In cooked hams, intramuscular fat can affect the
37 binding strength and consumer acceptability. In dry-cured hams, the fat content has a
38 great influence on the salt uptake during the salting process (Cierach, & Modzelewska-
39 Kapitula, 2011) and on the weight losses during drying (Čandek-Potokar, & Škrlep,
40 2012; Garcia-Gil et al., 2012). The development of online non-invasive technologies as
41 a means of predicting the fat content in green hams is of special interest for the meat
42 industry, since they would make it possible to classify the product into different fat
43 categories which would allow the elaboration processes to be optimized. **These**
44 **techniques need to be robust and cost-effective for being used in the industry.**

45 New techniques are being **tested for carrying out** the non-destructive determination of
46 the composition of the meat products. **For live animals and carcass inspection, reliable**
47 **ultrasonic devices are available for the measurement of lean and fat content (Miles,**
48 **Fisher, Fursey, & Page, 1987; Miles, Fursey, Page & Fisher, 1990), as well as the**
49 **depth of subcutaneous fat, in particular sites of the animal. Miles & Fursey (1977)**
50 **related the ultrasonic velocity to the fat content of meat muscles, comminuted tissue,**
51 **meat mixtures and dehydrated muscles.** In this regard, Koch et al. (2011a) estimated
52 the intramuscular fat content of porcine *Longissimus dorsi* muscle by using ultrasound
53 velocity and attenuation. Corona, García-Pérez, Ventanas, and Benedito (2014) and
54 Benedito, Carcel, Rosello, and Mulet (2001) have also used ultrasound to determine
55 the composition of a formulated dry-cured **pork** meat product (sausage) and raw **pork**
56 meat mixtures, respectively. Most of the aforementioned ultrasonic studies rely on the
57 measurement of the ultrasonic velocity, because it is the simplest and most reliable
58 ultrasonic measurement. However, each ultrasonic measurement provides information
59 on a reduced area of the sample which implies that, if large samples are to be
60 analyzed, multiple measurements are required. Moreover, the results are largely
61 dependent on the temperature **and anisotropy of meat tissues (Miles & Fursey, 1977).**
62 In this regard, other non-destructive techniques, such as X-Rays, do not require a
63 precise temperature control.

64 There are several X-Ray technologies that, based on the differential X-Ray attenuation
65 produced by the different tissue density, permit meat composition to be determined. X-
66 Ray computed tomography has been used to predict the lean/fat content in animal
67 carcasses (Vester-Christensen et al., 2009) and bone-in green hams (Picouet, Muñoz,
68 Fulladosa, Daumas, & Gou, 2014) and to determine the intramuscular fat content of

69 meat (Font-i-Furnols, Brun, Tous, & Gispert, 2013). Brienne, Denoyelle, Baussart, and
70 Daudin (2001) used Medical Dual Energy X-Ray Absorptiometry (DEXA) to predict the
71 fat content in pork meat/fat mixtures and beef muscles. Although a low correlation was
72 observed between the percentage of fat obtained through chemical analyses and the
73 percentage estimated from the Beer-Lambert equation, they proposed different
74 corrections and obtained an improvement. However, corrections are specific for each
75 sample format and DEXA equipment. Mercier et al. (2006) used the ratio between the
76 coefficients of attenuation of the two X-Ray energy levels obtained with a medical
77 DEXA to predict the fat content in legs of lamb carcasses. The predictions
78 underestimated the fat content, probably because dissected fat was used instead of
79 chemically analyzed fat for predictive model development. López-Campos, Larsen,
80 Prieto, Juárez, and Aalhus (2013) reported that DEXA technology may also be useful
81 for the objective estimation of the intramuscular fat content in beef. Nevertheless, the
82 medical devices used in the aforementioned studies are not suitable for working in
83 industrial environments at the required speed. In this sense, other authors
84 demonstrated that non-medical X-Ray instruments also allow the online determination
85 of the salt uptake in whole bone-in hams during the salting procedure (Fulladosa,
86 Muñoz, Serra, Arnau, & Gou, 2014) and the accurate estimation of the fat content of
87 boned and packaged meat trimmings (Hansen et al., 2003).

88 Nevertheless, more research is needed before using ultrasound and DEXA
89 technologies to determine the composition of products in which the fat is not uniformly
90 distributed or that contain bones. Fat content determination in whole pieces, such as
91 green bone-in hams, is still a challenge because among others the presence of bones
92 and the existence of different muscles with a high degree of heterogeneity in terms of
93 their fat content and distribution. Besides, combining the information obtained from
94 acoustic and electromagnetic waves as a means of achieving more accurate
95 predictions is worth investigating. Thus, the aim of the present study was to analyze the
96 ability of ultrasound and DEXA techniques to predict both separately and jointly the fat
97 content of green hams and to determine the feasibility of using them for industrial
98 classification purposes.

99

100 **2. MATERIAL AND METHODS**

101 **2.1 SAMPLES**

102 Thirty nine green hams from 'White' pigs (crosses containing Duroc (CDU) or Large
103 White (CLW)), average weight 11.1 ± 0.8 kg, and 39 green hams from 'Iberian' pigs

104 (crosses containing at least 50% Iberian breed (CIB)), average weight 10.6 ± 1.2 kg,
105 were purchased in 2 different slaughterhouses. The hams were taken to the pilot plant
106 in refrigerated storage and kept at 2 ± 2 °C for less than 2 days before the non-
107 destructive measurements were conducted. The different genetic source of the hams
108 allowed for a wide range of fat contents.

