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Ultrasound parameters used to characterize Iberian fresh pork loins of different feeding systems

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Abstract:	<p><p class="LO-Normal" style="text-align:justify;text-justify:inter-ideograph; line-height:200%">The objective of this work was to characterize different commercial categories of pork loin non-destructively by inspection using low-intensity ultrasound at different frequencies. To this end, such ultrasound parameters as the ultrasound pulse velocity (UPV) and variables related to the attenuation and to the frequency components obtained from the fast Fourier transform (FFT) were considered. The acoustic parameters and those obtained using traditional instrumental analyses (physicochemical and texture) were subjected to a Pearson correlation analysis. Several ultrasound parameters allowed the two categories explored to be distinguished. Also, the acoustic determinations yielded many significant correlations with the rest of the parameters studied. The results show that ultrasound can be postulated as an instrument for characterizing and distinguishing samples of different categories of loin, and that it has a promising predictive character.</p></p>

08/22/2021

Dear Editor Ferruh Erdogdu,

Thank you for appreciating the opportunity to resubmit our work for publication. Please find attached the file 'Detailed Response to Reviewers'. We hope the present version is suitable for publication in *Journal of Food Engineering*.

Yours sincerely,

Alberto González-Mohino Jiménez

PhD Food Science

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Reviewer #3

I recommend accept the manuscript.

- Thank you so much for your decision. We are pleased that our responses were satisfactory.

Reviewer #4

This is an interesting but not strikingly novel, well written short study.

However, a total of 8 loins, 4 of each type, is not enough for any degree of statistical confidence for a paper to be published in a recognised Peer Reviewed Journal. I find the authors explanation regarding problems with the cost of experimental material hard to believe.

Whole pork tenderloins at my local retail outlet cost under 6 euro per loin. So under 50 euro for materials. I would expect the authors to have good contacts with pork abattoirs and local suppliers and obtain the meat at wholesale cost or even free.

10 times the current number of samples would give a reasonable distribution to base confidence limits on and cost under 500 euro.

- Firstly, thank you for your response. Considering previous Peer-Reviewed papers about ultrasound inspection in meat products (de Prados et al. 2015 DOI - <https://doi.org/10.1016/j.jfoodeng.2014.12.024>; Fariñas et al. 2021 DOI - <https://doi.org/10.1016/j.jfoodeng.2021.110481>; Niñoles et al. 2011 DOI - <https://doi.org/10.1016/j.meatsci.2011.04.014>; González-Mohino et al. 2019 DOI - <https://doi.org/10.3813/aaa.919375>), and in other food matrices (Crespo et al. 2022 DOI - <https://doi.org/10.1016/j.foodcont.2021.108405>; González-Mohino et al. 2021 DOI - <https://doi.org/10.1016/j.lwt.2021.110882>; González-Mohino et al. 2019 DOI - <https://doi.org/10.1016/j.ultras.2019.02.010>), the sample size is enough for a robust statistical analysis. Regarding the price of the Iberian pork loin, our comment about the

cost was only a note not a justification, our apologies for the misunderstanding. We have based on these previous works to determine the sample size required; thus, we have considered this sample size for the present study. Likewise, we would like to give more information about the costs of the raw material used. The prices of these kinds of products can vary between regions. The purchased samples were close to 40 cm in length, where each piece loin of 'Cebo de Campo Ibérico' (CCI) cost 20€, while those of 'Bellota 50% Ibérico' (BI) the cost was 31.70€. Likewise, prior to the final ultrasound inspection measurements, the set-up was carried out with one pork loin of each type. Therefore, the total cost was 258.50€, considering only the raw material. The funding obtained came from a regional project of the Junta de Extremadura with reference IB16089. In addition, thank you for your suggestion, we are always looking for new ways and contacts with the meat industry to reduce research costs, and we will consider it in further works.

Highlights

- Different commercial categories of fresh pork loins were evaluated.
- Physicochemical, textural, and ultrasound inspection analyses were considered.
- Various ultrasound parameters have the capacity to distinguish among samples.
- Acoustic parameters were significantly correlated with several determinations.
- Ultrasound is postulated as being a feasible tool for the prediction of physicochemical parameters.

1 **Ultrasound parameters used to characterize Iberian fresh pork**
2 **loins of different feeding systems**

3

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21 **Abstract**

22 The objective of this work was to characterize different commercial categories of pork loin non-
23 destructively by inspection using low-intensity ultrasound at different frequencies. To this end,
24 such ultrasound parameters as the ultrasound pulse velocity (UPV) and variables related to the
25 attenuation and to the frequency components obtained from the fast Fourier transform (FFT) were
26 considered. The acoustic parameters and those obtained using traditional instrumental analyses
27 (physicochemical and texture) were subjected to a Pearson correlation analysis. Several
28 ultrasound parameters allowed the two categories explored to be distinguished. Also, the acoustic
29 determinations yielded many significant correlations with the rest of the parameters studied. The
30 results show that ultrasound can be postulated as an instrument for characterizing and
31 distinguishing samples of different categories of loin, and that it has a promising predictive
32 character.

33

34 **Keywords:** Ultrasound inspection; Pearson correlation; commercial pork loin; physicochemical
35 analysis; instrumental texture.

36

37 **1. Introduction**

38 Most of the traditional analytical methods used to characterize meat products alter the meat itself
39 (Xiong et al., 2017). For this reason, in recent years non-destructive methods have been explored
40 that would allow the quality parameters of meat and meat derivatives to be assessed reliably with
41 the ultimate goal being to put them forward as alternatives and/or complements to traditional
42 methods. Examples are near infrared spectroscopy (NIRS) (Cáceres-Nevado et al., 2021;
43 González-Mohino et al., 2018; Pérez-Palacios et al., 2019), hyperspectral imaging (HSI)
44 (Antequera et al., 2021; Barbin et al., 2013; Pu et al., 2014), and computer vision techniques
45 applied to magnetic resonance imaging (MRI) (Ávila et al., 2019; Caballero et al., 2016; Pérez-
46 Palacios et al., 2017). Among the different non-destructive methods, low-intensity ultrasound
47 inspection has been postulated as effective, fast, and cheaper than most modern instrumental
48 techniques (Gallo et al., 2018). Non-destructive ultrasound inspection has proven reliability for
49 pork products, using the wave propagation speed as a fundamental parameter for the prediction
50 of the quality parameters of meat and meat derivatives. The technique's viability has been verified
51 for raw meat mixtures (Benedito et al., 2001), 'sobrasada' (Llull et al., 2002), and hams (Corona
52 Jimenez et al., 2013). Isolated components of meat products such as the fat and fatty acids have
53 also been characterized using ultrasound (Niñoles et al., 2007), and even to predict the salt content
54 (de Prados et al., 2015). Apart from the speed of propagation, another parameter that some studies
55 have considered is attenuation, which has been applied to studying textural properties in
56 hamburgers (Fariñas et al., 2021) and to determining pastiness in ham (Contreras et al., 2020).

57 Although the calculation of speed and attenuation in the traditional way has been a very efficient
58 method for various works, other information that can be provided by the propagation of ultrasound
59 in the medium has been left unused. The frequency components related to the fast Fourier
60 transform (FFT) and diverse variables related to the attenuation (which are calculated when no
61 echoes are visible in the A-scans obtained in the non-destructive ultrasound inspections) are
62 parameters that have not as yet been considered for the assessment of the quality of meat and meat

63 derivatives. These parameters have, however, been found essential to monitor the curdling process
64 in milk (Jiménez et al., 2017), to characterize dry-cured products (Corona et al., 2013), to control
65 the quality of cheese maturation (Crespo et al., 2020), to distinguish between different varieties
66 of honey (González-Mohino et al., 2019a), and to study the influence of cooking on pork loins
67 (González-Mohino et al., 2019b).

68 Pork is regarded as one of the most important meats in the world because of both the volume of
69 its consumption and consumer preferences (García-Gudiño et al., 2021). The best valued pork
70 products in Spain are those deriving from the Iberian pig. Specifically, consumers consider Iberian
71 pork hams and loins to be high quality products, mainly because of their exceptional sensorial
72 attributes which depend on the characteristics of the raw material and processing conditions
73 (Ventanas et al., 2007).

