

1 **Monitoring ageing in beef samples using surface wave elastography: a feasibility**
2 **study**

3 Nicolás Benech^{1*}, Sofía Aguiar², Gustavo A. Grinspan^{1,3}

4 ¹Laboratorio de Acústica Ultrasonora, Facultad de Ciencias, Igua 4225, 11200, Montevideo, Uruguay

5 *nbenech@fisica.edu.uy

6 ²Instituto de Ensayo de Materiales, Facultad de Ingeniería, J. Herrera y Reisig, 565, Montevideo,
7 Uruguay

8 ³Sección Biofísica y Biología de Sistemas, Facultad de Ciencias, Igua 4225, 11200, Montevideo, Uruguay

9

10 **Abstract.**

11 The maturation process of beef improves meat quality. Several bio-physical-chemical
12 changes occur during ageing. Often, they emerge macroscopically as variations in the
13 mechanical properties of the tissue. Most of the actual testing methods are destructive.
14 Therefore, the number of tested samples is limited. Elastography was born as a medical
15 imaging modality to estimate noninvasively the elasticity of soft tissues. Thus, its
16 application to monitor ageing could help to overcome some drawbacks of destructive
17 methods. However, its use is minimum in industry. In this work, we study the feasibility
18 of using surface wave elastography (SWE) to monitor beef ageing. We tested 25 samples
19 for 21 consecutive days. The results show a decay close to 50% in the shear elasticity of
20 all samples. In addition, the rate of decay shows that the elasticity reduction is greater in
21 the first days for most samples. These results are consistent with previous knowledge in
22 this area, which encourages further research to improve the method and increase its
23 performance and reliability.

24 **Keywords:** ageing, elastography, surface waves, mechanical properties

25 **1. Introduction**

26 To obtain meat of high quality, post-mortem ageing at 4°C or lower for a certain
27 period is required. Tenderness and flavor are improved during this period
28 because biochemical and physicochemical processes occur during post-mortem ageing
29 [1-2]. These processes include the increase of membrane water permeability and the
30 weakening of connective tissue [3]. Thus, it is not surprising that ageing causes changes
31 in the mechanical properties of meat. These changes are evidenced by tensile tests [4],
32 shear force tests [5] or texturometers [6]. All these methods share the disadvantage that
33 they are destructive and therefore the samples tested lose their commercial value. In

1 addition, it is not possible to monitor the changes of a single piece at arbitrary time
2 intervals. Thus, one challenge facing the industry is to account with a reliable and non-
3 destructive method to estimate the mechanical properties of meat during post-mortem
4 ageing.

5 Ageing periods are usually more than 10 days for beef [7]. However, there is no
6 agreement on which is the best ageing time before commercialization, since there is a
7 large variation in both the rate and extent of post-mortem tenderization depending on
8 the muscle type and processing conditions [8]. It has been reported that different muscles
9 from the same carcass respond differently to the same ageing period [9]. Moreover,
10 there exist contradictory reports even for the same muscle. Some studies in longissimus
11 dorsi for example, found that there is little difference in shear values after 7 days ageing
12 [10]. However, other studies reported considerable tenderization for the same muscle
13 and in the same time period [11]. Thus, a method for noninvasive monitoring the
14 mechanical properties of beef samples could benefit the industry in decision making on
15 ageing periods and commercialization.

16 Elastography was born as a medical imaging modality to estimate the local
17 elasticity in soft tissues [12]. The goal is to improve the medical diagnosis of those
18 pathologies which affect the mechanical properties of some tissues. Examples include
19 breast tumor or liver fibrosis [13]-[14]. Within the past 30 years, several methods and
20 techniques were proposed including static and dynamic methods [15]-[17]. Most of
21 them are ultrasound-based techniques, due to the availability and portability of
22 ultrasound scanners. Despite the spreading of elastography in the medical field, its use
23 has been little studied in the food industry as a whole and particularly in beef industry.

24 Previous works including (static) elastography to study meat quality
25 characteristics of pork showed that elastographic results are significantly correlated to
26 shear force tests [18]. Dynamic elastography methods (transient elastography) were able
27 to quantify the viscoelasticity [19] and anisotropy [20] of beef samples. In addition, it
28 showed a linear correlation with pH during rigor mortis [21]. However, none of those
29 methods was translated into devices for industrial use. We believe that this fact is due to
30 the need for adapted ultrasound scanners and highly specialized personnel to operate
31 them. In addition, none of these methods is easily adapted to the production line.

32 Surface wave elastography (SWE) is an emerging elastographic method.
33 Applications include estimating the elasticity of soft tissues *in vivo* [22]-[23], the
34 elasticity of tendons *in vivo* [24] and even the elasticity of fruits [25]. The goal in SWE

1 is to estimate bulk elastic parameters from waves propagating at the surface of the
2 sample. Unlike the ultrasound-based methods mentioned above, SWE does not intend to
3 image the elasticity of tissues. Instead, it brings a mean value of their elasticity in a
4 region of interest (ROI). Therefore, SWE methods use simpler algorithms and
5 measuring devices. Consequently, the associated costs are lower, and the final user
6 requires a minimal training. Thus, SWE is a good candidate to monitor changes of mean
7 elasticity within a ROI in tissues as a function of time, temperature, environmental
8 conditions, etc.

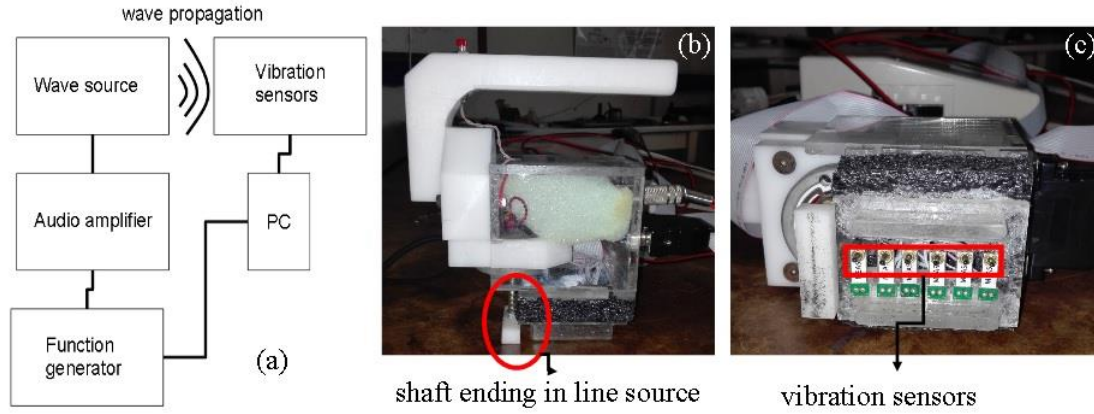
9 In this work, the main objective was to study the feasibility of using surface
10 wave elastography as a method to monitor the ageing process of beef samples. Recently,
11 we have developed a SWE device and method to estimate the elasticity of isotropic and
12 transversely isotropic tissues [26]. We named this method as *non-ultrasound surface*
13 *wave elastography* (NU-SWE). Using this device, we tested five hindquarter cuts during
14 21 consecutive days in order to study if the NU-SWE method is able to follow the
15 changes in their shear elasticity and provide information about its decay rate.