109

110 **2.2 ULTRASONIC MEASUREMENTS**

111 A specific device was designed and assembled for ultrasonic measurements; it mainly
112 consisted of a couple of narrow-band ultrasonic transducers (1 MHz, 0.75" crystal
113 diameter, A314S-SU model, Panametrics, Waltham, MA, USA), a pulser-receiver
114 (Model 5058PR, Panametrics, Waltham, MA, USA) and a digital oscilloscope
115 (Tektronix, TDS5034, Digital phosphor oscilloscope. Tektronix inc. Bearverton, OR,
116 USA). A digital height (192-633 Serie, Mitutoyo, Japan) gage was linked to the
117 computer by a RS 232 interface in order to measure the sample thickness (± 0.01 mm)
118 (Figure 1A).

119 The ultrasonic velocity was **calculated** from the time of flight (an average of 3 signal
120 acquisitions) and the sample thickness. In order to **assess** the ultrasonic velocity, the
121 system delay was taken into account, which was determined from the pulse transit time
122 measured across a set of methacrylate cylinders of different thicknesses. The delay
123 time was then obtained from the intercept on the y-axis of the time versus thickness
124 graph.

125 The ultrasonic measurements were taken in three zones of the ham (FC, BE and C), as
126 shown in Figure 1A. The number of experimental measurements carried out in each
127 zone depended on the hams' surface and weight. On average, 20 measurements were
128 carried out in the cushion (C) and 5 in the fore cushion (FC) and butt end (BE).
129 Measurements were carried out in triplicate. The hams were kept at 2 ± 2 °C for 24
130 hours before the ultrasonic velocity was measured in place. The ultrasonic velocity in
131 the ham was calculated as the average of the 30 ultrasonic velocities measured in all
132 the ham zones. The average ultrasonic velocity was correlated to the fat content of the
133 green hams.

134

135 **2.3 X-RAY ABSORPTIOMETRY MEASUREMENTS**

136 A commercially available X-Ray inspector model X20V G90 (Multiscan technologies,
137 S.L, Cocentaina, Spain) was used to scan the samples **at 2 °C**. X-Rays were emitted

138 from below the samples and the transmitted X-Rays were measured in the upper part
 139 of the equipment while a conveyor belt moves the sample through at 0.33 m s^{-1} (Figure
 140 1B). The device uses low-energy X-Rays to obtain images (matrixes of values, $4000 \times$
 141 1280 pixels) of the scanned object in the horizontal plane. Samples were scanned at
 142 three different voltages and intensities, specifically 90 kV and 4 mA , 70 kV and 8 mA
 143 and 50 kV and 15 mA , in exactly the same position and location in order to combine the
 144 information obtained from the three matrixes of values. Matrixes of attenuation values
 145 were imported and analyzed using a specific Matlab code (MATLAB, Ver. 7.7.0, The
 146 Mathworks Inc., Natick, MA, USA).

147 The global X-Ray attenuation value (A) for each sample and used energy was obtained
 148 by the following equation:

$$149 \quad A = -\ln \cdot \frac{\sum I_{(i,j)}}{\sum I_{o(i,j)}} \quad (\text{Eq. 1})$$

150 Where I is the of the radiation transmitted through each pixel of the matrix (i,j) ; I_o is the
 151 energy of the incident radiation to each pixel of the matrix (i,j) ; i ranges from 1 to 4000
 152 and j ranges from 1 to 1280 . Therefore, attenuation values for measurements carried
 153 out at 50 , 70 and 90 kV were obtained (A_{50} , A_{70} and A_{90}).

154 According to the Beer-Lambert law, X-Ray attenuation is proportional to the thickness
 155 and composition of the sample (n components):

$$156 \quad A = L \cdot \sum_{i=1}^n \epsilon_i \cdot c_i = \frac{L}{V} \cdot \sum_{i=1}^n \epsilon_i \cdot M_i \quad (\text{Eq. 2})$$

157 Where L is the sample thickness (m), V is the sample volume (m^3), ϵ_i is the absorptivity
 158 coefficient of component i ($\text{m}^2 \text{ kg}^{-1}$), which is dependent on the X-Ray energy, and c_i
 159 and M_i are the concentration (kg m^{-3}) and the mass (kg) of absorbing component i ,
 160 respectively.

161 Eq (2) can be converted into Eq (3) by dividing by the ham weight (M_t):

$$162 \quad \frac{A \cdot V}{L \cdot M_t} = \sum_{i=1}^n \epsilon_i \cdot X_i \quad (\text{Eq. 3})$$

163 Where X_i is the mass fraction of component i .

164 Since hams do not have a uniform thickness, an average thickness was estimated as
 165 the ratio between V and the sample surface in the scan (S). Then, a new parameter
 166 (A_T) can be calculated from Eq. (3):

$$167 \quad A_T = \frac{A \cdot S}{M_t} = \sum_{i=1}^n \epsilon_i \cdot X_i \quad (\text{Eq. 4})$$

168 The correlation between A_T , obtained at different voltages (A_{T50} , A_{T70} and A_{T90}), with the
169 fat content was analyzed.