74 Spanish legislation (BOE, 2014) considers different commercial categories of Iberian pork loins
75 based on the genetics and the feeding system. These categories are identified with different
76 coloured labels: 'white label' (at least 50% Iberian breed, fed on concentrates), 'green label' (at
77 least 50% Iberian, fed on natural resources and concentrates), 'red label' (at least 50% Iberian
78 breed, fed exclusively on natural resources), and 'black label' (100% pure Iberian breed, fed
79 exclusively on natural resources). These categories attain different prices on the market. For this
80 reason, a reliable classification system is necessary to discriminate between them. Until now,
81 there have been no studies characterizing each type of product for rapid, reliable, and non-
82 destructive classification.

83 For this study, on the one hand, physicochemical and instrumental texture analyses were carried
84 out, and on the other, non-destructive ultrasound inspection was performed in which the
85 propagation speed, frequency parameters related to the fast Fourier transform (FFT), and
86 parameters related to the attenuation of the sound waves were determined. It should be

87 emphasized that this work presents for the first time a study designed to characterize and classify
88 categories of Iberian loin based on acoustic parameters.

89 The main objective of the study was to characterize two commercial categories of fresh pork loins
90 by applying new ultrasound parameters for inspection, specifically, the propagation speed,
91 frequency parameters related to the fast Fourier transform (FFT), and parameters related to the
92 attenuation of the sound waves.

93 **2. Material and Methods**

94 **2.1. Samples and experimental design**

95 Two different commercial category pork loins were chosen to carry out the study: ‘Cebo de
96 Campo Ibérico’ (CCI), derived from 50% Iberian × Duroc cross-bred pigs reared extensively,
97 feeding on natural resources and concentrate, and ‘Bellota 50% Ibérico’ (BI), derived from 50%
98 Iberian × Duroc cross-bred pigs reared extensively, feeding exclusively on natural resources. Both
99 pork loin categories were purchased from Montesano Extremadura S.A. (Badajoz, Spain).

100 Four pieces of pork loin were acquired for each batch and were divided into two parts for the
101 subsequent analyses – one part was used for physicochemical and instrumental texture analyses
102 and the other for ultrasound inspection. **Thus, sixteen parts corresponding to eight pork loins (four**
103 **for CCI, and four for BI) were obtained.** Figure 1 is a diagram showing the procedure.

104 The ultrasound tests on each piece were carried out in three orthogonal directions at two
105 inspection frequencies (Figure 2). The different groups of parameters calculated were subjected
106 to a correlation study (acoustic vs destructive methods) as a step prior to studying the feasibility
107 of ultrasound inspection as a predictive tool for physicochemical and textural parameters.

108 **2.2. Physicochemical analyses and instrumental texture**

109 Water activity (a_w) was determined by a water activity measuring instrument (Lab Master-aw,
110 NOVASINA AG, Lachen, Switzerland). Instrumental colour of the pork loins was measured
111 using a Minolta CR-300 colorimeter (Konica Minolta, Osaka, Japan) that was calibrated with a
112 standard white calibration tile. The analysis was carried out according to the principles set out by
113 the Commission International d'Éclairage (CIE) (Billmeyer and Saltzman, 1981). Lightness (L^*),
114 redness (a^*), and yellowness (b^*) were the colour coordinates determined. Moisture content was
115 measured by drying the samples (5 g) at 102°C following the procedure of the official methods
116 of the Association of Official Agricultural Chemists (AOAC International reference method
117 935.29) (AOAC, 2000). Finally, the fat content was determined according to the method described
118 by Folch et al. (1957) with chloroform:methanol (2:1). All physicochemical determinations were
119 done in triplicate.

120 Instrumental texture was determined using a TA.XT plus texture analyser (Stable Micro Systems
121 Ltd., Surrey, UK). A texture profile analysis (TPA) was carried out, using for this method a
122 cylindrical probe 5 cm in diameter. For each batch and for each sample, five cubes (1 cm³) were
123 obtained and analysed. They were axially compressed to 50% of the original height. The
124 parameters calculated were: hardness (g), adhesiveness (g·s), springiness (g), gumminess (g),
125 cohesiveness (dimensionless), chewiness (g), and resilience ($J \cdot m^{-3}$).

126 2.3. Ultrasound inspection

127 2.3.1. Inspection

128 The ultrasound inspection instruments and procedures basically coincide with those described in
129 previous work (Cerrillo et al., 2014; Collell et al., 2012; Crespo et al., 2020; González-Mohino et
130 al., 2019b, 2019a) with some modifications. For this reason, here we shall limit ourselves to
131 describing them briefly, emphasizing those aspects which are most interesting and/or novel.

132 The pork loin samples were tested using contact ultrasound techniques in through-transmission
133 (TT) mode. Figure 3 shows the set-up used for the measurements. Two different pairs of

134 transducers were used: Panametrics V318-SU and A114-S, with nominal frequencies of 0.5 MHz
135 and 1 MHz, respectively. The intention with this was to check whether or not the inspection
136 frequency affects the calculated ultrasound parameters. The main characteristics of these two pairs
137 of transducers are listed in Table 1. **In addition, the transducers were excited at their resonance**
138 **frequency with a pulse repetition frequency (PRF) of 100 Hz, and a voltage of 200 V peak-peak.**

139 As mentioned above, in the case of the V318-SU pair, the inspection was carried out on each
140 piece in three orthogonal directions, while for the A114 pair it was carried out only in one
141 direction (Figure 2). The reason for this lies in the models' different values of the near field N
142 (Table 1). In particular, the value of N for the A114-S pair is 5.92 cm. Given that this value is
143 similar to, and in some cases greater than, the distance travelled by the ultrasound waves in the
144 inspections carried out in directions 2 and 3, and taking into account that the ultrasound
145 parameters obtained in inspections at distances shorter than N may be erroneous since they are
146 subject to interference phenomena, we decided not to carry them out with the A114S pair in the
147 aforementioned directions. There is no such problem when using the V318-SU transducers, so
148 these were used to inspect the samples in all three orthogonal directions.

149 A Panametrics-NDT Model 5077PR pulser-receiver was used for the loin characterizations. This
150 pulser-receiver was connected to an InfiniiVision DSO-X 3032A (KEYSIGHT) oscilloscope.
151 Finally, a coupling gel (Olympus) of fluid consistency was applied to the pork loin surfaces to
152 facilitate the transmission of the waves between them and the transducers. This gel is a non-toxic
153 water-soluble organic substance which is easy to apply and to remove. Ultrasound measurements
154 were carried out inside a temperature-controlled chamber (RIVACOLD RC325-45ED, Italy),
155 maintaining a constant temperature of $7.0\pm 0.2^{\circ}\text{C}$.

156 2.3.2. Parameters

157 Once all the equipment and instruments were in place, different ultrasound parameters were
158 obtained from the received signal (A-scan). By way of example, Figure 4 shows a representative

159 A-scan obtained with the V318 transducers. The ultrasound pulse velocity (UPV) calculation is
160 based on the time lapsed from the signal's emission until its reception (time of flight – TOF).
161 Since the distance is known for each inspection, it is possible to determine the UPV.

162 The frequency parameters are related to the fast Fourier transform (FFT) of the received
163 ultrasound signals. Figure 5 displays the FFT of the signals received in the inspection shown in
164 Figure 4. As can be seen in the figure, the frequency distributions are not at all close to Gaussian,
165 so that the largest amplitude of the FFT is not a priori a meaningful value with which to
166 characterize the inspected samples. **This fact is characteristic for the power spectrum of RF signals**
167 **(Mamou and Oelze, 2013)**. Hence, in order to facilitate the subsequent correlation analysis, the
168 frequencies corresponding to the 50th and 99th percentiles of the received signals in the
169 cumulative frequency periodograms of the FFT (FFT50, and FFT99, respectively) were
170 calculated, i.e., if the cumulative frequency corresponding to the 50th percentile is at f Hz for a
171 specific inspection, this means that 50% of the received signals had a frequency of less than f Hz.

