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WHCtrend, an up-to-date method to measure water holding capacity in meat

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Abstract

An up-to-date procedure to standardize the measurement of the water holding capacity of meat, including new parameters and a new instrument, are proposed to simplify and standardize the use of the filter paper press method. The new instrument “WHCtrend instrument” employed Video Image Analysis, and a video camera placed above a compression system to measure the area formed by 250 mg of homogenised meat. For measurements, an image was acquired at time = 0 s, and every 15 s for 10 min after compression with a 500 N force. The meat total area was easily distinguishable from the white background. A dynamic measurement of fluid release over time was obtained and was called “WHCtrend”. The new procedure was tested on different meats and the Pearson correlation coefficients between meat quality parameters and WHCtrend parameters were found to be significant. The “WHCtrend instrument” could be useful to rapidly screen meat water holding capacity and improve meat quality control.

Keywords: Water holding capacity; Meat quality; Filter paper press method; Instrument

1. Introduction

One of the most important factors affecting the economic value and quality of meat is the water holding capacity (WHC). WHC affects the weight change during transport and storage, drip loss during thawing, weight loss and shrinkage during cooking, juiciness and tenderness of the meat (Gault, 1985; Lawrie, 1985). Additionally, WHC is closely related to colour, texture and firmness of raw meat as well as the eating properties of cooked meat (Hughes, Oiseth, Purslow, & Warner, 2014). Drip loss originates from the spaces between muscle fibre bundles and the perimysial network, as well as the spaces between muscle fibres and the endomysial network. Fibers become less fluid with less ability to hold moisture tightly after the development of rigor, when muscles convert to meat. It is well known that excessive drip exudation and soft texture result from the combination of rapid pH decline and high temperature in post mortem muscle. This mostly occurs in pork which contains greater relative proportions of type II muscle fibres compared to beef or lamb (Joo, Kim, Hwang, & Ryu, 2013).

Many methods for measuring the WHC of meat have been employed (Kauffman, Eikelnboom, van der Wal, Engel, & Zaar, 1986; Honikel, 1998; Trout, 1988). The filter paper press method (FPPM) is a well-known method to test this parameter (Grau, & Hamm, 1956). It assumes that, as the meat is pressed, the fluid absorbed by the filter paper around the meat sample forms an outer ring zone (RZ), which is proportional to the quantity of loosely bound-water in meat. Later, Hofmann, Hamm, & Blüchel (1982) observed that the RZ does not correspond to the total amount of liquid exuded, but only to a fraction of it, as the the meat area (MA) also absorbs an amount of liquid. Therefore, they proposed to measure WHC as the ratio of meat film area to total area (TA = RZ + MA). This ratio accounts for the fluid under the meat film area and shows how much of the total area is covered by the MA independently of the sample weight.

The FPPM is advantageous as it is easy to carry out and can be applied to small meat samples. However, the FPPM has been interpreted and adapted in different ways (Zapotoczny, Kozera, Karpiesiuk, & Pawłowski, 2014; Jung et al., 2015). Some authors (Irie et al., 1996; Fiems, De Campeneere, Van Caelenbergh, De Boever, & Vanacker, 2003) utilized different types of filter paper and amounts of meat, which were placed under different pressures for different compression times. An important limitation is that the MA and TA are often measured using a planimeter (Irie, Izumo, & Mohri, 1996) and, at times, these areas are outlined using a pencil. Although this procedure enables measurements after filter paper has dried out; it is highly inefficient, time-consuming and imprecise. The use of an optical electronic system (Video Image Analyzer or VIA) to measure these areas represents a progress, since VIA is a rapid and accurate technique. However, it is difficult to make the procedure perfectly automatic because small changes in lighting can affect the measurements (Irie et al.,1996; Chmiel, Słowiński, & Dasiewicz, 2011; Zapotoczny et al., 2014). Further improvements have been evaluated by other authors who have applied different formulas to measure WHC (Wierbicki, & Deatherage, 1958; Hofmann et al., 1982; Van Oeckel et al., 1999) and developed an apparatus for controlling the pressure conditions (Wierbicki, & Deatherage, 1958).

2. Objective

The importance of having a fast and simple measurement of WHC led the author to modify, simplify and standardize the operating conditions of the press method by measuring samples with video image analysis.

An instrument was developed to standardize the FPPM, and a simple protocol was applied to speed up the WHC measurement. The present study describes the instrument and the protocol used to measure the meat film and total area. In addition, new parameters were applied on a large set of commercial meats to explore the wide range of characteristics that could affect the measurement, such as color and fluid content.

3. Materials and methods

The protocol, parameters and instrument were tested on 390 samples obtained from the *longissimus thoracis* of 390 animals (202 bulls, 105 calves, 51 steers, 32 pigs), different for species and commercial category. All meat samples were aged for 7 days, vacuum packaged and frozen (-20 °C). Before the analyses, which were conducted in triplicate, samples were thawed for 48 h at 2-4 °C.

The employed materials were: a machine to homogenize (600 rpm for 20 s; La Moulinette 800W, Moulinex); 125 mm ∅ filter paper (Whatman n° 1001) previously dried in the oven at 105 °C and maintained in a desiccator until the analysis started; Petri lids and dishes to prevent the homogenized meat from drying; a scale to weigh 250 mg homogeneized meat sample; an average processing capacity personal computer; a prototype instrument “WHCtrend instrument” and software assembled by the author.

The following parameters were measured. On raw meat, the total moisture content (% of water, oven-dried meat) thaw and drip loss (%). The free water (FW) was the percentage of water contained in the ring area out of the total moisture content (TMC), according to Wierbicki & Deatherage (1958). These authors reported the formula [1] where 0.09470518941 mg of water/mm^2 was the regression coefficient (TA_{600} compressed total area after 10 min, MA compressed meat area).