16 **2. Materials and methods**

17 *2.1 Surface wave elastography*

18 In this section, we briefly describe the NU-SWE method employed in this study.
19 Details about the device and theory of operation can be found in [26],[27].

20 The goal in NU-SWE is to estimate bulk shear elastic modulus from surface
21 wave measurements within a ROI. The challenge is to find the relationship between the
22 surface wave speed V_s and the bulk shear wave speed V_b because the latter has a simple
23 relation with the shear elastic modulus $\mu = \rho V_b^2$ [28]. In this expression ρ represents the
24 material density of the beef sample and a lossless elastic medium is assumed. The
25 relationship between V_s and V_b is obtained via a physical model for surface wave
26 propagation. Skeletal muscle is an anisotropic tissue. The orientation of muscle fibers
27 defines a symmetry axis. The elasticity is isotropic within a plane perpendicular to
28 fibers. Thus, skeletal muscle is assumed as a transversely isotropic tissue [20].
29 Therefore, there are two elastic constants related to shear wave propagation. $\mu_{\perp} =$
30 $\rho(V_b^{\perp})^2$ is the elastic constant related to the shear wave propagating perpendicular to the
31 fibers with perpendicular polarization and $\mu_{\parallel} = \rho(V_b^{\parallel})^2$ is related to the shear wave
32 propagating along the fibers direction with perpendicular polarization.



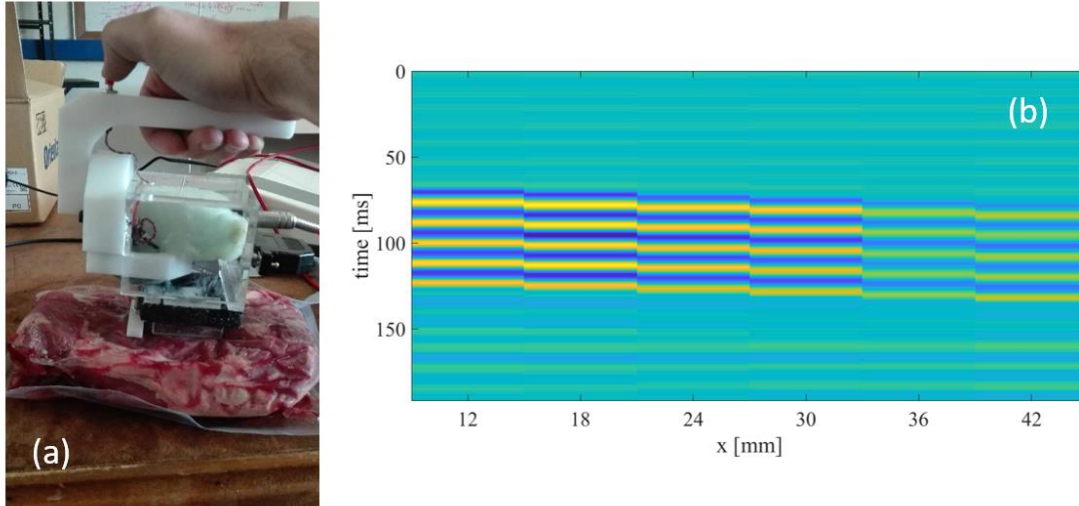
1
2 **Figure 1.** (a) Schematic diagram of the NU-SWE device. The PC controls the function generator and the
3 vibration sensors. (b) Side view of the prototype device. The shaft ending in a linear source is highlighted.
4 (c) Bottom view of the prototype. The piezoelectric vibration sensors are highlighted.

5 As is shown in figure 1, the main components of the NU-SWE device are the
6 wave generator and the vibration sensors. The wave generator is a vibrator with a shaft
7 attached to it, ending in a linear source. The source vibrates normally to the free surface
8 of the sample. If it is oriented perpendicular to the muscle fibers, it excites a wave that
9 propagates along the fibers with perpendicular polarization. The vibration sensors are
10 piezoelectric levers (bimorphs). They generate a voltage when deflected. A mass
11 attached to the levers offers sensitivity in the frequency range used in our experiments
12 (150 – 200 Hz). We use 6 sensors at known positions along the surface to record the
13 surface vibrations. By computing the phase difference between signals, we obtain the
14 phase speed of the surface displacement field V_s . Figure 2(a) shows the device being
15 applied to a sample of bottom round. In this sample, the muscle fibers are oriented
16 mainly along the longitudinal axis. The linear source acts perpendicular to the fibers and
17 the sensors record the displacement field along the fibers' direction. Figure 2(b)
18 displays the field recorded by each sensor, where the time-shift between them is clearly
19 visible.

20 Within soft tissues, a critical distance exists where only surface waves
21 propagate, without contribution from reflected waves from the opposite surface [27].
22 The different surface wave types interfere with each other on the surface. Therefore, the
23 phase speed of the recorded displacement field is dispersive. The dispersion relation
24 depends on the value of the bulk shear wave speed V_b . Using a fitting algorithm based on
25 least squares, the shear wave speed is retrieved. For the orientation of the linear source,
26 the value of V_b'' is retrieved. If the material density ρ is known, our device allows

1 computing $\mu_{//}$. We note here that the linear source can also be oriented parallel to the
2 fibers. This way, μ_{\perp} could also be measured. However, we choose to measure only $\mu_{//}$
3 because this value would be comparable to other methods like shear force tests. The
4 relationship between V_s and V_b is depicted in the appendix.