172 Finally, a series of parameters related to ultrasound attenuation were also calculated. These
173 parameters are obtained from the inspection of samples whose A-scan lacks clearly visible
174 reflections or echoes, as is the case of these loin samples (see Figure 4). Then, given that the
175 nature of pork loins makes it impossible to distinguish echoes, the attenuation was determined
176 from the signal's progressive energy loss. In this way, one obtains the mean value of the
177 parameters related to the attenuation as a function of time taken to receive certain energy
178 percentages – specifically, 30%, 50%, and 70% (AT30, AT50, and AT70, respectively), expressed
179 as multiples of TOF ($n \cdot \text{TOF}$).

180 2.4. Data analysis

181 Significant differences between the CCI and BI samples were studied. The normality of the
182 physicochemical, TPA, and ultrasound parameters were evaluated with the Shapiro-Wilk test.
183 The results of these normality tests were significant ($p < 0.05$), and therefore the data were

184 analysed by an ANOVA using the IBM SPSS v.22 (IBM Co., New York, U.S.A) statistics
185 software package.

186 The physicochemical, TPA, and ultrasound data were subjected to a principal component analysis
187 (PCA) and a linear correlation analysis based on the Pearson correlation coefficient (R) using the
188 XLSTAT software package (Addinsoft Pearson Edition 2014, Addinsoft, Paris, France).

189 **3. Results and Discussion**

190 **3.1. Physicochemical and texture analysis**

191 Table 2 lists the physicochemical data determined for CCI and BI. There were differences in a_w ,
192 in the colour parameters L^* and b^* , and in fat content between the two pork loin categories. Based
193 on the labelling, it can be seen that the fat content is an important parameter to discriminate
194 between the categories. This is also the case for lightness (L^*) and water activity (a_w), with higher
195 values in the CCI loins. On the contrary, yellowness (b^*) presented higher mean values in BI.
196 Although there have been numerous works focused on assessing how different Iberian pig feeding
197 regimes influence the chemical composition and sensory characteristics of ham (Cava et al., 2000;
198 Pérez-Palacios et al., 2011), there has been no previous study analysing loins from batches
199 established as in this study corresponding to the commercial categories established in the Iberian
200 quality standard (BOE, 2014). The significant differences in these physicochemical parameters
201 did not translate into differences in the texture of the products (Table 2) with the TPA parameters
202 showing no differences between CCI and BI samples. As mentioned above, there have been no
203 studies with the new commercial classification legislation. Also, comparisons of pig loins from
204 different rearing regimes are not usually approached from an instrumental rather than a sensorial
205 perspective (Jonsäll et al., 2002; Karpiesiuk et al., 2019). Figure 6 shows a factor score plot of the
206 PCA of the physicochemical and TPA data. The first two principal components accounted for
207 69.35% of the total variance (38.26% for F1, and 31.09% for F2). These parameters did not

208 achieve a homogeneous distribution that would allow discrimination of the loins in accordance
209 with their commercial category.

210 3.2. Ultrasound parameters

211 Table 3 lists the results of the ultrasound inspection of the CCI and BI pieces with the V318
212 transducer pair. The UPV values for both sample groups are quite similar to those determined in
213 other studies in the literature, with variations motivated by the different measurement conditions
214 (temperature, muscle, inspection thickness, inspection frequency). Measurements made in
215 muscles such as the biceps femoris (de Prados et al., 2015) determined the speed at approximately
216 1534 ± 4 m/s, while with other measurement conditions and sample types (Niñoles et al., 2011)
217 the speeds determined ranged between 1580 and 1590 m/s, both studies using 1 MHz transducers.
218 De Prados et al. (2015) estimated the UPV in pork loin at 1557 ± 6 m/s, again with a 1 MHz
219 transducer. As mentioned above, these variations in ultrasound speeds in pork muscle are due to
220 the measurement conditions, as can be observed in the study of ultrasound parameters in cooked
221 pork loins (González-Mohino et al., 2019b) which established the UPV at 1582 ± 8 m/s for fresh
222 pork loin using a 100 kHz transducer. With respect to the present results for the parameters related
223 to frequency components and attenuation, they are not comparable with other studies because
224 these parameters were not usually considered or, when they were, the transducers and inspection
225 geometries were of different characteristics (González-Mohino et al., 2019b), preventing
226 comparison with the present results.

227 We must also highlight the results for FFT50 and FFT99 (Table 3) in that their values in direction
228 1 are significantly lower than those in directions 2 and 3. The explanation for this must be sought
229 in the greater thickness of the direction 1 measurements compared to those in directions 2 and 3.
230 A greater thickness entails a greater transit length of the ultrasound waves through the sample,
231 and therefore greater attenuation, which is especially intense at high frequencies. For this reason,
232 the frequency components of the measurements made with greater thicknesses are lower. In this

233 same sense, the values of AT30, AT50, and AT70 are significantly greater in direction 1 than in
234 the other two directions. Given that the attenuation parameters are reported as a function of the
235 TOF, this result would indicate that, although the ultrasound waves are attenuated more in
236 direction 1 for the aforementioned reasons of traveling through a greater thickness, the rate of
237 attenuation in directions 2 and 3 is greater than that of direction 1. This result is consistent with
238 the fact that the muscle fibres are oriented longitudinally (Kim et al., 2018), which means that the
239 ultrasound waves have to pass through fewer fibres per unit length than when they do so in
240 directions 2 or 3. Since the signal must be attenuated when passing from one fibre to another, the
241 attenuation per unit length in directions 2 and 3 has to be greater than that in direction 1. Most
242 ultrasound parameters did not differ between the product categories. But, of the different
243 measurement directions tested, the UPV of direction 1 was indeed able to distinguish the samples,
244 with greater mean values for CCI than for BI. This may be due to the differences in their lipid
245 content. The behaviour of the ultrasound propagation speed with the fat content is antagonistic in
246 the sense that, as noted by Koch et al. (2011), the speed of propagation declines with increasing
247 fat content of porcine soft tissues. Benedito et al. (2001), however, found that a product with more
248 adipose tissue presented higher ultrasound speeds, although this was when the measurement
249 temperature was low so that the fat remained in a solid state. This is indicative of the major
250 influence of temperature on the measurement. In this present study, the measurement temperature
251 was 7°C, and therefore the pork fat at this temperature was in a solid state, so that the results can
252 be compared with those of the aforesaid workers. In any case, even though the temperature-
253 dependent state of the fat is an influencing factor, the ultrasound parameters, and in particular
254 UPV, are influenced by the feeding regime (Niñoles et al., 2007, 2011). Finally, and generally,
255 the attenuation parameters determined using V318 transducers showed no differences between
256 the pork loins.

257 Figure 7a shows the PCA score plot for the V318 ultrasound parameter results. The first two
258 principal components accounted for 57.69% of the total variance (33.35% for F1, and 24.34% for

259 F2). The PCA exploratory study of the data distributes the BI samples mostly in the upper
260 quadrants and the CCI in the lower quadrants, following a behaviour to be expected given the
261 significant results obtained.

262 Table 4 presents the ultrasound parameters obtained in the ultrasound inspection of the CCI and
263 BI samples using the A114 transducer pair. The velocity values are similar to those obtained with
264 the V318 transducer pair. Also, as expected, the FFT50 and FFT99 frequency components are
265 clearly greater than those obtained with the V318 pair simply because the nominal resonance
266 frequency of the A114 (1 MHz) is higher than that of the V318 (0.5 MHz). And the values of
267 AT30, AT50, and AT70 measured with the A114 transducers are lower than the corresponding
268 V318 values, which is logical in the sense that higher values of these attenuation parameters are
269 indicative of a lower attenuation of the ultrasound waves (Crespo et al., 2020). This must
270 obviously be the case with the waves emitted by the lower frequency transducer which, in the
271 present study, is precisely the V318.

272 As was the case with the speeds calculated for the V318 transducer, the mean values were
273 consistent and similar to those reported in other studies for fresh muscles (de Prados et al., 2015;
274 González-Mohino et al., 2019b), whereas, for the reasons mentioned above, there are no
275 comparable results in the literature for the frequency components and attenuation.