[1] $$FW = ((TA_{600} - MA) * 0.09470518941) / (\text{weight sample} * TMC) *100$$

On cooked meat cooking loss, cooling loss and residual water (%) obtained by the compression of the cooked sample during the measurement of hardness (Barbera, & Grigioni, 2014), Meat Cooking Shrinkage (MCS; %) (Barbera, & Tassone, 2006), hardness (N) assessed by Stress Resistance and Relaxation method (Prandi, & Barbera, 2009), colour by a Spectrophotometer CM-600d (Minolta

Camera Co., Tokyo, Japan) using a standard white tile (Cielab: L*, a*, b*, chroma and hue; ∅ 8 mm, Illuminant D65, 10° Observer).

The parameters described above were analyzed by SAS software version 9.4 (SAS, 2018). The procedures PROC CORR and PROC GLM were employed. Tukey test for multiple LSMeans comparisons, multivariate analysis, contrast and Pearson's correlation coefficients were performed. To measure the repeatability of the test, the same sample was measured three times (k = 3) and analyzed with GLM procedure using the following linear model $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ where: Y_{ij} were the measured parameters; α_i were the samples (390); ε_{ij} the residuals to obtain the variance of error which was used to calculate the standard deviation of repeatability. The repeatability of the parameters obtained by the proposed method was expressed as the within sample coefficient of variation (CV) equal to the standard deviation of error divided by the mean. Statistical analysis of data from the "WHCtrend instrument" is detailed later in the manuscript.

4. Results

4.1 The equipment

First results of the research were implementation of a prototype "WHCtrend instrument". The equipment consisted of the prototype, a computer, and a scale (Figure 1).

The "WHCtrend instrument" was a box (H = 70 cm; W = 40 cm; D = 37 cm) serving as a darkroom. It contained a video camera placed above the compression system, a light source, two removable translucent plexiglass plates, a press with a load cell incorporated to control the compression force, and a control lever attached to a disc mounted eccentrically under the load cell to enable compression (Figure 2). Externally to the box there were the load indicator, a slot to position the filter paper and cables to connect it to the computer. A scale was also connected to the computer.

To improve the precision of the measurement and increase image resolution, a high-resolution video camera, video card and monitor were preferred. The system used a display with a resolution of 1024x768 pixels. Analysis with the "WHCtrend instrument" required an initial quantitative and qualitative calibration session. Quantitative calibration consisted in measuring an image of an item of known surface area (2,500 mm^2) in order to assign a unit value to the pixels that formed the image. Qualitative calibration was run by acquiring an image of the empty filter paper in the press. This was used as background to optimize and simplify the separation of the TA from the white filter paper. The image of the empty white filter paper was subtracted from each image of total area; therefore, the system was independent from any fluctuations in lighting between one session and another. In addition, no special filters were necessary to process the image and determine the TA, unlike what has been proposed by other authors (Irie et al.,1996; Chmiel et al., 2011; Zapotoczny et al., 2014). In accordance with Honikel, & Hamm (1994), a 500 N load (about 51 kg) was applied to the sample and the compression force was kept constant during the test.

Operation was immediate and little affected by the quality of lighting, and the software showed on screen the graph of the increase in the total area. The sample was pressed for 10 min, instead of 5 min (as reported by Honikel, & Hamm, 1994), because according to our tests the trend of the TA continued to increase. With an average MA of 776 mm^2 (Table 1), the pressure applied was about 64.5 N/cm^2.

Dedicated software developed by the author allowed the user to insert the sample code; then it automatically recorded the weight of the meat sample from the scale, controlled and measured load, time, surface measurement, image capture and image processing. Storing of the data started automatically, as soon as the load cell exceeded the 400 N trigger point at time 0 s after manual activation of the control lever. As preliminary research showed at 500 N a halo, albeit slight, around the first image, the 400 N limit was used to prevent it and to compensate for the slight delay in the response of the instrument. This is important because the 0-time image is a TA but also the MA that will be subtracted from the TA of the successive images.

The user could set the trigger load to start the first image acquisition, the interval between images, and the analysis duration. The first image was obtained and measured at time = 0 s and subsequent images every 15 s for 10 minutes. The 15 s interval depended on the processing power of the computer. By the end of the procedure 41 images and areas were recorded.

During each measurement, the data were saved in a file created for each session. Each file included six variables: sample code, replication, load, time, MA and TA. The resulting file was in the ".txt" format and could be acquired by any software for further processing. A SAS program was written to acquire the “.txt” files and process the dataset to express the WHC by the selected parameters.

4.2 The protocol

The raw meat samples were obtained from the MCS protocol (Barbera, & Tassone, 2006), an experimental analysis to measure meat cooking shrinkage. External fat was trimmed off from raw meat (about 80 g), which was then chopped, homogenized (600 rpm for 20 s), and stored in a Petri dish at 2-4 °C before analysis.

On the scale, controlled by the dedicated software, 250±10 mg of homogenized meat sample was weighed on a dried filter paper sheet (Whatman n° 1001). The filter paper plus the meat were immediately placed between two removable translucent plexiglass plates in the “WHCtrend instrument” for the 10 min compression, until a repeated sound signalled the end of the analysis. The software did not permit the compression if the sample weight did not fall in the correct range. At the end of the procedure, the two removable plates were removed, cleaned and repositioned for a new analysis.

4.3 The traditional and new measured parameters

From each image collected during the 10 min test, 41 MA and TA (Figure 3) were measured to express WHC. The area (mm^2) which formed on filter paper at start (first image at 0 s), was the MA and after 10 min the largest area was the total area (TA_{600}). The MA remained constant throughout the test according to Honikel & Hamm (1994) which only pressed the sample for 5 min. At the end of the 10 min, the area of wet paper (in mm^2) around the MA was defined as the Ring (TA_{600} - MA) and in percentage as the Ring% [(TA_{600} - MA) / TA_{600}] (Grau, & Hamm, 1956). The MA / TA ratio (Hofmann et al., 1982) was here named the MA / TA_{600} ratio.