170

171 **2.4 DISSECTION AND CHEMICAL ANALYSIS**

172 After the ultrasound and X-Ray measurements, the lean and fat tissues for each ham
173 were dissected, weighed and minced together. Afterwards, the fat and moisture
174 contents of the mixture were determined. The moisture was analyzed by drying at
175 103 ± 2 °C until reaching constant weight (ISO 1442, 1997). The total fat content was
176 estimated by near infrared spectroscopy using a FoodScan™ Lab (Foss Analytical,
177 Dinamarca) according to AOAC (2007). All analyses were performed in triplicate. The
178 fat (X_f) and moisture (X_w) contents of the whole hams were calculated by referring the
179 mixture composition to the ham weight.

180

181 **2.5 DEVELOPMENT OF PREDICTIVE MODELS AND STATISTICAL ANALYSIS**

182 The green hams used in this study were split into two sets. The first set (Model
183 Calibration, MC) included 52 hams and was used to develop predictive models using
184 ultrasonic and X-Ray absorptiometry parameters. The rest of the hams (26) were used
185 for model validation (MV set). In order to cover a wide range of fat content in each set
186 of hams (Table 1), they were sorted according to the experimental fat content and for
187 each group of 3 hams, 2 hams were systematically included in the MC set and 1 in the
188 MV set. In addition, the hams of the MV set were divided into 3 groups according to
189 their fat content (low < 14%, medium 14-26% and high > 26% fat content level).

190 Predictive models were established to find single and multiple regression models
191 between the fat content and the ultrasonic and X-Ray variables. For that purpose, the
192 XLSTAT 2009 statistical package (Microsoft Office, Redmond, WA, USA) was used.
193 Regarding the ultrasonic measurements, only the ultrasonic velocity (v) was used
194 because other variables, such as attenuation and the frequency spectrum analysis, did
195 not provide relevant information. For X-Ray measurements, A_T values obtained at
196 different energies were used. The combination of US and X-Ray parameters was also
197 investigated. In this case, the independent variables of the model were selected by the
198 Stepwise method, the levels of significance to enter and keep the dependent variables
199 in the model being $p=0.05$ and $p=0.1$, respectively. The reliability of the predictive
200 models was given by the coefficient of determination (R^2) and the Root Mean Square

201 Error of Calibration (RMSEC). For the validation data set, the Root Mean Square Error
202 of Validation (RMSEV) was also calculated.

203

204 **3. RESULTS AND DISCUSSION**

205 **3.1 CHEMICAL COMPOSITION**

206 The chemical composition of the green hams used in this study is shown in Table 1.
207 The fat and moisture content ranged between 6.5-41.0% w.b. and 39.9-70.2% w.b.,
208 respectively. These ranges of fat and moisture contents cover the fat and moisture
209 contents of the majority of hams usually found on the market (Serra, & Fulladosa,
210 2011; Blasco et al., 1994).

211

212 **3.2 INFLUENCE OF FAT ON ULTRASONIC VELOCITY**

213 Figure 2 (A and B) shows the relationship between the ultrasonic velocity (v) and the
214 fat (X_f) and moisture contents (X_w) in the 78 green hams analyzed. It should be
215 highlighted that the v reported in each point of Figure 2 is the average ultrasonic
216 velocity of a whole ham (30 measurements distributed in the three zones, Figure 1A),
217 as explained in section 2.2. There is great experimental variation in the ultrasonic
218 response to differences in moisture and fat content, which is especially noticeable for
219 fat contents between 20 and 28% w.b. (Figure 2). This general variability could be
220 linked to the highly heterogeneous nature of the ham, which is a piece made up of
221 subcutaneous fat and different muscles, also containing a heterogeneous distribution of
222 intramuscular fat and connective tissue. In addition, the breed of the pig and feeding
223 system could significantly modify the v in the fatty fraction and affect the protein
224 content in the lean tissue, which would also determine the v in the muscles (Niñoles,
225 Mulet, Ventanas, & Benedito, 2011; Niñoles, Sanjuan, Ventanas, & Benedito, 2008).

226 As can be observed in Figure 2A, an increase in the fat content involved an increase in
227 the v measured at 2 °C. Thus, on average, an increase in the fat content of 5%
228 corresponded to an increase of 8.4 m s⁻¹ in the v . This result is explained by
229 considering that, at low temperatures, the ultrasonic velocity in the fatty tissue is higher
230 than in lean tissue. This fact has been previously reported by Benedito et al. (2001),
231 who found an ultrasonic velocity of 1610.0-1620.0 m s⁻¹ in fatty **pork** tissues and
232 1530.0-1555.0 m s⁻¹ in lean **pork** tissues at 4 °C. **Similarly, Miles & Fursey (1977)**
233 **reported ultrasonic velocities at 4 °C in intact beef muscles of around 1530 m/s and**
234 **significantly higher (1650 m/s) for beef adipose tissue.** The ultrasonic velocity in fatty

235 tissue is so high at this temperature because it depends on the solid/liquid ratio which
236 affects its textural properties; consequently, as the state of the fat at low temperatures
237 is mainly solid, in which ultrasound propagates faster, the v reaches its highest values.
238 In contrast, the ultrasonic velocity in lean tissue is lower because the main component
239 in raw meat is water and the ultrasonic velocity in water at 2 °C is 1412.8 m s⁻¹ (Kinsler,
240 Frey, Coppens, & Sanders, 1982). The ultrasonic velocity in the whole ham is lower
241 (1531.1-1586.9 m s⁻¹, Figure 2A) than in the fatty tissue because it is greatly influenced
242 by the water content of the lean tissue.