276 The UPV and attenuation presented significant differences among samples, while the frequency
277 parameters related to FFT did not. Again, the mean UPV values were significantly greater for
278 CCI than BI pork loins, probably due to the influence of the fat content (Benedito et al., 2001) as
279 was indicated above for the V318s. With regard to the attenuation parameters, each of them
280 showed differences between the two commercial categories, with greater mean values for the BI
281 than the CCI loins. This would indicate that the BIs attenuate the ultrasound waves less than the
282 CCIs, at least in direction 1. This effect was masked in the inspection carried out with the V318
283 transducers due to their lower emission frequency. Thus, the higher frequencies (in the present

284 case those emitted by the A114) have a shorter wavelength (see Supplementary Material 2), which
285 is why they are more sensitive to the detection of discontinuities or alterations in the medium.
286 These alterations could well have gone unnoticed with the V318, but are now detected with the
287 A114. In light of the data given in Tables 3 and 4, the lipid content is the most important factor
288 differentiating the two categories, being clearly greater in the CCIs. Therefore, the presence of
289 more infiltrated fat in this type of sample attenuates the ultrasound signal more than BI, with its
290 lower fat content. This result is consistent with that reported by Fariñas et al. (2021) for samples
291 of pork burgers, where a greater amount of fat lowers the attenuation values, with this parameter
292 understood as energy loss.

293 Figure 7b shows the PCA factor score plot of the A114 ultrasound parameter results. The first
294 two principal components accounted for 89.30% of the total variance (75.43% for F1, and 13.87%
295 for F2). The differences between the variances of the V318 and A114 transducers are probably
296 influenced by the number of variables considered. According to the significance results, the PCA
297 showed that the parameters calculated from the A114 transducer measurements discriminate the
298 two commercial categories, with the CCI samples being in the left-hand quadrants and the BI
299 samples in the right-hand quadrants. In addition, samples of the same commercial category were
300 located quite close to each other.

301 3.3. Correlation study

302 Table 5 lists the Pearson linear correlation coefficients (R) between the ultrasound parameters
303 determined with the V318 transducers and the physicochemical and TPA results. There were
304 several (positive and negative) significant correlations among the determinations studied.

305 Particularly interesting are the significant negative correlations between the fat content and the
306 attenuations in direction 2. As mentioned above, this is due to the increased attenuation of the
307 ultrasound waves as the amount of fat increases. Unfortunately, these results are not comparable
308 because attenuation is usually calculated as energy loss.

309 Significant negative correlation coefficients were obtained between cohesiveness and the
310 attenuations in direction 3. Since the cohesiveness corresponds to elastic deformation of the loin
311 sample, this result would indicate that the samples which experience the greatest deformation are
312 those with the lowest AT30, AT50, and AT70 attenuation coefficients, i.e., those that most
313 attenuate the ultrasound signal. This result coincides with the well-known behaviour that
314 attenuation experiences with the elasticity of a solid medium, in the sense that the more elastic
315 samples (those that suffer less deformation under the same stress) are less attenuating to sound
316 waves (Kinsler et al., 1999).

317 Additionally, there are other notable relationships between parameters, an example being a_w
318 which is positively correlated with UPV_1 and negatively with $FFT50_2$. Hence, if a pork loin has a
319 high a_w , this implies a greater speed and the passage of lower frequency components during the
320 propagation of the wave through the medium. It is difficult to establish any parallel between a_w
321 and a mechanical explanation for it. Nonetheless, the significant negative correlation of moisture
322 with $FFT99_3$ also seems to reflect the less favourable nature of the presence of water in the sample
323 in letting high frequency waves pass.

324 With respect to the physicochemical parameters related to colour, only b^* presented significant
325 correlations with $AT70_3$. Again, the lack of any mechanical meaning for the parameter b^* makes
326 it difficult to find an explanation for or against such a correlation.

327 Table 6 lists the Pearson linear correlation coefficients (R) between the ultrasound parameters
328 determined with the A114 transducers and the physicochemical and TPA results. In this case,
329 there are the same significant negative correlations that were found between fat content and AT30,
330 AT50, and AT70. Furthermore, fat correlates positively with the speed of propagation and the
331 frequency components of the sound waves, significantly in all cases. With respect to the
332 correlation with the speed, this was entirely expected based on the aforementioned results
333 obtained with the V318 and A114 transducers to differentiate the two commercial categories of

334 the study. Regarding the positive correlations with FFT50 and FFT99, this would indicate a
335 greater presence of high frequency components in the samples with higher fat contents. Given
336 that we had already concluded that the presence of fat favours the attenuation of the waves, this
337 result now shows that the said attenuation, although existing at any frequency, is proportionally
338 lower for higher frequencies. The negative and significant correlation of moisture with FFT99 is
339 also repeated, and this time also with FFT50. These results would again point to the fact that the
340 presence of moisture in the sample does not favour the passage of high frequency waves.

341 With respect to the colour parameters, L^* presented inverse correlations with all the attenuation
342 determinations. In the case of a_w , significant correlations with AT50 were also observed.
343 Although this is the only statistically significant case of correlation between a_w and any
344 attenuation parameter, it is necessary to note that the negative character of the correlation exists
345 for both transducers and for the measurement directions considered. **This result would be**
346 **consistent with that obtained by Lewicky et al. (2004), since the samples with higher a_w would**
347 **be more attenuating (lower ATxx values), which is logical due to these samples are more tender**
348 **and thus less crisp or brittle.**

349 Lastly, the fact mentioned above that samples undergoing less deformation under the same stress
350 are less attenuating to the sound waves is now reflected in the positive correlations between
351 springiness and the attenuation parameters, especially significant in the case of AT30. In fact, and
352 as expected, there is a positive and significant correlation ($R = 0.7803$) between the springiness
353 and the cohesiveness of the samples.

354 As a final discussion, and as mentioned above, most ultrasound inspection research on food has
355 focused exclusively on the determination of the speed, without exploring other parameters related
356 to the frequency components of the FFT, or the attenuation calculated without the observation of
357 echoes, which have proven their reliability as discriminating elements both in the current study

358 and in other scientific contributions. Not performing these types of calculations could be
359 considered to be a loss of information that might be decisive for various studies.

360 **4. Conclusions**

361 In view of the acoustic parameter results, one may conclude that ultrasound determinations have
362 the capacity to characterize these two commercial categories of pork loin, being sensitive to the
363 differences in composition of the two groups of samples. The A114 1000 kHz contact transducers
364 were those which demonstrated the better sensitivity in the inspection of the samples in their
365 single measurement direction, showing more significant differences between CCI and BI as well
366 as several significant correlations of the acoustic parameters with physicochemical and
367 instrumental texture parameters. As expected, the moisture and fat contents were the main
368 determinants differentiating the two categories, resulting in physical and chemical changes that
369 were detected by the various acoustic parameters calculated. Further analyses, such as
370 constructing predictive models, could improve the understanding of the relationships among these
371 parameters. The correlation study shed light on that possibility, in several cases finding
372 statistically significant high correlation coefficients.

373 Finally, this study shows the feasibility of using ultrasound measurements to differentiate
374 categories of Iberian pork loins, postulating this non-destructive technology as a sensitive and
375 rapid technique to characterize Iberian meat products. **This technology could be applied in the
376 production lines, characterizing and monitoring samples and processes, as other technologies
377 (NIRS) have tried.** This work has presented new contributions of ultrasound inspection for Iberian
378 meat products that may be of interest to the meat industry, either to assess quality rapidly,
379 efficiently, and non-destructively by detecting the properties that conform the product, or to avoid
380 possible cases of marketing fraud.

381

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389

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494 **Figure Legends**

495 **Figure 1.** Scheme of the procedure and data analysis.

496 UPV = ultrasound pulse velocity; FFT₅₀/FFT₉₉ = the 50th and 99th percentiles of the fast Fourier transform;
497 AT₃₀/AT₅₀/AT₇₀ = attenuation corresponding to receiving 30%, 50%, and 70% of the energy.

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498 **Figure 2.** Directions of the three orthogonal axes along which the samples were inspected with
499 the V318-SU and the A114S transducer pairs, and the corresponding thicknesses of measurement
500 (d).

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501 **Figure 3.** Set-up for the ultrasound measurements (1 = oscilloscope; 2 = pulse-receiver; 3 =
502 custom-designed metal structure; 4 = transmitter transducer; 5 = pork loin sample; 6 = receiver
503 transducer).