Measuring the total area every 15 s for 41 times allowed the opportunity to evaluate the dynamics of fluid release. To recognize a faster or slower fluid release trend could help understand, in terms of quality, the consumer's perception of meat goodness. A new parameter was proposed: the WHCtrend. It represents the fluid that was squeezed out of the meat over time, as described by Hofmann et al. (1982), and described by the equation [2]

[2] $$\text{WHCtrend} = k_0 + k_1 * \text{time} + k_2 * \text{Ln}(\text{time})$$

where: the time ranged from 0 to 600 s and was expressed as a natural logarithm (base e); "k_0" or intercept, estimated the MA at time = 0 s; "k_1" was the linear coefficient or slope; "k_2" was the coefficient that indicated the concavity of the curve until the maximum height. Coefficients were estimated for each of the three replications.

Two other new parameters were analysed: the trend of the ring expressed in mm^2 (Rt) and as a percentage of TA (Rp). As for the WHCtrend, the same equation [2] was applied but the intercept (k_0) was set to zero and for each repetition the two respective coefficients (Rt_1, Rt_2; Rp_1, Rp_2) were estimated.

The three applied models had R^2 coefficients of determinations of 0.97 to 0.99.

4.4 The repeatability

The mean values and CV for traditional and new parameters are reported in table 1. The CV was the residual variability of the three replications for the same sample resultant from sample preparation, operator, meat and instrument variability. The protocol to prepare the sample and the image-processing technique showed a repeatability ranging from 1.6 CV% for Load to 30.4 CV% for "WHCtrend k_1" and comparable to other parameters normally applied to meat quality analysis. A very high CV value was reported for the "Ring% trend Rp_1" (109.9 %) due to the mean value (0.007828) being close to zero. In this case, the coefficient of variation is very sensitive to small changes in the mean.

4.5 The application

Since the FPPM has been interpreted and adapted in several different ways (Zapotoczny, Kozera, Karpiesiuk, & Pawłowski, 2014; Jung et al. 2015) no comparison was made with a particular method. The "WHCtrend instrument" was used to verify its effectiveness in discriminating among species and commercial categories (Table 2). The different groups were selected as they present different WHC, meat and fluid colour. Since fluid colour is particularly pale for pork and chicken, identifying MA and TA with traditional software was particularly challenging for these types of meat.

Although sample weight and compression force were in the imposed tolerance range, they were significantly different among groups (Table 2). Thus, for the analysis of the remaining parameters, these two parameters were included in the model as covariates to improve precision in determining the group's effect.

The MA was significantly greater in pork than in beef by about 7 % despite pork being tougher than beef (hardness: pork 127.8 *vs* beef 112.1 N, $P < 0.0001$). The opposite outcome would be more likely, as the 500N load was constant throughout the analysis. However, the MA was measured on raw meat while the hardness on cooked meat. Cooking might change meat structure and its constituents differently in pork and beef; this might explain the unexpected outcome (Joo et al., 2013).

Other parameters in table 2 (MA/TA_{600}, Ring%, Ring, FW, Rp_2) confirmed the reduced availability of free fluid in pork compared to beef, with a distinction for steer (k_2, Rt_1, Rt_2, Rp_1).

The coefficients describing the three parameters WHCtrend, Ring and Ring% trend were also significantly different among groups and their curves are shown in figure 4 a, b, c, respectively. To decide which of the three new coefficients of parameters were more efficient in discriminating between the groups, a multivariate analysis of variance (MANOVA) for each group of coefficients was applied and the differences among three contrasts were measured: beef *vs* pork, bull *vs* steer and bull *vs* calf (Table 4). The best parameter to discriminate all the three contrasts was the WHCtrend, thus its use is suggested (Figure 4a).

The Pearson correlation coefficients between meat qualitative parameters and the “WHCtrend instrument” parameters were calculated (Table 3) to understand the correlations and the meaning of new proposed parameters estimating the content of free fluid in raw meat and the way it was released.

MA and k_0 correlated in the same direction, because MA measures and k_0 estimates the area of the compressed meat, and they also correlated positively with hardness. The meaning of this last correlation was not clear as a tougher meat would be expected to expand less under compression. Ring, ring%, FW, and k_2 estimate the content of free water in a similar way and positively correlated with drip loss, MCS, cooking and cooling loss and colour. The same qualitative parameters negatively correlated with k_1 to indicate the way water was released: lower meat qualitative parameters indicate a lower availability of free water at cooking and a faster release of available fluid, so a poor WHC.

Also, TA_{600} was positively correlated with MCS, cooking loss, cooling loss, residual water, hardness, L*, b*, and hue; negatively with thawing loss. The wider the surface, the greater were the values of quality parameters related to moisture loss with the exception of thawing.

MA negatively correlated with drip loss, cooking and cooling loss, a*, and chroma indicating that a larger MA was associated with a less red meat and lower losses in raw and cooked meat. Positive correlation with the residual water confirmed a greater WHC.

The colour attributes were associated to the pigment myoglobin and the lightness was related to the structural attributes of the muscle. These together determined that the reflected light and dark muscles were associated with a higher WHC (Table 3). This was confirmed in Table 3. A positive correlation was between L*, b* and Hue and parameters TA_{600}, Ring, Ring%, FW and k_2 to indicate a poor ability to retain free water, also confirmed by the negative correlation with k_1.

5. Discussion

5.1 The equipment

To verify the functionality of the method, the prototype, and the protocol, 390 commercial meat samples were measured. An issue was the presence of some unusual measures due to images being altered by electromagnetic fields from other instruments in the laboratory. A suitable shielding of transmission cables could be useful. To cope with this problem, the software was modified to show values and trend of the curve on the screen. As a result, it was possible to delete any erroneous measurement clearly visible from the graph on the screen.

Lighting quality was improved. To avoid the reflection of light on the translucent plexiglass plate an electroluminescent sheet was used. The lighting was not uniform (Figure 3), but light reflections were avoided. The qualitative calibration session neatly solved the problem and made the colour measurement unnecessary.