5



6

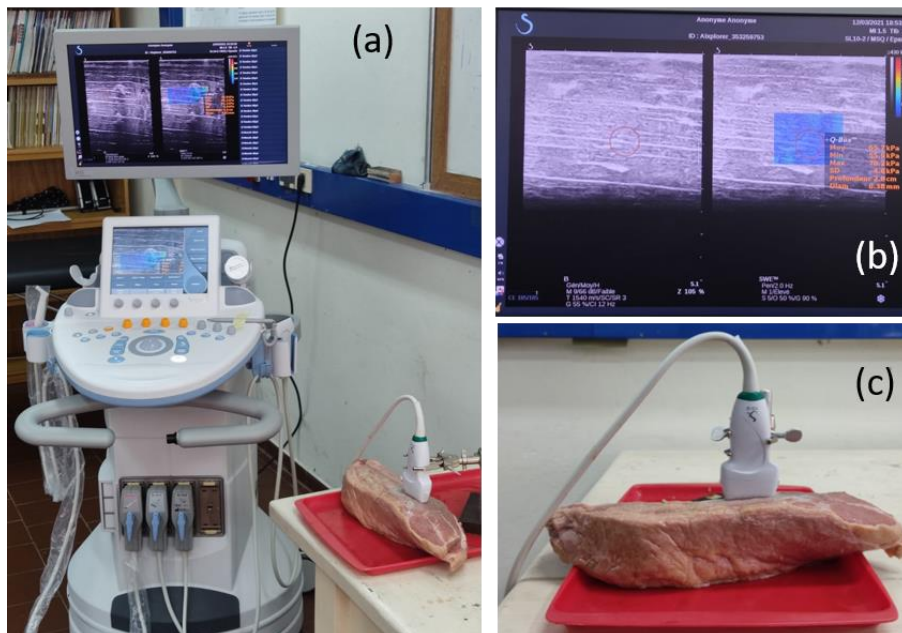
7 **Figure 2.**(a) Example of application of the device over a sample of bottom of round. The linear source is
8 aligned perpendicular to the muscle fibers. (b) Vibration field recorded by the sensors. The time shift
9 between sensors is clearly seen.

10 The “sensing” depth of the surface wave is related to its wavelength. In our
11 experiments, we use frequencies in the range 150-200 Hz. Typical values of the shear
12 wave speed in beef samples are between 3 and 8 m/s. Thus, the wavelength varies
13 between 1.5 and 5 cm. These values are much larger than the plastic film bags used for
14 vacuum sealing. In previous works [29], we tested the device with “naked” beef
15 samples. Then, the samples were vacuum sealed and tested again. The measurement of
16 the shear elasticity was unaltered by the plastic bag. Thus, our device not only is
17 nondestructive, but the beef samples can be tested without even touching them,
18 preserving the sanitary conditions in a slaughterhouse.

19 2.2 Shear Wave Elastography

20 The surface wave method described above estimates the bulk shear elasticity of
21 the beef sample from surface sensors. In order to compare the estimated value with an
22 independent reference method, we used an Aixplorer V12 (SuperSonic Imagine, Aix-
23 en-Provence, France). This device remotely generates a shear wave in the bulk of the

1 sample, via acoustic radiation force, and tracks its propagation speed in real time using
2 ultrafast frame rates. The method is termed as supersonic shear imaging (SSI) [30]. The
3 screen displays a 2D image of the shear elasticity (in kPa), within the selected ROI.
4 Figure 3 displays images of the experimental setup. We used a linear array probe (SL
5 10-2) with a 2-10 Mhz bandwidth which allows elasticity imaging up to 4 cm depth.
6 The sample is a piece of bottom round from a local butcher shop. Its approximate
7 dimensions (large×width×depth) are 18×5×6 cm³. The array is positioned parallel to the
8 muscle fibers. Therefore, the shear wave propagation is parallel, and the polarization is
9 perpendicular to the muscle fibers. Thus, both the surface and bulk methods estimate
10 $\mu_{//}$.



11
12 **Figure 3.** Experimental setup for shear wave elastography measurements. (a) The AixplorerV12 device
13 and the linear array over a sample of bottom round. (b) Detail of the screen of the Aixplorer device. The
14 screen displays two images. On the left, a sonogram of the sample where the fiber orientation is clearly
15 seen. On the right, the elasticity map is superimposed to the sonogram in the ROI. (c) Detail of the array
16 positioned over the beef sample. The array is positioned parallel to the muscle fibers.

17 2.3 Sample preparation and ageing conditions

18 In this work, a total of 25 beef samples were analyzed. The batch consisted of 5
19 samples of 5 different beef cuts: strip loin (SL), thick flank (TF), tail of rump (TR), eye
20 of round (ER) and bottom round (BR). The samples were provided by a local
21 slaughterhouse. All samples came from animals of the same breed, gender, age and type
22 of feeding. They were vacuum sealed and arrived at our laboratory after three days post-
23 mortem and were kept in a cold chamber at 0°C. The cold chamber is spacey enough to

1 allow performing the experiments inside it as shown in figure 4. Nevertheless, due to
2 door opening and varying external conditions, we registered temperature fluctuations
3 (between 0 °C and 3 °C) during the experiments.



4
5 **Figure 4.**(a) Cold chamber with beef samples inside. (b) The tests were performed inside the cold
6 chamber.

7 Ten measurements were performed in each sample per day. All samples were
8 measured every day during 21 consecutive days. In the first day of experiments, the
9 ROI was marked in the plastic bag. This way, we ensured that the experiments were
10 carried out within the same ROI.

11 *2.4 Statistical analysis*

12 For comparison between the surface wave and the bulk shear wave
13 measurements acquired with the NU-SWE and SSI, respectively, we performed a
14 Bland-Altman plot [31] and analyzed the mean difference using the one sample t-test
15 [32].

16 Regarding the ageing process, we performed the Welch's t -test [32] to test the
17 hypothesis of two cut types have equal mean of the shear elastic modulus at the end of
18 the measurements. We used the set of fifty measurements for each cut type to test the
19 hypothesis (ten measurements per sample and five samples for each cut type). We also
20 tested the hypothesis of the same cut type have equal mean before and after the 21-day
21 ageing process.