243 It should be **emphasized** that the influence of the fat content on the v in ham is highly
244 temperature dependent. In this regard, the v in pure fat decreases with the rise in
245 temperature (McClements, & Povey, 1992). This fact has also been observed in
246 different meat products, where velocity was measured at between 2 and 38 °C (Corona
247 et al., 2014; Koch et al., 2011b; Niñoles et al., 2008; Chanamai, & McClements, 1999),
248 the reduction in velocity being mainly ascribed to the fat melting as the temperature
249 rises. The temperature used (2 °C) is appropriate for fat content assessment, since
250 there is a remarkable difference between the v in the fatty and lean tissues. As the
251 temperature increases, the ultrasonic velocity in fat falls and that of lean tissue goes
252 up, leading to similar v values for both tissues, which hinders the fat content estimation.

253 The moisture content was found to have the opposite effect on v to that reported in the
254 case of fat (Figure 2B). Thus, in average terms, an increase in the moisture content of
255 the green ham of 5% corresponded to a decrease of 9.6 m s⁻¹ in v . As previously
256 mentioned, the v in water is lower than the velocity in the other components of ham (fat
257 and protein+others) (Benedito et al., 2001); therefore, as the water content increases,
258 the v in the ham decreases. The influence of the moisture content on v has also been
259 reported in the curing process of *Biceps femoris* and *Longissimus dorsi* muscles and
260 sobrassada (a dry-cured minced meat product), where the v increased due to the
261 dehydration (Niñoles, 2007; LLull, Simal, Benedito, & Roselló, 2002). Likewise, Koch et
262 al. (2011a) indicated that the water loss in thawed *Longissimus dorsi* muscle entailed
263 an increase in v .

264 The water and fat contents of hams have the opposite effect on the v and, at the same
265 time, they show a high negative correlation in fresh hams (non-dried hams). Therefore,
266 it is expected that, although both fat and water affect v , there will be a relationship
267 between the v and each component. For a low correlation between fat and water
268 contents, the influence of both water and fat contents on the v should be assessed.

270 3.3 INFLUENCE OF FAT ON X-RAY ABSORPTIOMETRY PARAMETERS

271 Figure 3A shows the relationship between X-Ray attenuation values (A) obtained at
 272 different energies and the measured fat content of the hams. There was an increase in
 273 A values when the X-Ray energy decreased. This fact is linked to the greater
 274 absorption phenomena which exist at low energies than at high ones (Kalender, 2005).
 275 Whatever the energy considered, an increase in the percentage of fat content involved
 276 a decrease in A. Non-significant differences in the slope of the A vs X_f were detected
 277 ($p>0.05$), due to the large experimental variation of attenuation not explained by the fat
 278 content. It has been described that X-Ray attenuation at low energies is dependent on
 279 both fat content and the product thickness (Hansen et al., 2003), which is not constant
 280 in hams. The variation in ham weight could also increase the variation in A values.

281 A_T is proportional to the attenuation (A) and to the ratio between sample surface in the
 282 scan (S) and the ham weight (M_t) (Eq. 4). This ratio is related to the composition but
 283 also to the shape of the ham. As shown in Figure 3A, although a drop in the fat content
 284 produces an increase in A, it simultaneously increases the density and consequently,
 285 for a constant sample surface in the scan, it decreases the ratio S/M_t . Therefore, a
 286 decrease in the fat content has an opposite effect on the two factors of Eq. (4), and the
 287 resulting effect on A_T is unknown. In the present study, A_T was found to be positively
 288 correlated to the fat content at the three different voltages and intensities studied,
 289 specifically 90 kV and 4 mA ($R^2=0.57$), 70 kV and 8 mA ($R^2=0.53$) and 50 kV and 15
 290 mA ($R^2=0.34$) (see Figure 3B).

291

292 3.4 PREDICTIVE MODELS

293 A linear model was established for MC set between the fat content and the ultrasonic
 294 velocity (Eq. 5); the RMSEC being 2.90% (Table 2) and $R^2=0.89$ (Eq. 5). This could be
 295 considered a robust model because very different samples were used in the study.

$$296 \quad X_f (\% \text{ w. b.}) = 0.54 \cdot v - 821.99 \quad (\text{Eq. 5})$$

297 Where X_f is the fat content and v is the ultrasonic velocity. The slope of Eq. (5)
 298 indicates that an increase of 1 m s^{-1} in the ultrasonic velocity led to an increase of
 299 0.54% in the fat content.

300 Miles & Fursey (1977), using the reciprocal of the squared ultrasonic velocity ($1/v^2$) at 0
 301 °C, reported less satisfactory predictive models of fatness for comminuted beef
 302 muscles ($R^2<0.536$). These authors assessed the fat composition in meat muscles and

303 mixtures of lean and fatty tissues. However, in the present work the fat content
304 assessment is conducted on a much complex medium (whole bone-in ham), which
305 includes different types of muscles, connective tissue and subcutaneous fat, which
306 highlights the relevance of the results for implementing quality control systems in the
307 meat industry. Miles & Fursey (1977) reported that the best temperature for conducting
308 the ultrasonic measurements was 37°C, however in the present work the temperature
309 chosen was 2°C since it is the most commonly one used for refrigeration of green
310 hams prior to classification and processing. When analysing fresh pork *Biceps femoris*
311 at 0 °C, Niñoles et al. (2011) found that an increase of 1 m s⁻¹ in the ultrasonic velocity
312 implied an intramuscular fat content increase of 0.34%. The different coefficient value
313 found by Niñoles et al. (2011) (0.34 compared to 0.54 of the present work) could be
314 due to the great experimental variability (R²=0.59) found by these authors, which
315 greatly increases the standard error of the estimated coefficient. However, Park,
316 Whittaker, Miller, and Hale (1994) suggested that the increase of 1 m s⁻¹ in the
317 ultrasonic velocity measured at 22 °C led to a reduction of 0.21% in the fat content of
318 *Longissimus dorsi* beef muscle, which may be explained by considering the fact that
319 the fat melts at high temperatures.