According to Honikel, & Hamm (1994), a 500 N load (about 51 kg) was applied, but in this study the compression time was doubled to 10 min because Honikel, & Hamm (1994) found that the fluid trend release increased for up to 7 min. Wierbicki, & Deatherage (1958) used a pressure of 345 N/cm^2 and found that the free moisture area increased for about 4 min. In this study, with a pressure of about 64.5 N/cm^2 the fluid trend release was still growing after 10 min (Figure 4a); therefore, it could be useful to increase the load to reach a plateau in the trend more rapidly.

5.2 The protocol

The WHCtrend was an automatized analysis and, once started, lasted for 10 min. Three replications were done in about 35 min (total time of analysis), including sample preparation. The procedure run by the “WHCtrend instrument” was not labour-intensive. In fact, the experimenter’s presence was only needed to initiate a new operation, when a sound would signal the end of the previous one. Homogenizing at 600 rpm for 20 s was important in order to easily weigh 250 mg and therefore have quantitatively comparable measures, to prevent the operator from choosing specific parts, and to avoid over-heating of the meat and fluid separation. Sometimes weighing 250±10 mg would be troublesome and time-consuming. This could cause meat dehydration and thereby could affect the measurement. For this reason, a Petri lid and dish was used to keep the homogenized meat in a closed environment and prevent dehydration. The samples were placed in a refrigerator at 2-4 °C. When analyzing multiple samples, it was recommended to analyse the first replicate for all available samples before making the second one and so on. This protocol was established to avoid an increase in release of fluid in the Petri dish during the waiting time. Unlike Grau, & Hamm (1956), 250 mg of meat were used instead of 300 mg, because the latter amount gave a too large and irregular total area. In a previous work, the behaviour of filter papers was analysed, highlighting how the Whatman 1001 was the most constant and regular in absorbing the released fluid (Tassone, Barbera, & Battaglini, 2004).

Measuring each image with a planimeter takes 25-64 s. Irie et al. (1996) established that the operating time for measuring one area by VIA is 15-16 s. This very long time was due to the old technology and filters used to process and measure the image and separate it into the two areas. The same could be done in less than 1 s using the “WHCtrend instrument”. In addition, newer hardware could reduce the acquisition time between two frames, improving measurement accuracy.

5.3 The traditional and new measured parameters

The measures of traditional parameters obtained with the “WHCtrend instrument” are standardized and constant, making it possible to obtain comparable results among different studies on the subject. The possibility of obtaining intermediate data also offers the opportunity to study the dynamics of fluid release which, as already indicated, could be associated with the qualitative perception of meat by the consumer.

Among the three new proposed parameters (WHCtrend, Ring trend and Ring% trend) the best parameter was the WHCtrend (Table 4). The meaning of the three coefficients was: intercept (k_0) or MA at the beginning of meat compression; k_1 was the tendency to give up the free water either slowly ($k_1 \to 1$) or immediately in the first minutes ($k_1 \to 0$); positive k_2 indicated the concavity of the curve upwards and greater ($k_2 \to 100$) or lesser ($k_2 \to 0$) availability of free water.

5.4 The repeatability

The repeatability (CV) of the parameters measured with the “WHCtrend instrument” is comparable with that obtained from other authors and is reported in table 5.

The repeatability (CV) varies between 1.4 and 70%. Particularly interesting is the data of Zapotoczny et al. (2014) which obtained a value of 2.6 %, using a planimeter to measure the water holding capacity expressed as the ratio of juice area to MA in pork samples. Bickerstaffe, Bekhit, Robertson, Roberts, & Geesink (2001), measured shear force on beef using a MIRINZ tenderometer and found a CV ranging from 11 to 45 %.

5.5 The application

The parameters measured with the 'WHCtrend instrument' have been found to be different among groups and, as expected, pork was different from beef. Wierbicki, & Deatherage (1958) measured the effect of pressure on the free moisture area trends after 1 min of compression, while varying the pressure from 69 (100 lb/inch^2) to 690 N/cm^2 (1000 lb/inch^2). They obtained a smaller free moisture area in pork than beef at 69 N/cm^2, similarly to the results obtained in the present work at 65 N/cm^2. When increasing the pressure up to 690 N/cm^2, the results were the opposite with larger free moisture area in pork than in beef. Therefore, pork needs more pressure to release free moisture. Authors selected a constant pressure of 345 N/cm^2 (500 lb/inch^2), for the determination of the water-binding capacities of fresh meats.

Since MA was significantly larger for pork (Table 2) while similar for the three beef samples, it is likely that the two meats have a different “sponge effect” (Puolanne, & Halonen, 2010; Farouk, Mustafa, Wu, & Krsinic, 2012).

Hughes et al. (2014) found no or poor correlation between the parameters related to the WHC and the water lost during cooking. However, Hughes et al. (2014) observed that the correlation between the WHC of the raw muscle and cooking loss can be quite high, but this correlation is dependent on cooking temperature. This indicates that the ability of raw meat to retain fluid is not the same as that of cooked meat. Muscle tissue is known to shrink laterally and longitudinally by different degrees at different temperatures indicating that not one, but several proteins are involved in the shrinkage and water expulsion observed during cooking. In the modelling of WHC in relation to cooking, Kondjoyan et al. (2014) attributed the increase in protein density and deformation between 38 and 54 °C to myosin, but denaturation and acceleration in deformation beyond 60°C to collagen. However, they state that in their model the anisotropic deformation (shrinkage) of beef is not explained by the corresponding meat ‘contraction’ associated with juice expulsion. Bouhrara, Clerjon, Damez, Kondjoyan, & Bonny (2012) again postulated a role for collagen in temperature-induced muscle shrinkage. This may explain why MCS and cooking loss are positively correlated ($r = 0.46$, $P < 0.0001$), whereas cooking loss is negatively correlated with some “WHCtrend instrument” parameters.

The work of Wierbicki, & Deatherage (1958) focused on the effect of pressing time on the meat film and the fluid trend release, but, due to operational difficulties, it was not thought of as a parameter of the trend itself. With the “WHCtrend instrument” it is possible to take into consideration also this parameter which, as previously mentioned, has been shown to be discriminating.