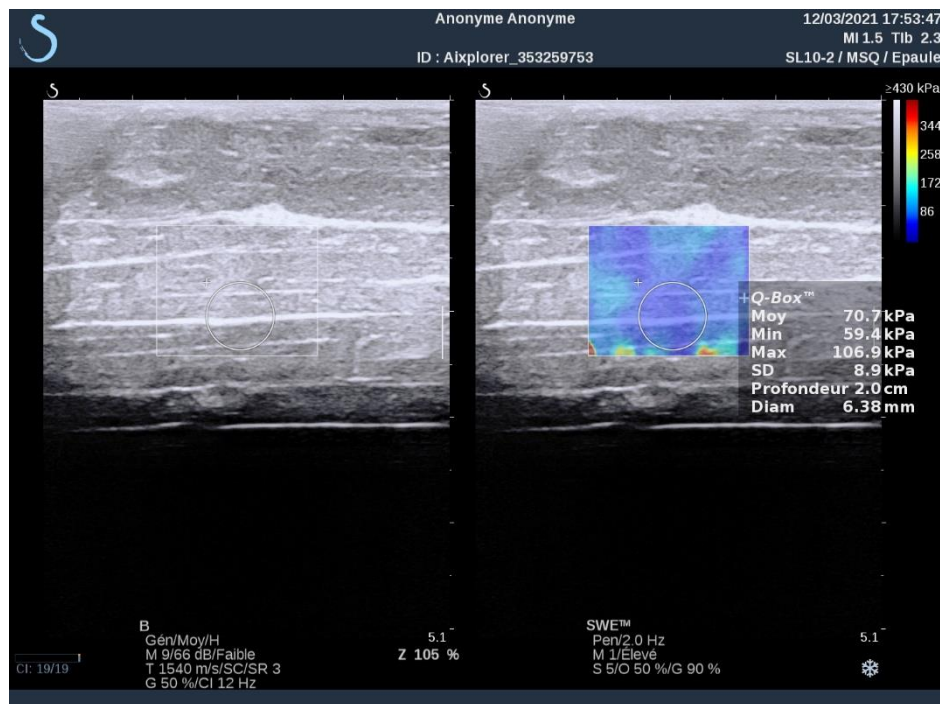
1 Our data allow studying the rate of decay of the shear modulus by computing
2 locally the slope of the shear modulus curve as a function of time. We performed a one-
3 way ANOVA [32] to compare the slope values intra samples of the same cut type. In
4 addition, we tested the hypothesis that each cut type has an equal mean of the slope
5 before and after the process.

6 In all cases we used a level of significance $p = 0.05$.

7 3 Results

8 3.1 Comparison between surface and bulk methods

9 Before beginning the ageing monitoring, we performed measurements on a
10 single sample of bottom round, in order to compare the surface wave method with the
11 shear wave method. The sample was acquired in a local butcher shop and kept at 4°C
12 for 24 hs before measurements.

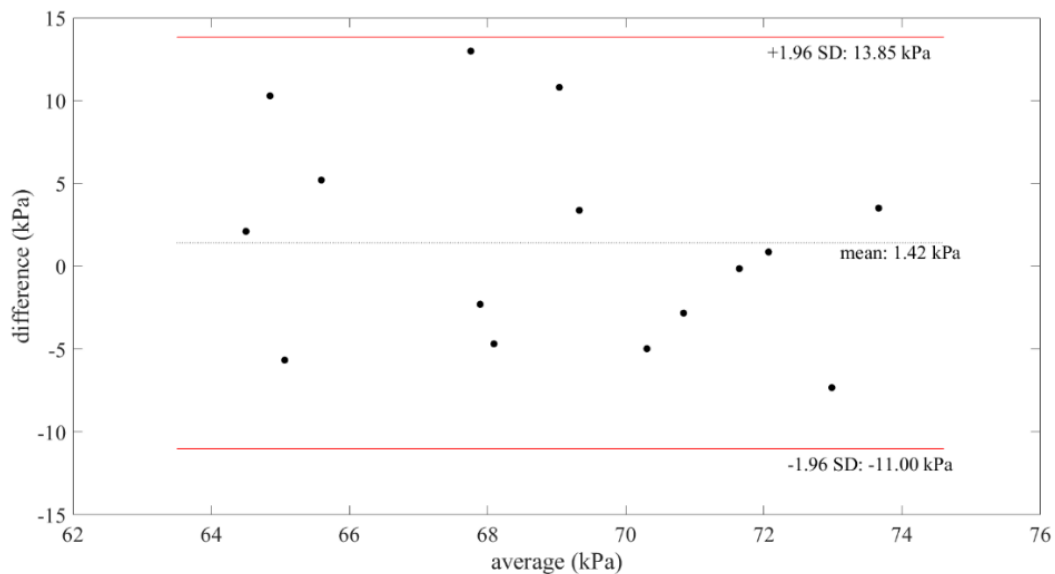


13
14 **Figure 5.** Screen capture of the Aixplorer V12 device while measuring the shear elasticity of a
15 beef sample. The left image is a sonogram of the sample where the fiber orientation is clearly seen. The
16 right image shows the superimposition of the elasticity map on the sonogram.

17 For the surface wave measurements, we marked 3 different ROIs on the surface
18 of the sample and performed a set of 5 measurements in each ROI, totaling 15
19 measurements. The result was $V_b = 8.1 \pm 0.6$ m/s, where the uncertainty represents one
20 standard deviation over the 15 measurements. Assuming a material density $\rho = 1.0 \times$
21 10^3 kg/m³, the estimated shear elasticity was $\mu_{//} = 65.6 \pm 9.7$ kPa.

1 For the bulk shear wave measurements, we used the Aixplorer V12 with the
 2 musculoskeletal preset. Figure 5 displays a screen capture of the Aixplorer device
 3 during the experiments. The image is split in two. The left side is a sonogram (B mode)
 4 of the sample, where the fiber orientation is clearly visible. The right side shows the
 5 superimposition of the elasticity map on the sonogram. The white circumference is the
 6 selected ROI for which the mean shear elasticity value is displayed. The box also
 7 displays the measuring depth, the standard deviation, and the minimum and maximum
 8 values within the ROI. We repeated this procedure in 15 different positions in the
 9 volume of the sample while positioning the ultrasonic probe at the marked ROIs of the
 10 surface wave measurements. The estimated shear elasticity is $\mu_{//} = 69.2 \pm 8.4$ kPa.

11 Figure 6 displays the Bland-Altman plot comparing the surface and bulk
 12 methods. The mean difference was 1.42 kPa, higher on the bulk method. The standard
 13 deviation of the difference was 6.3 kPa. There were no data points out of the confidence
 14 interval, showing there was no influence of outliers. The one sample t-test of the mean
 15 difference indicated that it did not differ significantly from 0 ($p > 0.4$).