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504 **Figure 4.** A-scan obtained with the Panametrics V318 transducers for BI (a), and CCI (b) pork
505 loin with direction 1.

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506 **Figure 5.** FFT of the A-Scan from Fig. 4a (a), and Fig.4b (b).

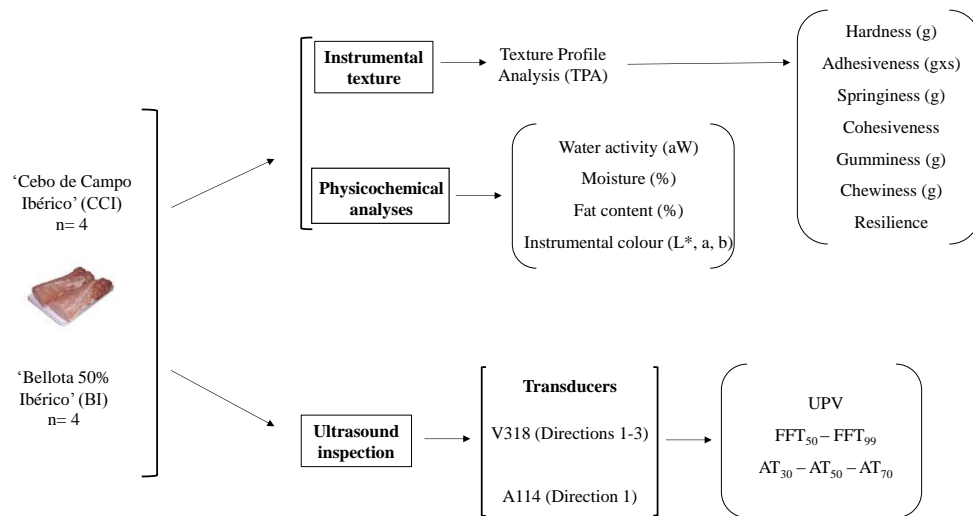
507 =====

508 **Figure 6.** PCA factor score plots of physicochemical and TPA results for two different
509 commercial category pork loins. CCI= Cebo de Campo Ibérico (green label); BI= Bellota 50%
510 Ibérico (red label).

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511 **Figure 7.** PCA factor score plots for the V318 transducer (a) and A114 transducer (b) parameter
512 results for two different commercial category pork loins. CCI= Cebo de Campo Ibérico (green
513 label); BI= Bellota 50% Ibérico (red label).

514 =====

Figure 1. Scheme of the procedure and data analysis

UPV= ultrasound pulse velocity, FFT₅₀/ FFT₉₉= Fast Fourier Transform corresponding to the 50th and 99th percentiles, AT₃₀/ AT₅₀/ AT₇₀ = attenuation corresponding to reaching 30%, 50%, and 70%, of the energy.

Figure 2. Directions of the three orthogonal axes along which the samples were inspected with the V318-SU and the A114S transducer pairs, and the corresponding thicknesses of measurement (d)

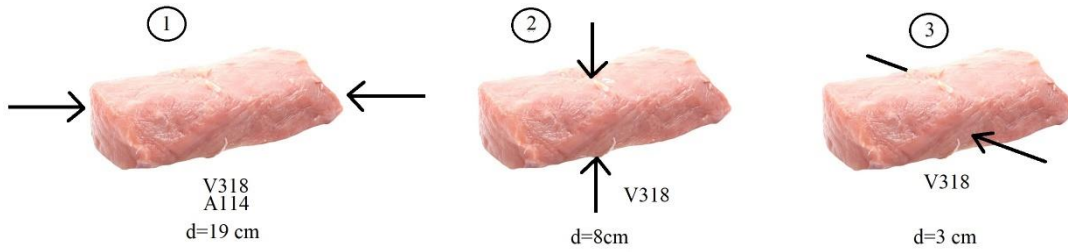


Figure 3. Set-up for the experimental ultrasonic measurements (1= Oscilloscope, 2= Pulse-receiver, 3= Custom-design metal structure, 4= Transmitter transducer, 5= Pork loin sample, 6= Receiver transducer)

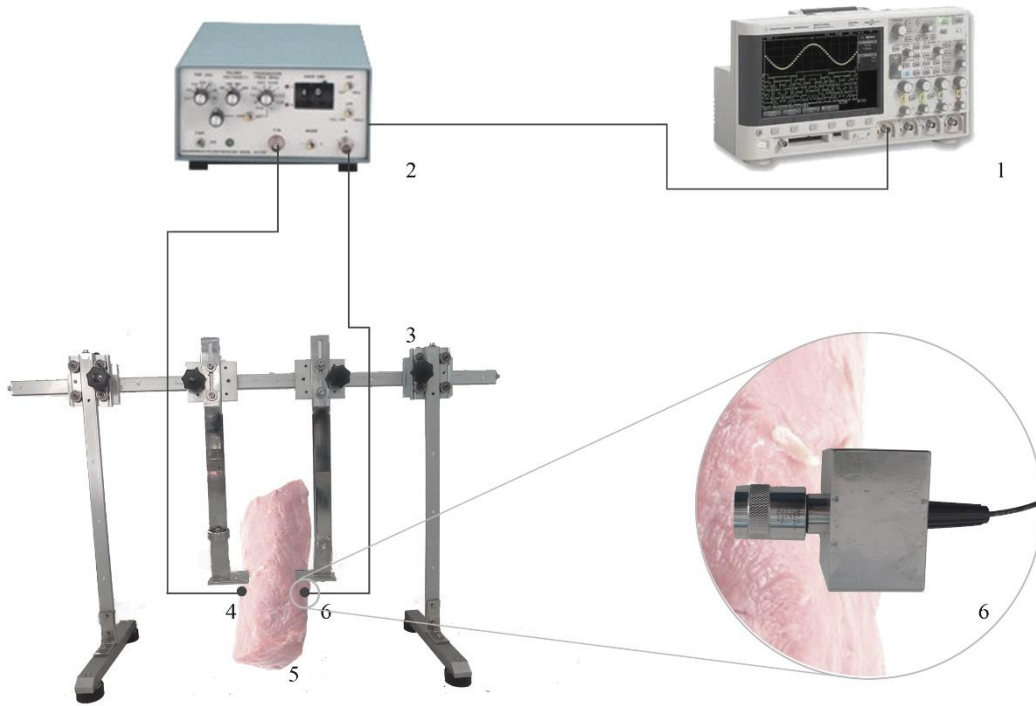


Figure 4: A-scan obtained with the Panametrics V318 transducers for BI (a), and CCI (b) pork loin with direction 1.