6. Conclusion

Many methods and different operating conditions of filter paper press method have been published in the literature, causing confusion in interpreting data in this field. The filter paper press method has the advantage of being rapid and simple but needed to be standardized. The “WHCtrend instrument” has solved this issue and has made it easier to uniformly compress the meat over time, while measuring the water release in a dynamic way. The adopted protocol clearly defines: meat quantity, filter paper type, applied load, compression time, area measurement method and the dependent variables used to express it. In this study, the test parameters have been controlled, so it was possible to define and standardize the working conditions and to automate the procedure. The application and the instrument were tested on different species and categories of meat and using the “WHCtrend instrument” made it easier to uniformly compress the meat over time and to differentiate meats that are indistinguishable from the colour of the fluid area. Last but not least, results obtained by a standardized procedure can be directly compared across studies. This could be useful for rapid screening of meat WHC and for improving meat quality control.

Water holding capacity is a very complex characteristic of meat and many factors influence its development, thus new parameters may help understand the underlying mechanism.

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Table 1. Means and coefficients of variation (CV) of the measured parameters by the “WHCtrend instrument”.

Parameters		Mean	CV %
Load	N	500.4	1.6
Sample weight	mg	251.8	1.7
Meat area (MA)	mm^2	775.6	7.3
Total area after 600 s (TA_{600})	mm^2	1348.1	4.9
MA / TA_{600}	%	57.8	7.4
Ring%	%	42.2	10.1
Ring	mm^2	572.5	12.2
Free water	%	28.6	12.3
WHCtrend k_0	mm^2	732.9	7.4
WHCtrend k_1	mm^2/s	0.2704	30.4
WHCtrend k_2	mm^2/s	73.870	19.8
Ring trend Rt_1	mm^2/s	0.3141	28.1
Ring trend Rt_2	mm^2/s	63.642	23.5
Ring% trend Rp_1	mm^2/s	0.007828	109.9
Ring% trend Rp_2	mm^2/s	6.022	21.5

Degree of freedom for error = 761;
k_0: intercept estimating the meat area at time = 0 s;
k_1, Rt_1, Rp_1: linear coefficient or slope;
k_2, Rt_2, Rp_2: coefficient indicating the concavity of the curve until the maximum height.

Table 2. LSMeans and comparison to test the effectiveness of the parameters measured by the "WHCtrend instrument" in discriminating among 4 meat categories on the *longissimus thoracis*.

Parameters		Bull	Calf	Pig	Steer	MSE
Load	N	500.3	501.2	500.9	498.7	41.17
Sample weight	mg	251.8^{A}	252.4^{A}	252.6^{A}	249.9^{B}	8.043
Meat area	mm^2	776.5^{B}	761.7^{B}	825.7^{A}	772.2^{B}	5317
Final total area (TA_{600})	mm^2	1351.9^{B}	1383.4^{A}	1268.3^{C}	1306.5^{C}	5076
MA/TA_{600}	%	57.7^{aBC}	55.3^{bC}	65.7^{A}	59.2^{B}	42.72
Ring%	%	42.3^{bAB}	44.7^{aA}	34.3^{C}	40.8^{B}	42.71
Ring	mm^2	575.5^{B}	621.7^{A}	442.6^{C}	534.4^{B}	11071
Free water	%	29.2^{bAB}	30.8^{aA}	23.3^{C}	27.2^{B}	26.80
WHCtrend k_0	mm^2	732.5^{B}	724.1^{B}	783.0^{A}	724.1^{B}	4324
WHCtrend k_1	mm^2/s	0.262^{bAB}	0.244^{bB}	0.358^{aA}	0.312^{bAB}	0.031
WHCtrend k_2	mm^2/s	75.4^{AB}	82.9^{A}	45.9^{bC}	65.4^{aBC}	772.7
Ring trend Rt_1	mm^2/s	0.307^{b}	0.283^{b}	0.402^{a}	0.360^{ab}	0.036
Ring trend Rt_2	mm^2/s	64.8^{bAB}	73.8^{aA}	35.7^{cC}	53.9^{bBC}	905.1
Ring% trend Rp_1	mm^2/s	0.0070^{B}	0.0032^{bB}	0.0222^{A}	0.0127^{aAB}	0.0004
Ring% trend Rp_2	mm^2/s	6.12^{bA}	6.79^{aA}	3.58^{B}	5.42^{bA}	6.144

Degree of freedom for error = 384;
A B C D = $P<0.01$ and a b c = $P<0.05$ on the same row;
k_0: intercept estimating the meat area at time = 0 s;
k_1, Rt_1, Rp_1: linear coefficient or slope;
k_2, Rt_2, Rp_2: coefficient indicating the concavity of the curve until the maximum height.

Table 3. Pearson correlation coefficients and probability between WHC parameters and meat qualitative parameters measured on the four meat categories.

Parameters	Means	N°	MA	TA_{600}	Ring	Ring%	FW	k_0	k_1	k_2
Thawing Loss (%)	5.5	308	NS	-0.13^{a}	NS	NS	NS	NS	0.14^{a}	NS
Drip loss (%)	3.8	336	-0.28^{A}	NS	0.26^{A}	0.28^{A}	0.27^{A}	-0.25^{A}	-0.23^{A}	0.25^{A}
Meat Cooking Shrinkage (%)	16.4	390	NS	0.16^{A}	0.13^{A}	0.12^{a}	0.14^{A}	NS	NS	0.12^{a}
Cooking loss (%)	17.3	371	-0.13^{A}	0.15^{A}	0.19^{A}	0.20^{A}	0.22^{A}	-0.15^{A}	-0.17^{A}	0.21^{A}
Cooling loss (%)	4.9	371	-0.23^{A}	0.12^{a}	0.23^{A}	0.24^{A}	0.22^{A}	-0.20^{A}	-0.11^{a}	0.18^{A}
Residual water (%)	24.2	195	0.25^{A}	0.35^{A}	NS	NS	NS	0.22^{A}	NS	NS
Hardness (N)	103.8	369	0.23^{A}	0.13^{a}	NS	-0.13^{A}	NS	0.23^{A}	NS	NS
L*	44.9	390	NS	0.24^{A}	0.19^{A}	0.13^{A}	0.18^{A}	NS	-0.16^{A}	0.18^{A}
a*	23.6	390	-0.11^{a}	NS	NS	0.11^{a}	NS	-0.18^{A}	NS	NS
b*	9.1	390	NS	0.21^{A}	0.20^{A}	0.19^{A}	0.23^{A}	-0.13^{A}	-0.10^{a}	0.19^{A}
Chroma	25.4	390	-0.12^{a}	NS	NS	0.13^{a}	0.12^{a}	-0.18^{A}	NS	NS
Hue	21.0	390	NS	0.28^{A}	0.24^{A}	0.19^{A}	0.26^{A}	NS	-0.15^{A}	0.22^{A}