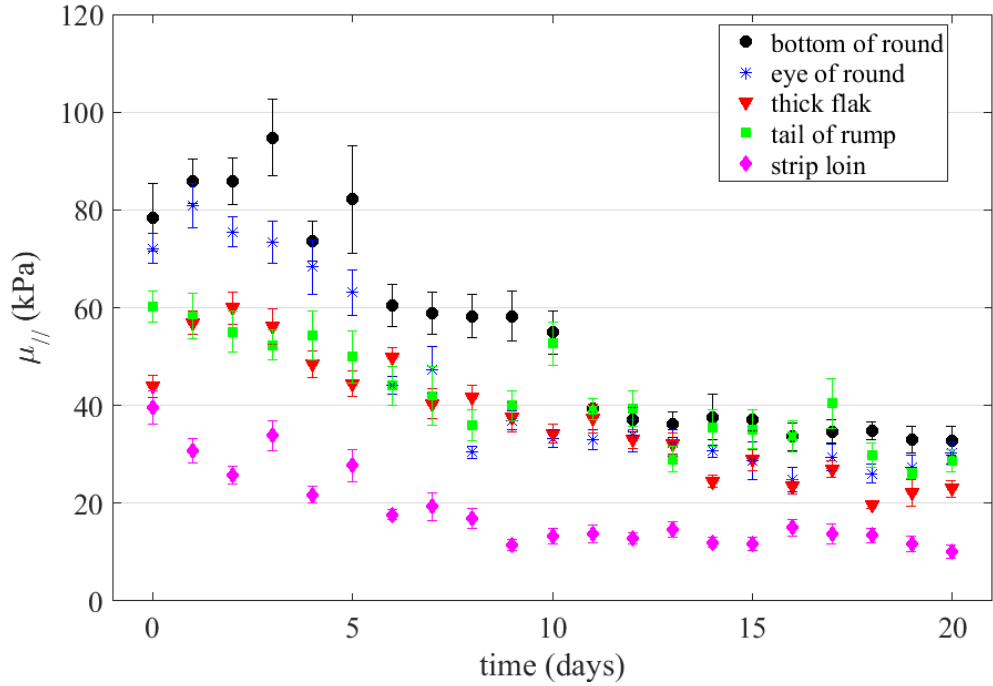


16
 17 **Figure 6.** Bland-Altman plot comparing the data of the surface wave and the shear wave methods. The
 18 black dotted line indicates the mean value of the difference and the red lines indicate the 95% confidence
 19 interval (1.96 SD).

20 *3.2 Monitoring ageing with surface wave elastography*

21 Figure 7 displays the mean shear elastic modulus $\mu_{//}$ for each cut type as
 22 function of the maturation time measured with the surface wave method. The mean for
 23 each cut type is computed using the mean of each sample over the ten measurements.

1 The bars in this plot indicate one standard deviation. After initial fluctuations, a
 2 systematical decrease in the elastic modulus can be observed for all cut types. Some of
 3 them reach an almost constant elastic modulus well before the 21 days of ageing while
 4 others continue decreasing until the end of measurements.



5
 6 **Figure 7.** Mean elastic modulus for each cut type as function of the maturation time. The errorbar
 7 represents one standard deviation over the 5 samples for each cut type.

8 **4. Discussion**

9 The results displayed in figure 7 show that all the cut types studied here achieve
 10 a significant variation in their shear modulus after ageing ($p < 0.01$ in all cases). The
 11 decrease in μ_{ij} is of the order of 50% compared to the initial values. They agree with
 12 those reported in other studies where it was found that ageing times between 8 and 14
 13 days is enough for most cut types to achieve constant values of their mechanical
 14 properties [9]. However, we emphasize that the method used here is nondestructive and
 15 the samples do not lose their commercial value.

16 When comparing the cut types studied here, the results show that there is no
 17 significant difference in the shear elastic modulus of ER, BR and TR ($p > 0.3$). On the
 18 other hand, the elasticity of SL and TF have a significant difference between them ($p <$
 19 0.01) and with all other cut types ($p < 0.001$ and $p < 0.01$ respectively).

20 Regarding the ER, the TR and the BR, these three cut types are located on the
 21 external and posterior face of the leg. Particularly, the ER is in the posterior face of the

1 thigh and the primary muscular plane that comprises it is the semitendinosus muscle.
2 The TR is located on the external face of the thigh region, and its primary muscular
3 plane is the biceps femoris muscle. On the other hand, the BR is a piece that is
4 comprised between the ER and the TR. Thus, it is located on the external and posterior
5 face of the thigh region, and its main muscular planes are the biceps femoris and
6 semitendinosus muscles[33]. Therefore, from this point of view, it makes sense that the
7 elasticity values of these cuts have not reflected significant differences between them.

8 The TF is also located in the thigh region, but its location is different from that
9 of the ER, BR and TR. The TF is in the anterior face of the thigh, being its primary
10 muscle plane the femoral quadriceps muscle [33]. Therefore, although TF also belongs
11 to the thigh region, it is much less interrelated, both regionally and functionally, with
12 the ER, BR and TR. It is therefore reasonable that the measurements of shear elasticity
13 would be significantly different between these cut types and TF.

14 SL is located away from the thigh. It is in the region of the lumbar back and the
15 muscular planes that compose it are mainly the iliocostalis, longissimus dorsi,
16 multifidus dorsi and serratus dorsalis muscles (according to distance of the cut from the
17 eye muscle) [33]. From a functional point of view, these muscles act in the posture
18 maintenance, unlike the muscles mentioned above which are related to locomotion.
19 Thus, it is not surprising that the shear elastic modulus is significantly different between
20 SL and all other samples. This result agrees with previous works which conclude that
21 the tenderness of beef samples is correlated to their anatomical location. [34]-[36].
22 Among other factors, this is related to the distribution of different fiber types due to the
23 muscle role in the live animal, which influences its mechanical properties. The muscles
24 involved in posture are more oxidative than those involved in movement [37], [38].

25 The rate of change of the shear modulus is a property of interest for industry
26 because its value allows describing the ageing time for each cut type. In addition, it
27 could be coupled with other factors for decision making about the commercialization
28 times and therefore affects the commercialization value.

29 When taking the derivative of the data displayed in figure 7, the result is noisy,
30 and conclusions are hard to take. This is because the derivative operation amplifies the
31 noise of the original data [39]. The uncertainty in our data came from different sources.
32 Prior to the study reported here, a set of measurements were performed in order to
33 estimate the incidence on the uncertainty of the inherent electronic noise and the
34 algorithm that computes the surface wave speed. We took 5 hindquarter samples

1 (different from those of the aging study) and performed 64 measurements on each one.
2 They were performed in a single day and under the same conditions of temperature,
3 applied stress and the device's position over the sample. The applied stress was
4 controlled by placing the device on a mechanical arm coupled to a stepper motor. A
5 strain gauge at the bottom of the device allows measuring the applied stress. Under
6 these controlled experimental conditions, the standard deviation represents between
7 0.5% and 1% of the mean value in all cases depending on the cut type.

8 Other sources must be added when tracking the samples over time. Ideally, the
9 samples should all be measured under the same conditions of the controlled experiments
10 mentioned above, regarding mainly the temperature and applied stress (i.e., the pressure
11 exerted over the sample). However, as mentioned before, there are temperature
12 fluctuations due to door opening and varying external conditions. In addition, as
13 measurements were made freehand as shown in figure 4(b), the applied stress conditions
14 are not necessarily the same in each measurement. The boundary conditions affect the
15 internal stress of the sample and, as a consequence, the surface wave speed value. When
16 the device is manually operated, the strain gauge avoids sharp variations in the
17 boundary conditions. Nevertheless, it is not possible to ensure constant external pressure
18 for each measurement. Therefore, the final uncertainty is greater than the obtained in the
19 controlled experiments. In future versions of the device, we will address this issue.
20 Finally, due to the small number of samples for each cut type, the influence of the
21 variations of a single sample on the average is relevant.