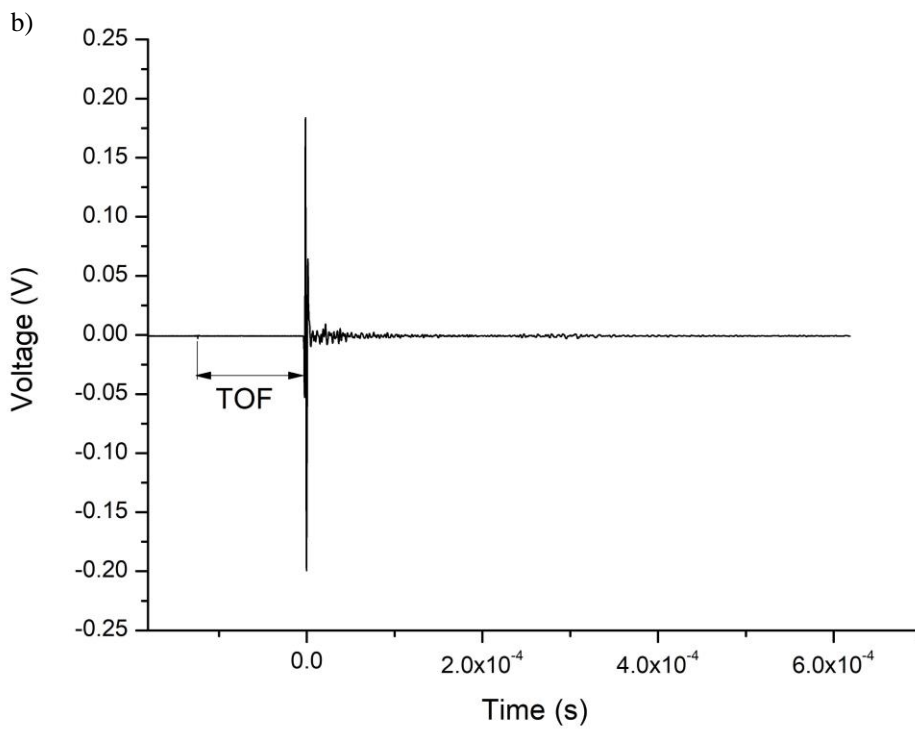
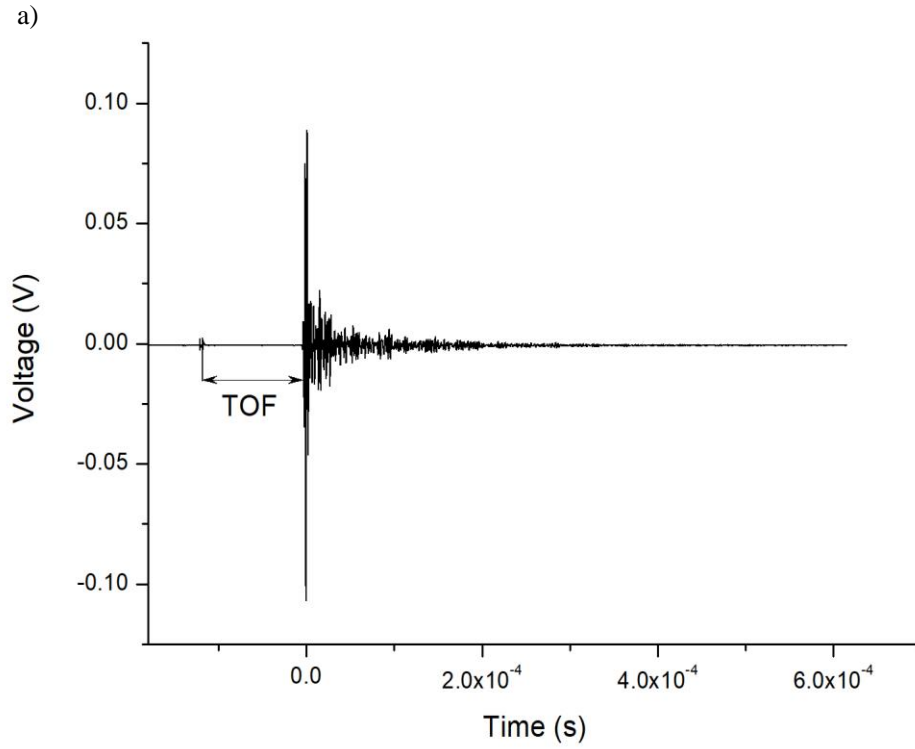


Figure 5. FFT of the A-Scan from Fig. 4a (a), and Fig.4b (b).

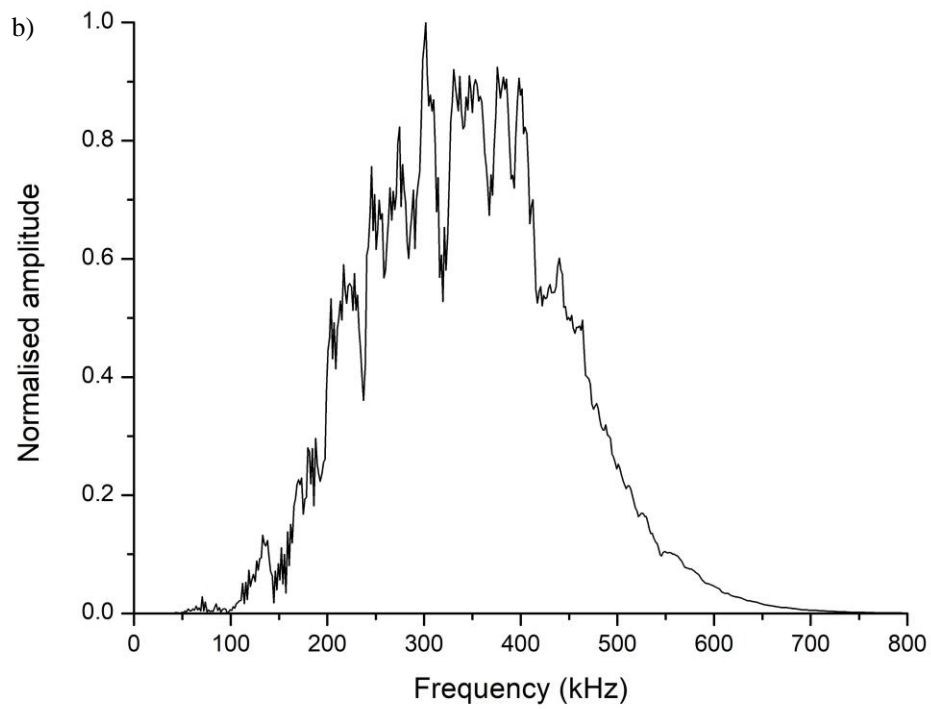
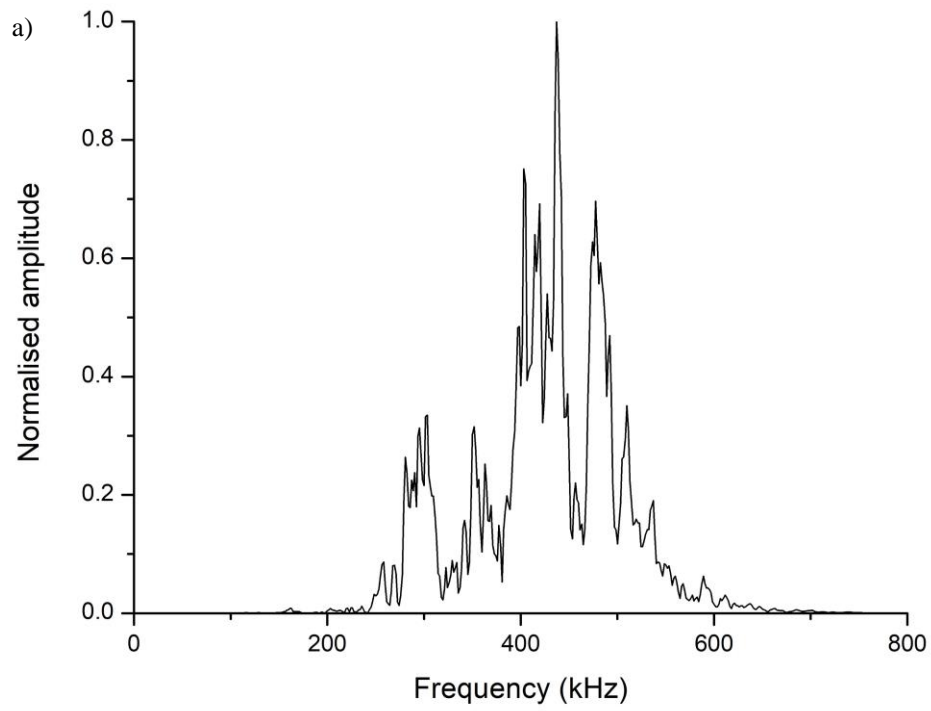


Figure 6. PCA with factor score plots of physicochemical and TPA results, for different commercial category pork loins. CCI= Cebo de Campo Ibérico (green label), BI= Bellota 50% Ibérico (red label).