A = $P<0.01$; a = $P<0.05$;
N°: number of observations; MA: meat area; TA_{600}: final total area; FW: free water; k_0: intercept estimating the meat area at time = 0 s; k_1: linear coefficient or slope; k_2: coefficient indicating the concavity of the curve until the maximum height.

Table 4. Multivariate analysis and contrast to test (Wilks' Lambda, Pr > F), by the regression coefficients, the best parameter among WHCtrend, ring trend, and ring% trend, to discriminate among meat categories.

Parameters	Cattle *vs* Pig	Bull *vs* Steer	Bull *vs* Calf
WHCtrend: k_0, k_1, k_2	<0.0001	<0.0001	0.0009
Ring trend: Rt_1, Rt_2	<0.0001	0.0251	0.0001
Ring% trend: Rp_1, Rp_2	<0.0001	NS	0.0054

Degree of freedom for error = 384;

k_0: intercept estimating the meat area at time = 0 s;

k_1, Rt_1, Rp_1: linear coefficient or slope;

k_2, Rt_2, Rp_2: coefficient indicating the concavity of the curve until the maximum height.

Table 5. Range of repeatability (CV) in a number of parameters obtained from other authors.

Authors	Year	Parameters	CV %
Bickerstaffe et al.	2001	shear force	11±45
Denoyelle & Lebihan	2003	tenderness	15±20
Denoyelle & Lebihan	2003	compression	15±24
Modzelewska-Kapituła et al.	2015	moisture content	1.4
Modzelewska-Kapituła et al.	2015	expressible water	12.8
Moelich et al.	2003	cooking loss	13±14
Moelich et al.	2003	peak shear force	11±13
Moelich et al.	2003	tenderness	8±31
Otto et al.	2004, 2006	drip loss	47±70
Palka & Daun	1999	fibre diameter	13±24
Palka & Daun	1999	sarcomere length	3±8
Târnâuceanu & Pop	2016	WHC	10
Zapotoczny et al.	2014	WHC	2.6

Figure 1. The “WHCtrend instrument” and the equipment (pc and scale) to improve the Filter Paper Press Method.