320 A multiple regression analysis was performed to study the relationship between the fat
321 and moisture contents and the ultrasonic velocity. The analyses detected a severe
322 collinearity between both variables (the fat and moisture contents), due to the VIF
323 being higher than 5 (VIF_{f-w}=8.2), caused by the inherent relationship between the fat
324 and moisture contents in the green hams. Therefore, including the moisture content in
325 the model does not lead to a better explanation of the experimental variability observed
326 in the ultrasonic velocity.

327 The fat content was also predicted by means of X-Ray parameters (A_T) at three
328 different energies. The predictive model (Eq. 6) showed a RMSEC of 4.20% and a R²
329 of 0.80.

330
$$X_f (\% \text{ w .b.}) = - 279.643 - 0.00473 \cdot A_{T50} + 0.00806 \cdot A_{T70} - 0.00103 \cdot A_{T90} \text{ (Eq. 6)}$$

331 Predictive errors were high in comparison to what occurs using technologies in which
332 the thickness of the sample is not critical, such as the ham grading system based on
333 electromagnetic induction measurements (Serra, & Fulladosa, 2011), or in technologies
334 in which thickness determination is inherent to the measurement, such as in the case
335 of US. There are only slight X-Ray attenuation differences between fatty and lean
336 tissues and a more accurate thickness correction (including a laser volume sensor)
337 could help to obtain better models.

338 The use of hams from different animal breeds increases the robustness of the models,
339 but may also have an adverse effect on the predictive errors. Figure 4 shows the
340 typical geometry of scanned ham surfaces from different crossbreeds. CLW hams
341 exhibited a different shape from CDU and CIB hams. In Eq. (4), an average thickness
342 was used instead of the real thickness. The error of this approximation may depend on
343 the dimensional conformation of the hams. Therefore, new models were developed by
344 discarding the CLW hams and, thus, considering only the hams with a similar
345 geometry. From this approach, the errors (RMSEC=2.23%) were smaller than the ones
346 obtained using all the hams (RMSEC=4.20%), pointing to the importance of the
347 homogeneous conformation of the hams. In contrast, since the geometry is not
348 important in US technology, the error is similar (3.02% vs 2.90%) when using ν .

349 When using all the hams, the stepwise regression analysis including both the US and
350 X-Ray parameters, showed that the parameter which provided the most relevant
351 information for fat content prediction (Table 2) was the ν . The addition of X-Ray
352 parameters to the model did not decrease the prediction error. In contrast, when
353 discarding CLW hams, the most relevant information is provided by A_{T50} and A_{T70} and ν
354 is not included in the model.

355

356 3.5 VALIDATION AND CLASSIFICATION TESTS

357 Figure 5 depicts the relationship between the fat contents measured and predicted
358 using ultrasound (A, Eq. 5, $R^2=0.90$) and X-Ray (B, Eq. 6, $R^2=0.67$) models (Table 2).
359 RMSEV were 2.97% and 4.65% for ultrasound and X-Rays, respectively, both
360 providing a reliable, non-destructive measurement of the fat content of green hams
361 over a wide range of fat content (from 6.5 to 41.0% w.b.). The number of validation
362 errors decreases when CLW hams are excluded from the model for X-Rays
363 (RMSEV=3.27%). Miles et al. (1987) reported standard deviations of the residuals of
364 around 1.85 for the ultrasonic estimation of the fat content in specific sites of the beef
365 carcass. In other studies, the ultrasonic velocity has been used to estimate the fat
366 content of green meat mixtures and fish (Benedito et al., 2001; Ghaedian, Coupland,
367 Decker, & McClements, 1998) obtaining a better correlation than in the present study,
368 ($R^2=0.99$, in both cases). In all likelihood, this fact could be explained by considering
369 that highly homogeneous samples were tested in the former studies. The green hams
370 used in the present study, however, are heterogeneous; this is due to several factors,
371 the fat distribution within the samples, the connective tissue characteristics, the
372 different moisture and fat profiles and the existing bones and skin, among others.

373 In order to evaluate the feasibility of using the ultrasonic and X-Ray models to classify
374 the hams into different categories according to their fat content, the MV set hams
375 (Table 1) were classified into three groups: low (<14%), medium (between 14 and
376 26%) and high (>26%) fat content levels (Table 3). Once the estimated fat content was
377 calculated from Eq. (5) and (6) and compared with the measured one, the classification
378 performance was assessed. In average terms, whereas the ultrasonic model classified
379 88.5% correctly, the X-Ray model only classified 65.4% of the MV ham set (Table 3).
380 The ultrasonic model was able to correctly classify 87.5 and 100.0% of the ham pieces,
381 in the low and high fat content groups, respectively. However, for a medium fat content,
382 the percentage of correctly classified hams was reduced to 75.0% (Table 3). In
383 contrast, the X-Ray model provided similar percentages for every category.