22 One way to filter out the noise amplification in the time derivative operation is
23 to take larger time steps to compute the difference of the shear elastic modulus. We took
24 a time step of five days to compute the derivative. The result of this operation is
25 displayed in figure 8 for each cut type. The rate of change decreases as a function of
26 time.

27 We performed the one-way ANOVA analysis intra sample at the beginning and
28 at the end of measurements. For each time interval, the results show that there is no
29 significant difference in the slope of the shear elastic modulus between samples of the
30 same cut type ($p > 0.3$ in all cases). This result is consistent with the origin of the
31 samples used in this study, where all belong to the same breed, age, gender and feeding
32 type. It is known that the mechanical properties depend on all these parameters [8],[9].
33 Our research does not include yet these variables, but this point will be addressed in
34 future works.

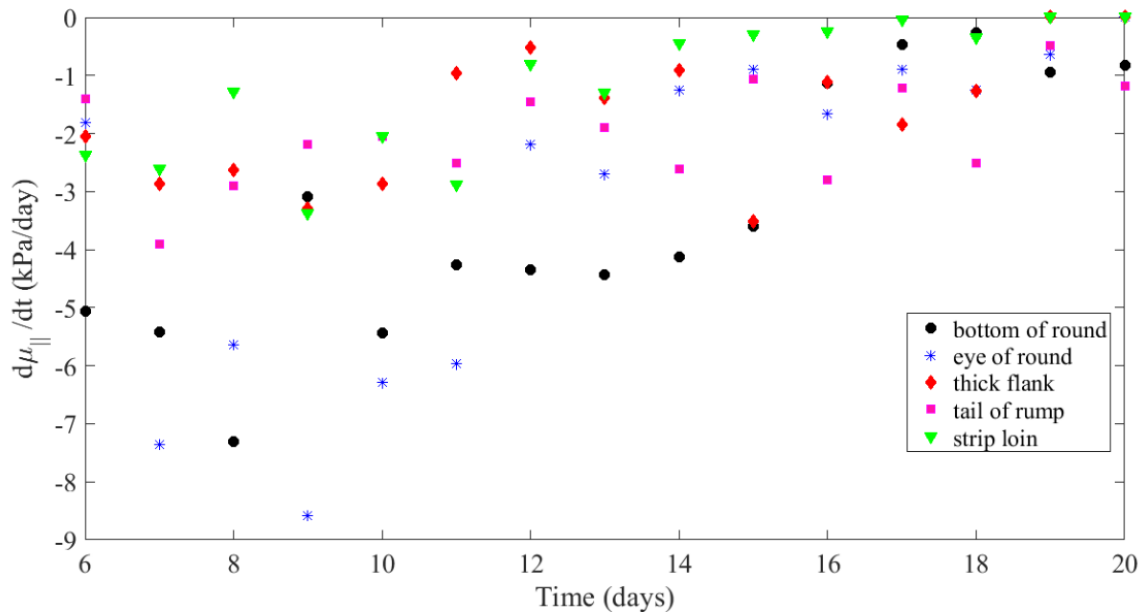


Figure 8. Rate of change of the shear modulus for different cut types.

The statistical analysis show that the slope at the end of measurements is significantly different from the initial slope ($p < 0.03$) for all cut types, except for the TF ($p > 0.3$). Indeed, the rate of decay for TF has not significant differences throughout the whole period, meaning that its shear modulus decreases with the same rate. Presumably, this sample will continue to decrease its elasticity value beyond the 21 days covered by this study. The number of samples should be increased to know if this is a casuistic result found in this work or if it is a characteristic behavior of this cut type.

For the other cut types, we tested the hypothesis of the final slope be equal to the slope at previous days. The results show that the slope of the SL does not differ significantly from itself from day 13 onwards ($p > 0.7$). Regarding the ER, BR and TR, their slope remains unchanged from day 14 ($p > 0.4$), 18 ($p > 0.5$) and 16 ($p > 0.5$) respectively. Thus, the results show that the cut types analyzed in this work have different maturation times. This agrees with previous studies showing that different muscles need different maturation times to reach minimum values in shear force tests [40].

5. Conclusion

To the best of our knowledge, this is the first time that surface wave elastography is employed in monitoring the mechanical properties of beef samples during aging. The NU-SWE method used in this study assumes that the beef is a lossless elastic medium. Thus, the only mechanical property monitored was the shear

1 elasticity. We performed a comparison between the surface wave method and a bulk
2 shear wave method on a single sample. The results show good agreement between them
3 although it is necessary to increase the number of samples in the study to provide more
4 reliable results.

5 The results show that, after 21 days of ageing time, the samples achieve a
6 significant reduction of their shear elasticity. The reduction is close to 50% for all
7 samples. In addition, we show that the shear elasticity of the cut types studied is
8 different depending on the functionality of the muscle in the live animal.

9 Regarding the rate of change of the shear elasticity, the results show that the
10 slope is greater in the first days of ageing. It decelerates at the end of measurements.
11 The SL is the first to reach a constant decaying rate after 13 days of maturation. The
12 other cut types require a longer time. The behavior of the TF is different. Its shear
13 elasticity decreases at the same rate throughout the whole period covered.

14 The results reported here are reasonably well consistent with previous
15 knowledge in this area, considering that there is no general agreement about the
16 maturation time required for beef samples or about the rate of change in their
17 mechanical properties. Nevertheless, the surface wave elastography device needs
18 improvements in order to reduce the uncertainty on the measurements when operated
19 freehand.

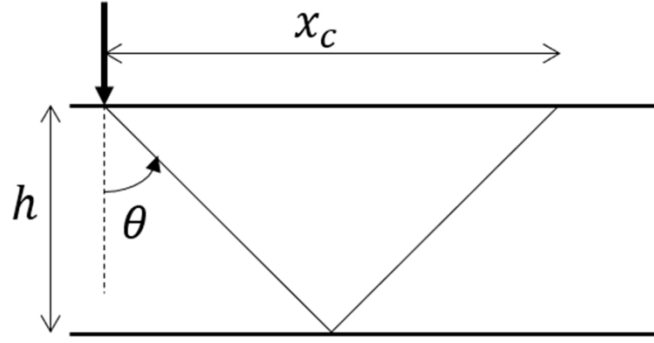
20 **Acknowledgments**

21 We thank Patricia Lema for letting us use the coldchamber at her lab in the Engineering
22 Faculty and Rafael Piriz for providing us the beef samples. We also thank Project ANII
23 FMV_1_2019_1_155527 for let us use their Aixplorer device. This work was supported
24 by Comisión Sectorial de Investigación Científica(CSIC, Uruguay) and by ITP Ltd.