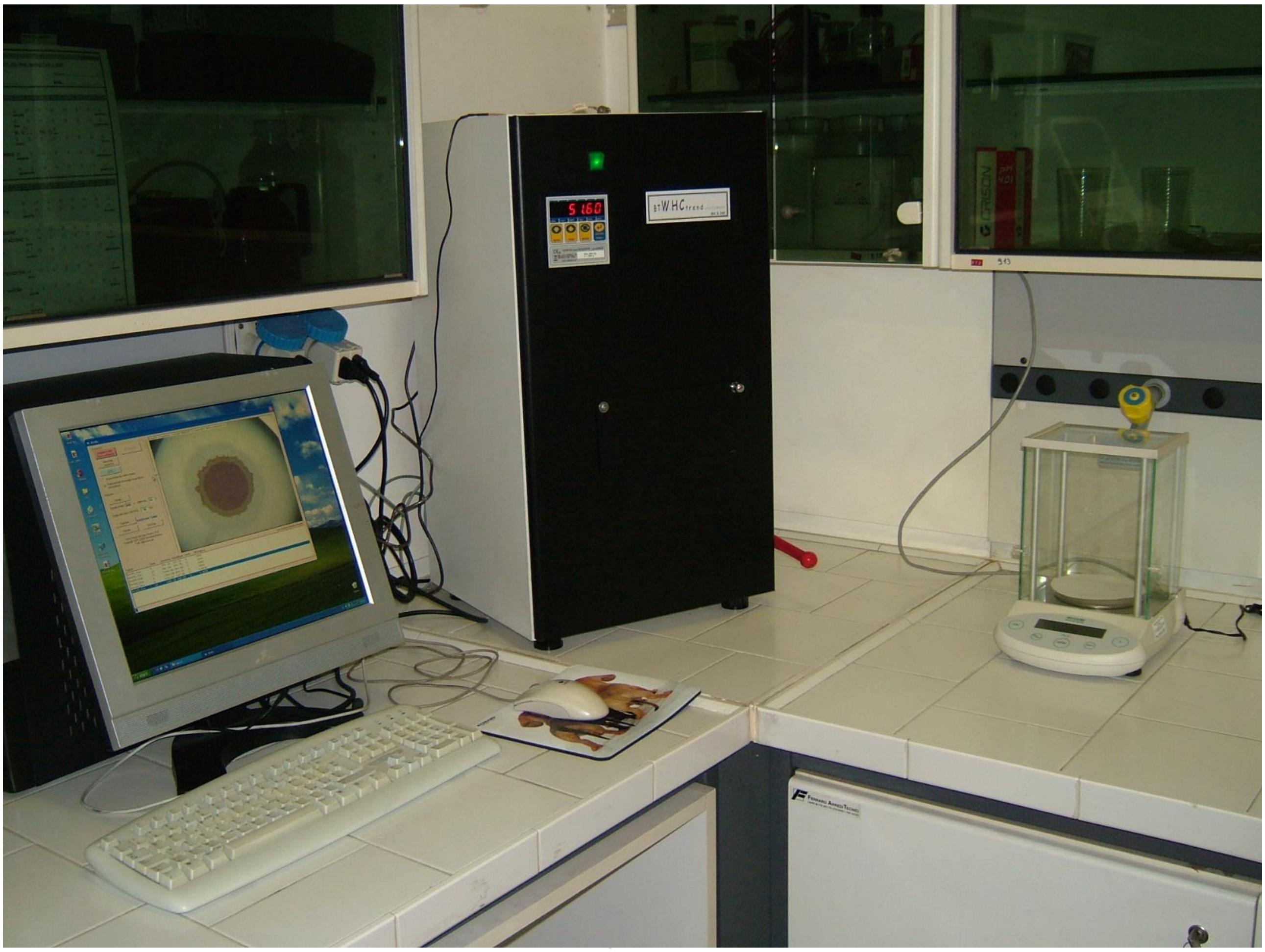
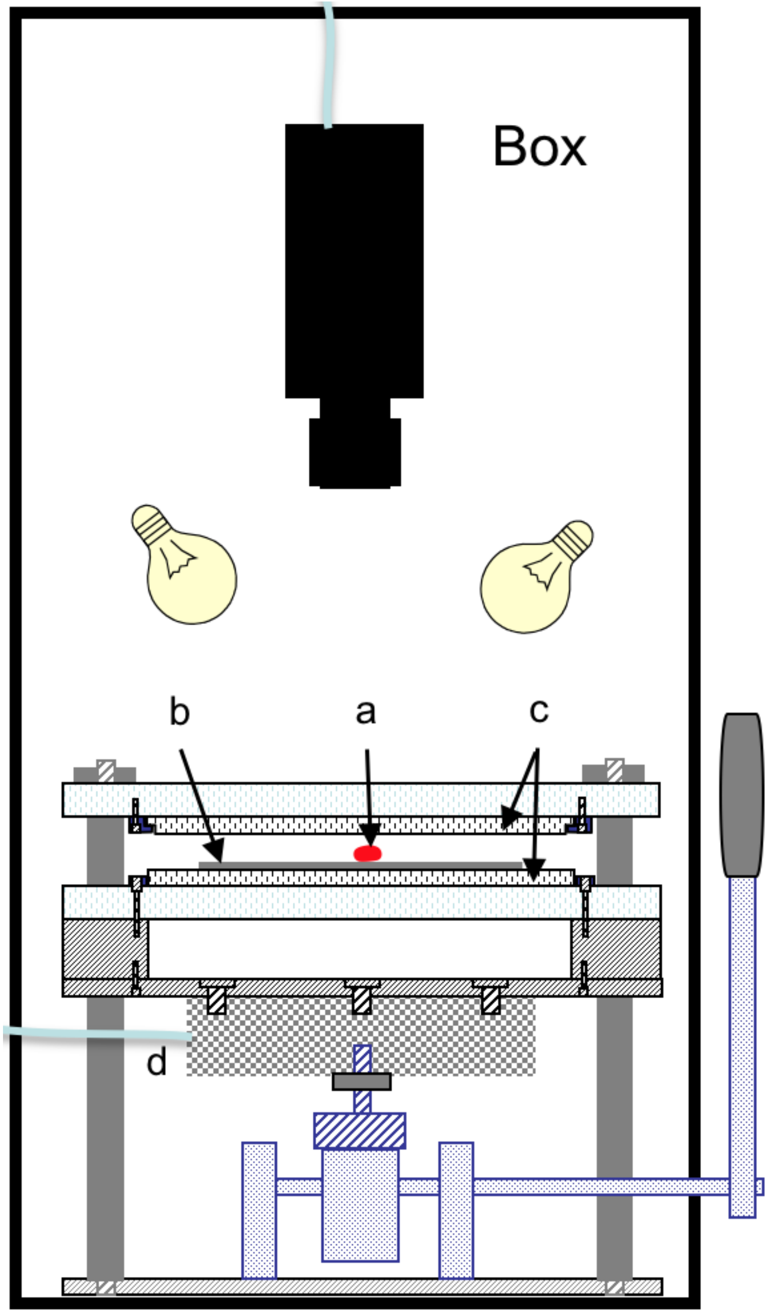
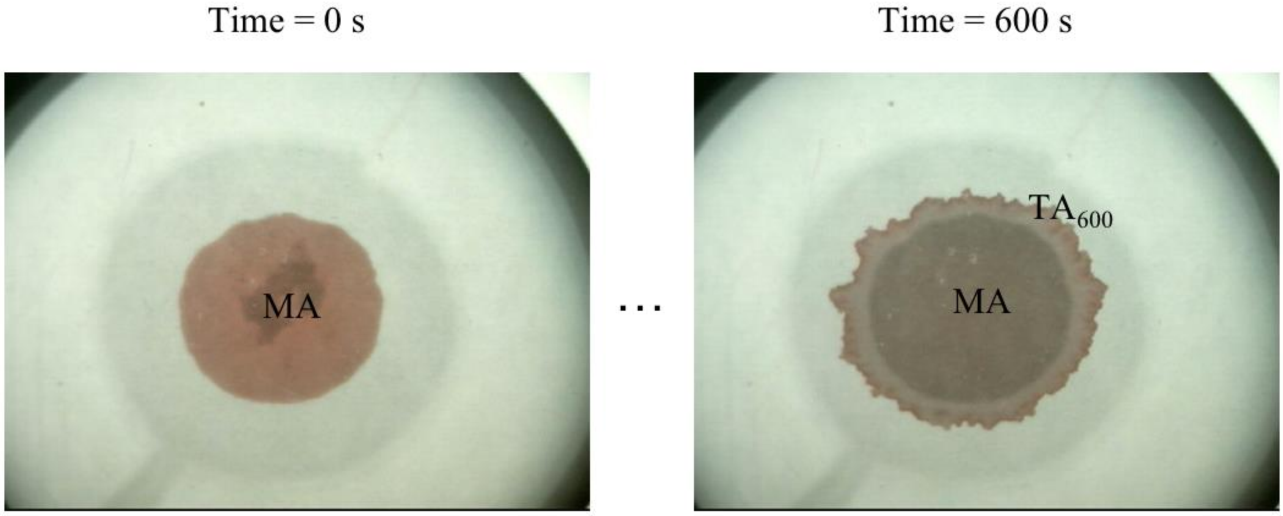

51.60
BT WHC trend

Figure 2. WHCtrend instrument’s details.



a: 250 mg of ground meat; b: filter paper sheet; c: removable translucent plexiglass plates; d: load cell.