384 Thus, US could better classify hams into different groups of fatness, which would be
385 highly relevant for industrial quality control purposes. It would be necessary to develop
386 a prototype which permits a rapid measurement before implementing this technology
387 industrially as a means of easily and rapidly sorting and processing the raw material
388 according to the fat content. X-Rays could also be useful, especially if a specific
389 calibration is developed for each kind of raw material in order to overcome the
390 variability produced by the different conformation of the hams. In this case, the device
391 is already suitable for industrial conditions and works at production speed.

392 As previously explained, it is not worth combining X-Ray and US sensors together in an
393 instrument because it does not offer a significant improvement and it would increase
394 the cost of the device.

395

396 **4. CONCLUSIONS**

397 Ultrasound velocity and X-Ray attenuation are influenced by the composition of the
398 hams, allowing predictive models to be developed for the fat content with errors of
399 2.97% and 4.65% for US and X-Ray, respectively, when all the hams are used. When
400 discarding hams with a different geometry (CLW hams), the X-Ray predictive error
401 improved, decreasing to 3.27%. Nevertheless, in no case did the combination of
402 parameters obtained from both technologies improve the prediction accuracy. These
403 predictive models permitted a satisfactory classification of the hams into three fat levels
404 (<14, 14-26 and >26% fat content), demonstrating the feasibility of these non-
405 destructive techniques for ham classification purposes. Research should be conducted
406 in order to include accurate sample geometry corrections in the X-Ray technique and to
407 develop fast ultrasonic devices to be used online.

408

409 **ACKNOWLEDGEMENTS**

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412

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506

507 **Figure 1.** The experimental set-up used in the ultrasonic **(A)** and X-Ray **(B)**
508 measurements and the location of ultrasonic measurement zones. C. Cushion, FC.
509 Fore cushion and BE. Butt end.

510 **Figure 2.** Relationship between the ultrasonic velocity and the fat (X_f) **(A)** and moisture
511 contents (X_w) **(B)** of raw hams.

512 **Figure 3.** Relationship between X-Ray parameters, A **(A)** and A_T **(B)**, obtained at
513 different X-Ray energies (50, 70 and 90 kV) and the fat content (X_f) of raw hams.

514 **Figure 4.** Scanned surface of hams from crosses containing Large white and Landrace
515 (A), Duroc (B) or Iberian (C) breeds.

516 **Figure 5.** Validation of the predictive model for the estimation of the fat content (X_f) of
517 raw hams based on ultrasonic (A) and X-Ray absorptiometry (B) measurements.

518

519

Figure 1

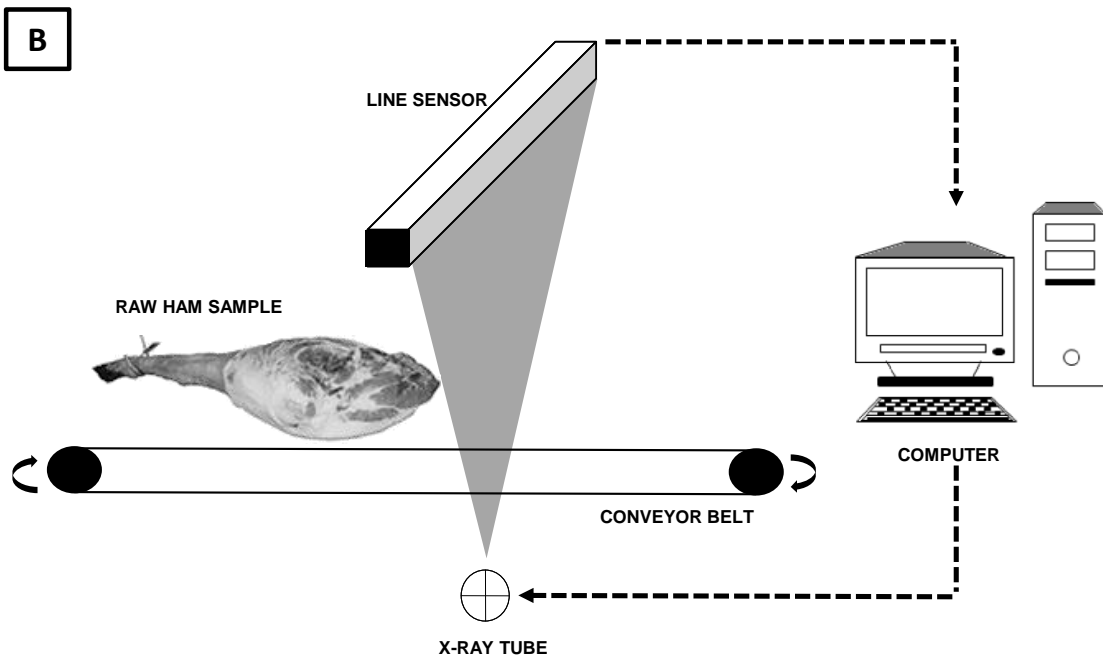
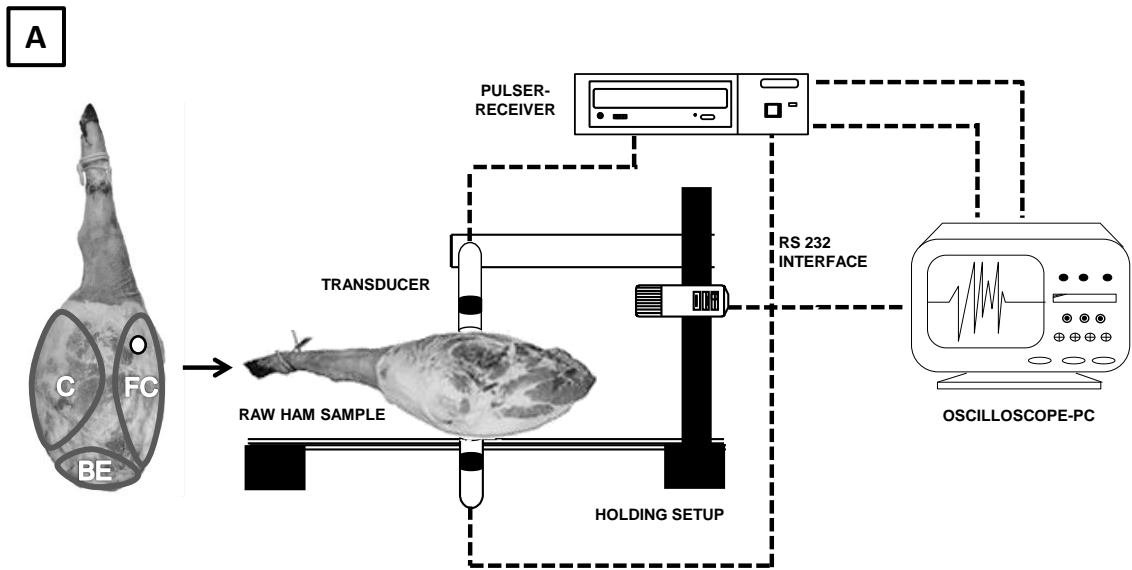