26 **Appendix**

27 Guided wave propagation in finite plates takes place after reflection back and
28 forth on the bounded surfaces. Often, reflections give birth to mode conversion between
29 P and SV waves which generate a complicated pattern of interference within the plate
30 and dispersion curves for each mode with their respective cutoff frequency. However,
31 due to the difference in orders of magnitude on the speed of P and S waves in soft
32 tissues, there is no mode conversion [27]. In addition, due to the dipole characteristic of

1 the line source for shear waves, most part of the energy is carried in a specific angle
 2 which depends on the symmetry of the tissue [26].



3
 4 **Figure A.1** Schematic representation of wave propagation in soft tissues. Within the volume of the
 5 sample, most part of the energy is carried as an SV wave which propagates in a specific angle with
 6 respect to the source direction. Consequently, there exists a distance x_s on the surface where only surface
 7 waves propagate.

8 Figure A1 shows schematically this situation. There exists a distance $x_c =$
 9 $2h \tan(\theta)$ over the surface of the sample where only surface waves propagate. For
 10 transversally isotropic solids, like skeletal muscle, $\theta \cong 60^\circ$. The mean depth in our
 11 samples is $h = 4$ cm. Therefore, the distance $x_c \cong 12$ cm. Thus, for $0 < x < x_c$, the
 12 vertical displacement of the surface is composed by the interference of the Rayleigh and
 13 the leaky surface wave: [27], [40].

14
$$u_z(x, t) = A_R [e^{-ikx} + A_L e^{-\zeta x} e^{-iqx}] e^{i\omega t} \quad (\text{A. 1})$$

15 where A_R is the amplitude and k the wave number of the Rayleigh wave, A_L is the
 16 relative amplitude between the leaky wave and the Rayleigh wave, ζ and q are the
 17 imaginary and real parts of the leaky wave wavenumber, respectively. The phase $\phi(x)$
 18 of the quantity between brackets is $\phi(x) = \text{atan}(N(x)/D(x))$ where:

19
$$N(x) = -[\sin(kx) + A_L e^{-\zeta x} \sin(qx)] \quad (\text{A. 2})$$

20
$$D(x) = \cos(kx) + A_L e^{-\zeta x} \cos(qx) \quad (\text{A. 3})$$

21 The phase speed is, therefore:

22
$$V_s(\omega) = \omega \left(\frac{\partial \phi}{\partial x} \right)^{-1} = \omega \left[\frac{D^2 + N^2}{N'D - ND'} \right] \quad (\text{A. 4})$$

23 After some computation, the expression for the phase speed is:

$$\begin{aligned}
& V_s(\omega) \\
& = \frac{\omega}{k} \left\{ \frac{1 + A_L^2 + 2A_L \cos((k - q)x - \epsilon)}{1 + A_L \left[(\chi + 1) \cos((k - q)x - \epsilon) + \left(\frac{\delta}{k}\right) \sin((k - q)x - \epsilon) + \chi A_L \right]} \right\} \quad (A.5)
\end{aligned}$$

where $\chi = q/k$, $\delta = \zeta k$, and ϵ is a phase constant between the Rayleigh and leaky waves. Experimentally, we measure the phase speed of the surface wave within the frequency bandwidth of the source. The experimental curve is fitted, in a least squares sense, with the theoretical phase speed computed from equation (A.5). Thus, V_b is obtained as the value which produces the best fit.

References

- [1] K. Takahashi, "Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: The non-enzymatic mechanism of meat tenderization", *Meat Science* 43, No. 5, S67-S80, (1996).
- [2] E. H. Lonergan, W. Zhang and S. M. Lonergan, "Biochemistry of post-mortem muscle. Lessons on mechanisms of meat tenderization", *Meat Science* 86, 184-195, (2010).
- [3] C. E. Byrne, D. J. Troy and D. J. Buckley, "Post-mortem changes in muscle electrical properties of bovine M-longissimus dorsi and their relationship to meat quality attributes and pH fall", *Meat Science*, 54, 23-34, (2000).
- [4] M. C. Lanari, A. E. Bevilacqua and N. E. Zaritsky, "Changes in tenderness during aging of vacuum packaged beef", *J. Food Processing and Preservation*, 11 (2), 95-109, (1987).
- [5] C. L. Bratcher, D. D. Johnson, R. C. Littell and B. L. Gwartney, "The effects of quality grade, aging and location within muscle on Warner-Bratzler shear force in beef muscles on locomotion", *Meat Science*, 70, 279-284, (2005).
- [6] K. Palka, "The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility bovine semitendinosus muscle", *Meat Science*, 64, 191-198, (2003).
- [7] J. Lepetit and J. Coulioli, "Mechanical properties of meat", *Meat Science*, 36, 203-237, (1994).
- [8] D.L. Hopkins and G.H. Geesink, "Protein degradation post-mortem and tenderization" chapter 8 of "Applied muscle biology and meat science" Edited by M. Du and R. J. McCormick, CRC Press, Boca Raton, FL, (2009).
- [9] M. Stuby-Souva, "Tenderness and ageing response of beef muscles of different quality grades before and after freezing", Doctoral dissertation, Oklahoma State University, Chap 2, (1994).