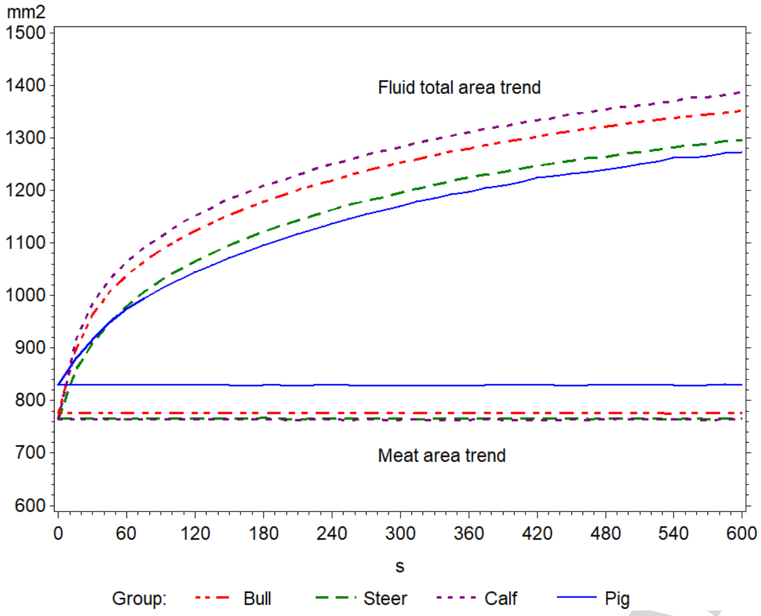
Figure 3. Measured parameters at time 0 and 600 s.



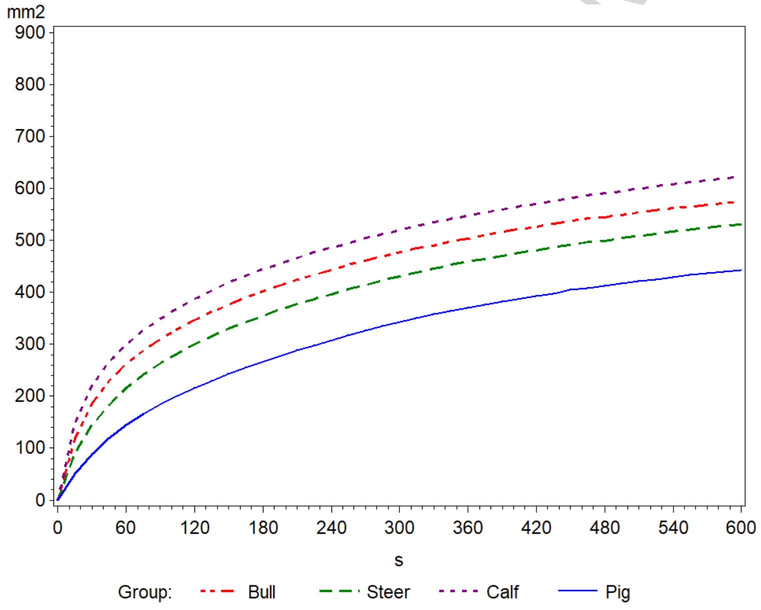
MA = meat area; TA_{600} = final total area.

Figure 4. "WHCtrend instrument" parameters for meat under compression in 4 categories in the *longissimus thoracis*.

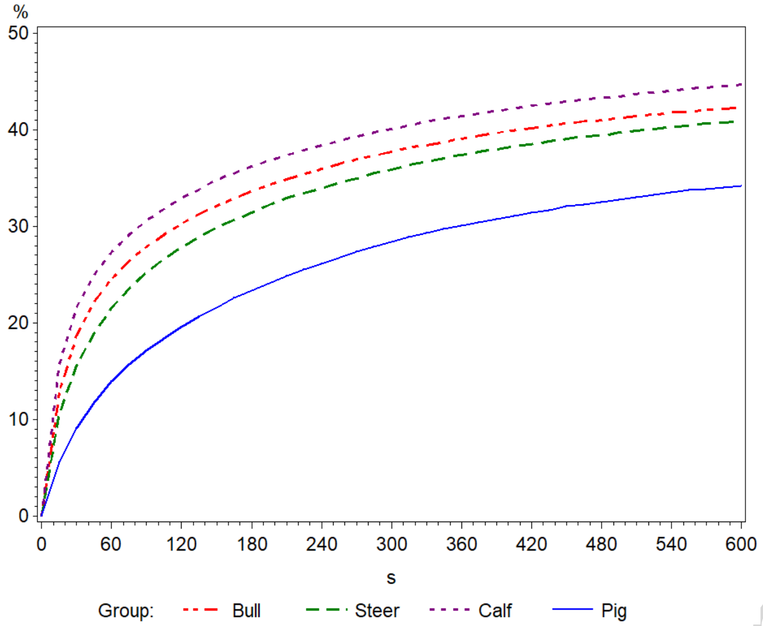
a. Trend of fluid release (WHCtrend) and meat area



b. Ring trend area (TA-MA)



c. Ring% trend [(TA-MA)/TA*100]



HIGHLIGHTS

- A new instrument “WHCtrend instrument” to standardize the measurement of fluid release in meat.
- A new parameter “WHCtrend” to measure the trend in fluid meat release.
- Video Image Analysis applied to the measurement of water-holding capacity.
- Correlations between meat qualitative parameters and “WHCtrend instrument” parameters.