Figure 2

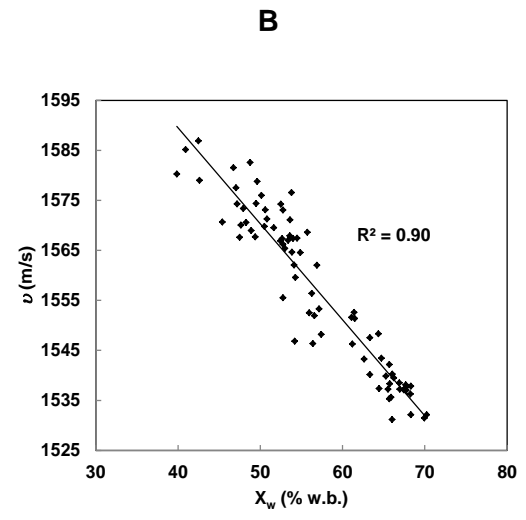
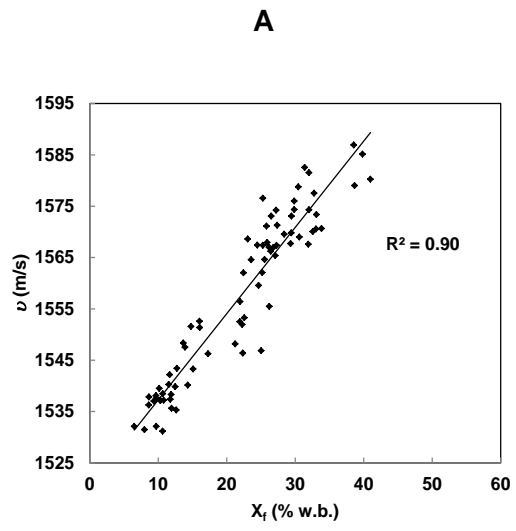


Figure 3

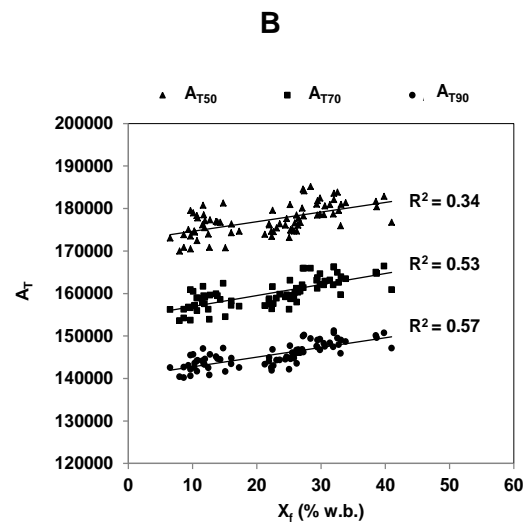
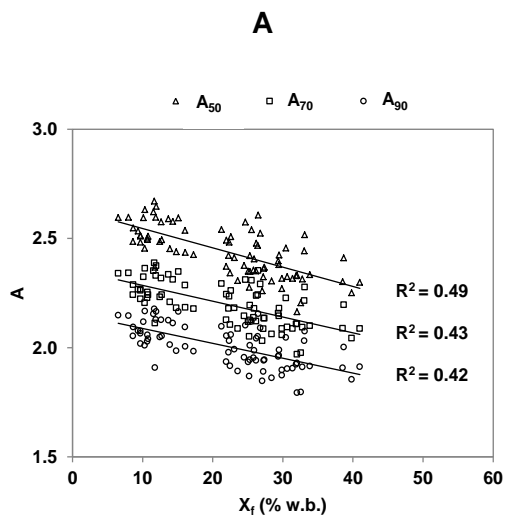