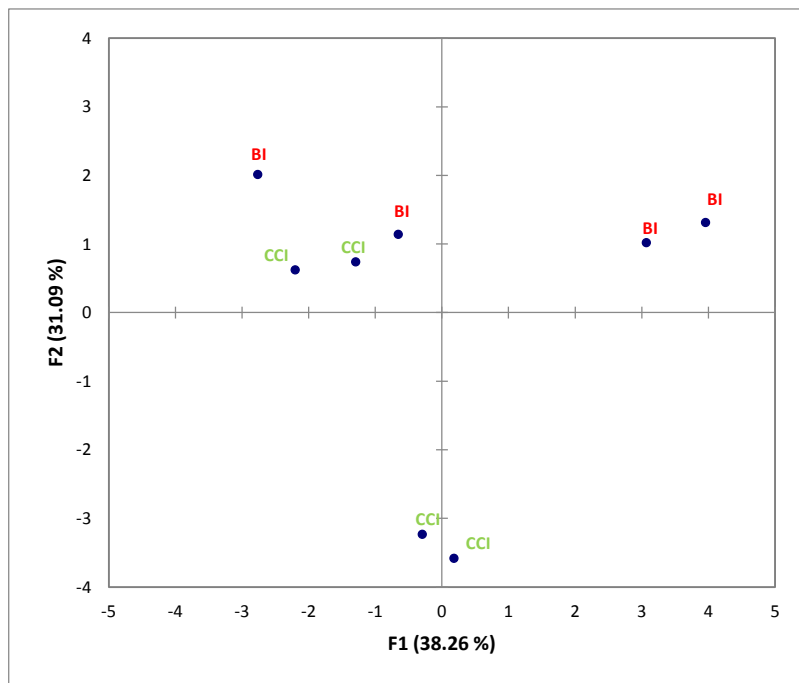


Figure 7. PCA with factor score plots from V318 transducers (a) and A114 transducers (b) parameters results for different commercial category pork loins. CCI= Cebo de Campo Ibérico (green label), BI= Bellota 50% Ibérico (red label).

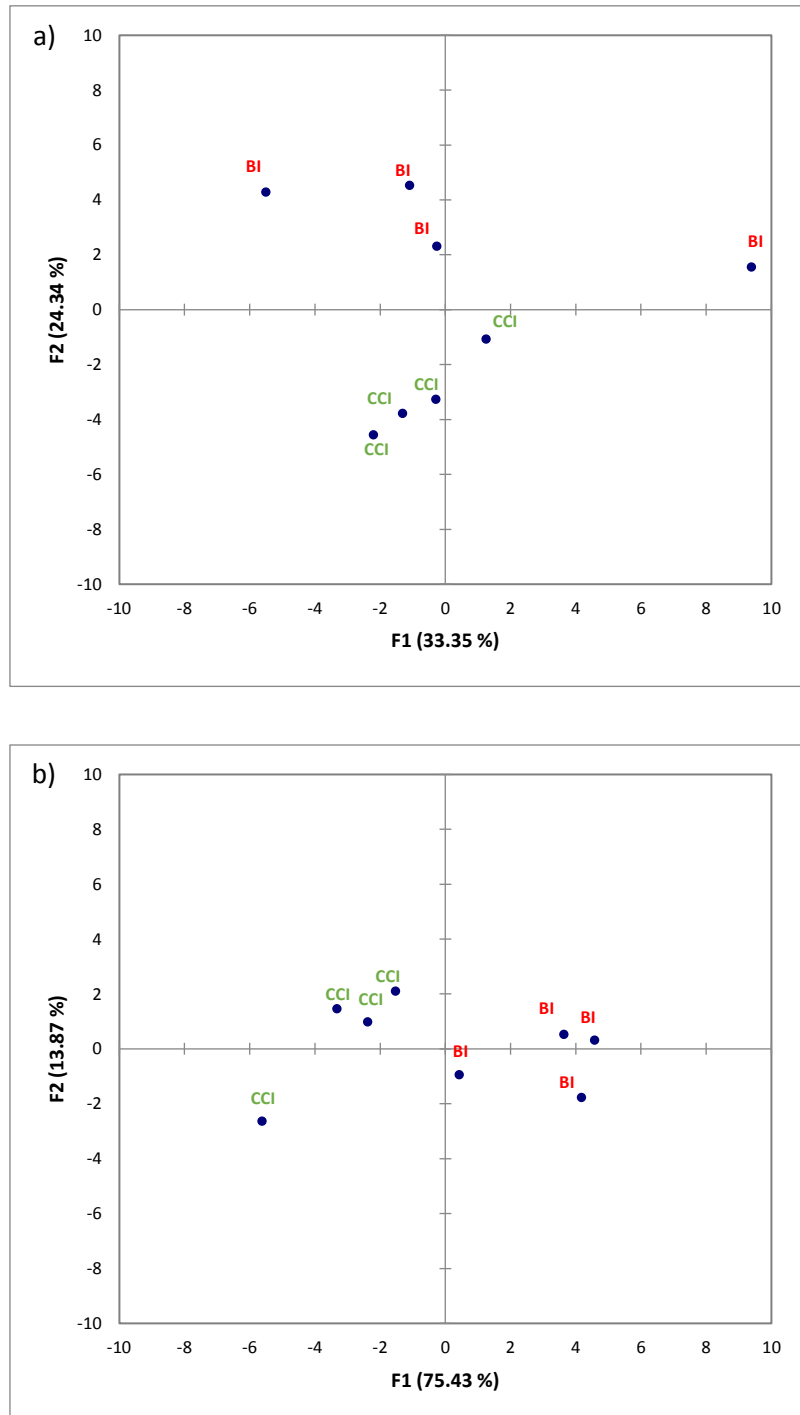


Table 1. Characteristics of the transducer models used.

Model	Diameter (cm)	Frequency (kHz)	Wavelength (mm)	-6 dB bandwidth (%)	N (cm)	ϕ (°)	Waveform duration (μ s)		
							14dB	20 dB	40 dB
Panametrics V318-SU	1.9	500	3.0	61.93	2.96	11.29	3.680	4.888	16.600
Panametrics A114S	1.9	1000	1.5	64.59	5.92	5.62	2.540	2.900	6.079

UPV=1525 m/s was taken for the calculation of wavelength, N, and ϕ

N= near-field length

ϕ = beam angle

The bandwidth and waveform direction values were obtained according to the following conditions:

Damping: 500 ohms; Attn: 36 dB; Gain: 40 dB

Table 2. Physicochemical and TPA results (mean \pm SD), and *p*-values obtained in the statistical analysis comparing the two pork loin categories.

	CCI	BI	<i>p</i>
aw	0.981 \pm 0.003	0.977 \pm 0.004	0.03
L*	48.41 \pm 1.57	45.18 \pm 2.24	0.00
a*	9.41 \pm 1.56	10.55 \pm 1.80	0.06
b*	3.09 \pm 1.06	4.27 \pm 0.98	0.01
Moisture (%)	67.94 \pm 5.17	69.23 \pm 2.17	0.44
Lipids g/100 (%)	8.27 \pm 1.62	6.08 \pm 1.73	0.00
Hardness (g)	1934 \pm 771	1612 \pm 744	0.19
Adhesiveness (g·s)	-68.70 \pm 49.50	-66.48 \pm 33.48	0.70
Springiness (g)	0.37 \pm 0.08	0.44 \pm 0.18	0.55
Cohesiveness	0.37 \pm 0.03	0.38 \pm 0.07	0.48
Gumminess (g)	723 \pm 330	620 \pm 349	0.21
Chewiness (g)	274 \pm 137	287 \pm 205	0.81
Resilience ($J \cdot m^{-3}$)	0.17 \pm 0.05	0.18 \pm 0.05	0.35

CCI = Cebo de Campo Ibérico; BI= Bellota 50% Ibérico.
aw= water activity, L*= lightness, a*= redness, b*= yellowness.

2

Table 3. Ultrasound parameters determined with the V318 transducers (mean values \pm SD), and *p*-values obtained in the statistical analysis comparing the two pork loin categories.