- 1 [10] R. Field, "Effect of castration on meat quality and quantity", *J. Animal Science*, 32,
2 849-858, (1971).
- 3 [11] M. Koohmaraie, S. Seideman, J. Schollmeyer, T. Dutson and A. Babiker, "Factors
4 associated with the tenderness of three bovine muscles", *Food Science*, 53, 407-410,
5 (1988).
- 6 [12] J. Ophir, I. Céspedes, H. Ponnekanti, Y. Yazdi and X. Li, "Elastography: A method
7 for imaging the elasticity of biological tissue", *Ultrason. Imaging*, 13, 111-134, (1991).
- 8 [13] B. S. Garra, I. Céspedes, J. Ophir, S. Spratt, R. Zuurbier, C. Magnant and M.
9 Pennanen, "Elastography of breast lesions: initial clinical results." *Radiology*, 202, 79-
10 86, (1997)
- 11 [14] A. Athanasiou, A. Tardivon, M. Tanter, B. Sigal-Zafrani, J. Bercoff, T. Deffieux, J-
12 L. Gennisson, M. Fink and S. Neuenschwander, "Breast lesions: Quantitative
13 elastography with supersonic shear imaging – Preliminary results", *Radiology*, 256,
14 297-303, (2010).
- 15 [15] L. Castera, X. Forns and A. Alberti, "Non-invasive evaluation of liver fibrosis
16 using transient elastography", *J. Hepat.*, 48, 835-847, (2008).
- 17 [16] J. Ophir, S. Alam, B. Garra, F. Kallel, E. Konofagou, T. Krouskop and T.
18 Varghese, "Elastography: Ultrasonic estimation and imaging of the elastic properties of
19 tissues", *Proc. Inst. Mech. Eng. Part H.: J. Eng. Medicine*, 213, 203-233, (1999).
- 20 [17] J. Bercoff, M. Tanter and M. Fink, "Supersonic shear imaging: A new technique
21 for soft tissue elasticity mapping", *IEEE Trans. Ultras. Ferroelec. Freq. Control*, 51,
22 396-409, (2004).
- 23 [18] J. Ophir, R. Miller, H. Ponnekanti, I. Céspedes and A. Whittaker, "Elastography of
24 beef muscle", *Meat Science*, 36, 239-250, (1994).
- 25 [19] S. Catheline, J-L. Gennisson, G. Delon, M. Fink, R. Sinkus, S. Abouelkaram and J.
26 Culioli, "Measurement of viscoelastic properties of homogeneous soft solid using
27 transient elastography: An inverse problem approach", *J. Acoust. Soc. Am.*, 116, 3734-
28 3741, (2004).
- 29 [20] J-L. Gennisson, S. Catheline, S. Chaffai and M. Fink, "Transient elastography in
30 anisotropic medium: Application to the measurement of slow and fast shear wave speed
31 in muscles", *J. Acoust. Soc. Am.*, 114, 536-541, (2003).
- 32 [21] A. Ayadi, J. Culioli and S. Abouelkaram, "Sonoelasticity to monitor mechanical
33 changes during rigor and ageing", *Meat Science*, 76, 321-326, (2007).
- 34 [22] X. Zhang and J. Greenleaf, "Estimation of tissue's elasticity with surface wave
35 speed", *J. Acoust. Soc. Am.*, 122, 2522-2525, (2007).
- 36 [23] G. Grinspan, S. Aguiar and N. Benech, "Optimization of a surface wave
37 elastography method through diffraction and guided wave effects characterization", *J.*
38 *Phys: Conference Series*, 705, 012014, (2016).

- 1 [24] J. Martin, S. Brandon, E. Keuler, J. Hermus, A. Ehlers, D. Segalman, M. Allen and
2 D. Thelen, “Gauging force by tapping tendons”, *Nature communications*, 9:1592,
3 (2018).
- 4 [25] T. Ikeda, P-K. Choi, T. Ishii, I. Arai and M. Osawa, “Firmness evaluation of
5 watermelon flesh by using surface wave elastic waves”, *J. Food Eng.*, 160, 28-33,
6 (2015).
- 7 [26] N. Benech, G. Grinspan, S. Aguiar and C. Negreira, “Surface wave elastography:
8 Device and method”, *Meas. Sci. and Tech.*, 30 (3), 035701, (2019).
- 9 [27] N. Benech, J. Brum, G. Grinspan, S. Aguiar and C. Negreira, “Analysis of the
10 transient surface wave propagation in soft solids elastic plates”, *J. Acoust. Soc. Am.*,
11 142, 2919-2932, (2017).
- 12 [28] D. Royer and E. Deulesaint, “Elastic waves in solids I: Free and guided
13 propagation”, Springer-Verlag, Berlin, (2002).
- 14 [29] N. Benech and R. Piriz, “Study of mechanical properties of vacuum sealed beef
15 samples using surface wave elastography” 2ndIberoamerican Congress on Food
16 Engineering (in Spanish), CIIAL, Punta del Este, Uruguay, (2016).
- 17 [30] J. Bercoff, M. Tanter and M. Fink, “Supersonic shear imaging: a new technique for
18 soft tissue elasticity mapping”, *IEEE Trans. Ultras. Ferroelec. Freq. Control*, 51, 396-
19 409, (2004).
- 20 [31] D. G. Altman and J. M. Bland, “Measurement in medicine: The analysis of method
21 comparison studies”, *The Statistician*, 32, 307-317, (1983).
- 22 [32] D. Yates, D. Moore and D. Starnes, “The practice of statistics”, W.H. Freeman &
23 Co. New York, (2008).
- 24 [33] Manual de Carnes Bovina y Ovina. Handbook of Uruguayan Meat. Instituto
25 Nacional de Carnes, available
26 at:<http://www.inac.uy/innovaportal/v/1852/4/innova.front/manual-de-carnes-bovina-y-ovina> ,
27 INAC, (2004).
- 28 [34] D.W Zinn, C.T. Gaskins, G.L. Gann, and H.B. Hendrick. “Beef muscle tenderness
29 as influenced by days on feed, sex, maturity and anatomical location”. *J. Anim. Sci.* 31,
30 307-309, (1970).
- 31 [35] F.K. McKeith, D.L. DevoL R.S. Miles. P.J. Bechtel. and T.R. Carr. “Chemical and
32 sensory properties of thirteen major beef muscles”. *J. Food Sci.* 50, 869-872, (1985)
- 33 [36] J.B.Morgan, J.W. Savell, D.S. Hale. R.K. Miller. D.B. Griffin. H.R. Cross and S.D.
34 Shackelford, “National beef tenderness survey”*J. Anim. Sci*, 69, 3274-3283, (1991).
- 35 [37] G. K.Totland and H.Kryvi, “Distribution patterns of muscle fibre types in major
36 muscles of the bull (*Bos taurus*)”. *Anatomy and Embryology*, 184, 441-450, (1991).
- 37 [38] P. Henckel, “Perimortal metabolic events and consequences for meat quality.
38 Proceedings 2nd Dummerstorf Muscle Workshop Muscle Growth and Meat Quality”,
39 Restock, 17-19 May 1995, 77-82, (1995).

- 1 [39] F. Kallel and J. Ophir, “A least-squares strain estimator for elastography”, *Ultras.*
- 2 *Imaging*, 19, 195-208, (1997).
- 3 [40] M. A. Stuby-Souva, “Tenderness and aging response of beef muscles of different
- 4 quality grades before and after freezing” (Doctoral dissertation, Oklahoma State
- 5 University). CHAP. 3, (1994).
- 6 [41] C. Schröder and W. Scott Jr., “On the complex conjugate roots of the Rayleigh
- 7 equation: The leaky surface wave”, *J. Acoust. Soc. Am.*, 110, 2867-2877, (2001).