Figure 4

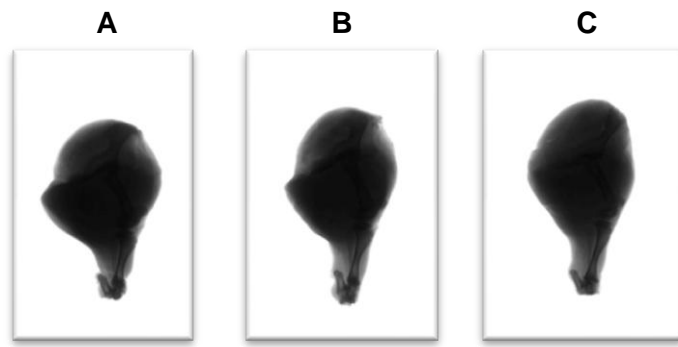
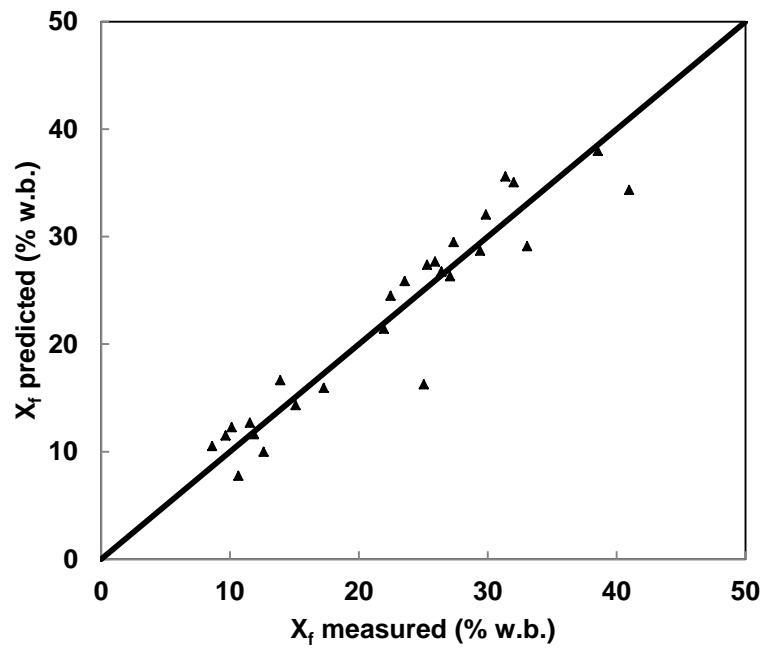


Figure 5

A



B

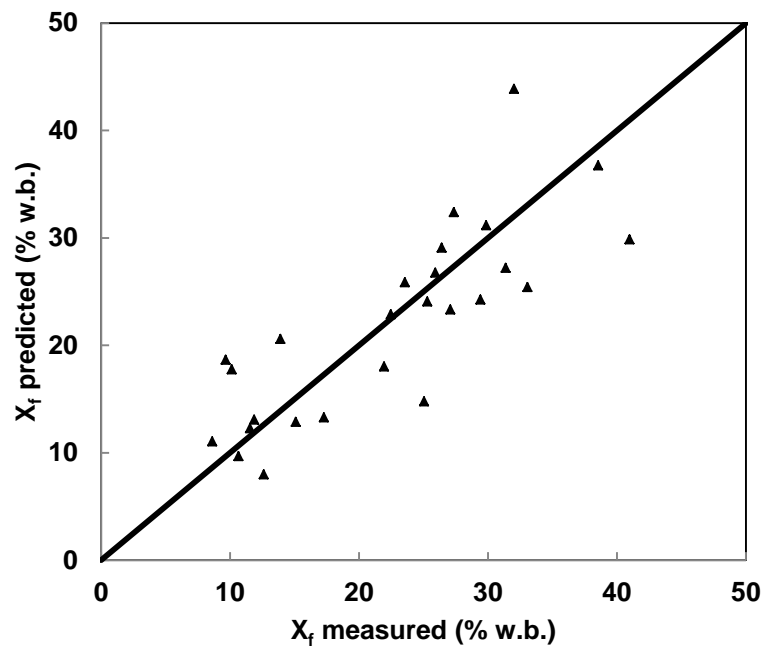


Table 1. Average, minimum and maximum values of moisture (X_w % w.b.) and fat (X_f % w.b.) contents for validation and calibration ham sets.

	n	X_w (% w.b.)			X_f (% w.b.)		
		MEAN	MIN	MAX	MEAN	MIN	MAX
CALIBRATION (MC)	52	57.0	40.9	70.2	21.7	6.5	39.8
VALIDATION (MV)	26	56.4	39.9	68.3	22.4	8.6	41.0

Table 2. Parameters of predictive models for fat content of raw hams using X-Ray and ultrasound measurements.

Crossbreeds used	Technology	MODEL VARIABLES	RMSEC(%)	R ²	RMSEV(%)
CLW, CDU, CIB	US	ν	2.90	0.89	2.97
CLW, CDU, CIB	X-Rays	$A_{T50}, A_{T70}, A_{T90}$	4.20	0.80	4.65
CDU, CIB	US	ν	3.02	0.59	3.29
CDU, CIB	X-Rays	$A_{T50}, A_{T70}, A_{T90}$	2.23	0.79	3.27

Table 3. Classification of raw hams according to the fat content (X_f) (low $X_f < 14\%$, medium $14 \leq X_f \leq 26\%$ and high $X_f > 26\%$) by using the predictive model based on ultrasonic and X-Ray measurements.

FAT LEVEL	X_f (% w.b.)	% CLASSIFICATION	
		US	X-Rays
LOW	<14	87.5	70.0
MEDIUM	14-26	75.0	62.5
HIGH	>26	100.0	75.0
TOTAL		88.5	65.4