V318	CCI	BI	p
UPV ₁	1550 \pm 12	1491 \pm 33	0.02
UPV ₂	1546 \pm 9	1552 \pm 15	0.52
UPV ₃	1532 \pm 28	1563 \pm 8	0.08
FFT50 ₁	403 262 \pm 37 820	387 968 \pm 39 561	0.60
FFT99 ₁	626 708 \pm 11906	636 239 \pm 69 140	0.79
FFT50 ₂	427 110 \pm 31496	466 763 \pm 18 093	0.07
FFT99 ₂	734 458 \pm 42054	738 087 \pm 32 649	0.90
FFT50 ₃	451 277 \pm 10829	451 765 \pm 16035	0.96
FFT99 ₃	726 754 \pm 10407	750 773 \pm 39513	0.28
AT30 ₁	1.092 \pm 0.091	1.278 \pm 0.211	0.33
AT50 ₁	1.209 \pm 0.224	1.409 \pm 0.305	0.33
AT70 ₁	1.306 \pm 0.316	1.583 \pm 0.411	0.48
AT30 ₂	1.043 \pm 0.003	1.121 \pm 0.100	0.15
AT50 ₂	1.052 \pm 0.004	1.193 \pm 0.185	0.25
AT70 ₂	1.061 \pm 0.002	1.322 \pm 0.325	0.07
AT30 ₃	1.104 \pm 0.004	1.099 \pm 0.018	0.70
AT50 ₃	1.116 \pm 0.005	1.113 \pm 0.015	0.69
AT70 ₃	1.138 \pm 0.003	1.135 \pm 0.013	0.76

CCI= Cebo de Campo Ibérico; BI= Bellota 50% Ibérico.

UPV= ultrasound pulse velocity (m/s); FFT50/ FFT99= fast Fourier transform corresponding to the 50th and 99th percentiles; AT30/ AT50/ AT70 = attenuation corresponding to reaching 30%, 50%, and 70% of the energy.

Subscript numbers refer to the directions of the measurements.

Table 4. Ultrasound parameters determined with the A114 transducers (mean values \pm SD), and *p*-values obtained in the statistical analysis comparing the two pork loin categories.

	CCI	BI	<i>p</i>
UPV	1542 \pm 11	1513 \pm 13	0.01
FFT50	789 362 \pm 53 435	728 805 \pm 29 234	0.09
FFT99	1 072 887 \pm 67 217	1 022 223 \pm 42 581	0.25
AT30	1.019 \pm 0.005	1.063 \pm 0.028	0.02
AT50	1.023 \pm 0.005	1.142 \pm 0.026	0.02
AT70	1.027 \pm 0.004	1.253 \pm 0.068	0.02

CCI= Cebo de Campo Ibérico; BI= Bellota 50% Ibérico.

UPV= ultrasound pulse velocity (m/s); FFT50/ FFT99= fast Fourier transform corresponding to the 50th and 99th percentiles; AT30/ AT50/ AT70 = attenuation corresponding to reaching 30%, 50%, and 70% of the energy.

Table 5. Linear correlation coefficients between the V318 transducer ultrasound parameters (UPV= ultrasound pulse velocity; FFT50/ FFT99= fast Fourier transform corresponding to the 50th and 99th percentiles of the received signal; AT30/ AT50/ AT70 = attenuation corresponding to reaching 30%, 50%, and 70% of the energy) and the physicochemical and TPA parameters. Subindices (1, 2, or 3) mean the measurement direction. Boldface numbers mean significant correlations.

	Physicochemical parameters						TPA parameters						
	aw	L*	a*	b*	Moisture	Lipid content	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
UPV ₁	0.7245	0.6407	-0.5286	-0.4045	0.0869	0.2974	0.3703	0.0026	-0.3282	0.0752	0.4312	0.1700	0.0093
UPV ₂	-0.2666	-0.2093	0.1340	0.2673	0.5350	-0.6795	-0.4611	-0.3832	0.2168	0.3491	-0.2670	-0.0559	-0.0386
UPV ₃	-0.3516	-0.5388	0.6156	0.6121	-0.4226	-0.2227	-0.2518	0.5585	-0.0037	-0.0409	-0.2497	-0.1691	0.1760
FFT50 ₁	0.6239	0.1147	-0.3510	-0.1014	0.1081	-0.1193	0.1634	0.6518	-0.5369	-0.0019	0.2380	-0.0643	0.1393
FFT99 ₁	0.0124	-0.0183	0.0492	0.2011	0.2200	-0.4006	-0.5913	0.0157	-0.2881	0.0328	-0.4454	-0.4525	-0.2523
FFT50 ₂	-0.7497	-0.2773	0.4580	0.2796	0.1818	-0.3320	-0.7013	-0.3516	0.2626	0.0070	-0.6473	-0.3473	-0.2639
FFT99 ₂	-0.3333	0.0487	0.6234	0.4419	-0.8419	0.4713	-0.1986	-0.0828	0.1294	-0.1565	-0.2917	-0.1861	-0.1147
FFT50 ₃	-0.1067	-0.2743	-0.4009	-0.5567	0.5328	-0.2950	-0.2111	-0.2539	-0.1830	-0.3195	-0.2100	-0.1606	-0.4441
FFT99 ₃	-0.3283	-0.5590	-0.0765	-0.2847	0.1404	-0.2462	-0.5124	0.1680	-0.3881	-0.6125	-0.5917	-0.5642	-0.5592
AT30 ₁	-0.6685	-0.5753	0.1920	-0.1454	0.0060	-0.1335	-0.5313	-0.1684	-0.0230	-0.5305	-0.6449	-0.4438	-0.5337
AT50 ₁	-0.6305	-0.4098	0.0847	-0.2921	0.0961	-0.0519	-0.4832	-0.3551	0.0165	-0.5040	-0.5886	-0.3815	-0.5837
AT70 ₁	-0.3734	0.0467	0.1906	0.1571	0.1770	-0.0698	-0.2620	-0.1878	0.2585	0.1335	-0.2619	-0.1341	0.0132
AT30 ₂	-0.4844	-0.6657	0.3302	0.3065	0.3844	-0.7457	-0.1515	-0.1695	0.4287	0.3136	-0.0043	0.3100	0.2020
AT50 ₂	-0.4740	-0.6496	0.3138	0.2909	0.4027	-0.7478	-0.1617	-0.1689	0.4124	0.3108	-0.0107	0.2977	0.1907
AT70 ₂	-0.4794	-0.6651	0.3214	0.3013	0.3929	-0.7568	-0.1989	-0.1456	0.3826	0.2870	-0.0496	0.2535	0.1674
AT30 ₃	-0.0627	-0.1248	-0.1747	-0.5554	-0.4782	0.5455	-0.1234	-0.0140	-0.4578	-0.8780	-0.3528	-0.4384	-0.7020
AT50 ₃	-0.1202	-0.1039	-0.0439	-0.4043	-0.6131	0.6083	-0.1688	0.0275	-0.4170	-0.8562	-0.4166	-0.4962	-0.6602
AT70 ₃	-0.0255	-0.2058	-0.3817	-0.7395	-0.1169	0.3410	-0.0184	0.0119	-0.4321	-0.8068	-0.2175	-0.3059	-0.6214

Table 6. Linear correlation coefficients between the A114 transducer ultrasound parameters (UPV= ultrasound pulse velocity; FFT50/ FFT99= fast Fourier transform corresponding to the 50th and 99th percentiles of the received signal; AT30/ AT50/ AT70 = attenuation corresponding to reaching 30%, 50%, and 70% of the energy) and the physicochemical and TPA parameters. Subindices (1, 2, or 3) mean the measurement direction. Boldface numbers mean significant correlations.

	Physicochemical parameters						TPA parameters						
	aw	L*	a*	b*	Moisture	Lipid content	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
UPV	0.4963	0.6752	-0.2981	-0.4652	-0.5331	0.8902	0.4254	-0.0007	-0.3146	-0.3133	0.3006	0.0562	-0.1797
FFT50	0.0967	0.5136	0.1368	-0.1414	-0.7617	0.7924	-0.0679	-0.2873	-0.2220	-0.3696	-0.1506	-0.2050	-0.4452
FFT99	0.0761	0.4079	0.2351	-0.0429	-0.8661	0.8046	0.0522	-0.0162	-0.2054	-0.3544	-0.0523	-0.1281	-0.2694
AT30	-0.6757	-0.8061	0.4851	0.4835	0.4141	-0.8192	-0.0207	-0.1851	0.7415	0.4927	0.1029	0.5094	0.4571
AT50	-0.7390	-0.8749	0.5262	0.5080	0.3103	-0.8121	-0.4025	0.0211	0.4193	0.2021	-0.3147	0.0257	0.1678
AT70	-0.6710	-0.7835	0.5378	0.5911	0.3469	-0.8597	-0.4405	0.0410	0.4016	0.3068	-0.3090	0.0125	0.2178

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AUTHOR STATEMENT

Authors declare the following:

Alberto González-Mohino: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing.

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Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: