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A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle

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INTRODUCTION

Meat quality includes wholesomeness, nutrient content, palatability traits, attractiveness and the capacity of the muscle to retain fluids during handling and processing (Kauffman et al., 1969). Even though each of these traits is extremely important, the variations in some are considerably greater than others. In particular, water-holding capacity (WHC), which may vary considerably among muscles originating from animals of the same species, breed, sex, weight, age and ante- and post mortem treatment, affects both quality and composition. Muscles releasing excess fluids are dryer tasting and lose more weight during processing, storage, transit and display, thus altering composition, quantity of product for merchandising, and visual appearance.
Most post-rigor muscles contain about 70 % water, depending primarily on lipid content and the physiological maturity of the muscle (Kauffman et al., 1964). This water is either very tightly bound chemically to other molecules (i.e. proteins), is loosely associated with other charged molecules or is in the extracellular spaces free to move into the external environment. Only very severe treatment (i.e. drying at high temperatures) affects the bound water, but the retention or loss of the other two forms are affected by several phenomenon. The availability of charges is associated with the ultimate pH of muscle. At pH levels considerably above (< 6.0) or below the isoelectric point (~ (5.0), the number of available charges is enhanced thus increasing WHC. This has been described by Gault (1985). Also, Offer and Trinick (1983) have provided new insights on the swelling responce that accounts for a proportion of a muscle's capacity to retain fluids.
Because WHC is such an important qualitative and quantitative characteristic of muscle, and because the magnitude of its variation is large, there is need to assess it both in the field and in the laboratory. Speed and economics is especially important for the former application whereas precision and accuracy is particularly critical for the latter. Although numerous methods have been described in the literature, they have never been compared collectively for accuracy and reproducibility under a relatively standard set of conditions. Where it is rather simple to compare such traits as time required and cost of equipment, the originators of the methods usually failed to test the reproducibility and quantitative accuracy when muscle, temperature, time post mortem and anatomical location were defined and when large variations in WHC were insured through selection criteria. Therefore, it was the objective of this investigation to assemble all methods proclaimed to quantify WHC and for which materials or equipment were available or could be developed in this laboratory.
Consequently three distinctly different categories and a total of fifteen methods including an additional ten modifications were tested, using a rigorous randomized design and a critical statistical analysis. It was our intent to determine which methods for assessment of WHC could be used routinely to insure the greatest accuracy and reproducibility. Of course, the selection criteria is also dependent on cost, time, simplicity, adaptability and purpose of the measurement.

MATERIALS AND METHODS

Since in most studies on pork quality, samples of the longissimus are used for practical reasons, it was chosen for the comparisons. To insure the presence of adequate variation in WHC, nine dark, firm and dry (DFD), nine pale, soft and exudative (PSE) and ten 'normal' (N) pork loins, 24 hr. post mortem (P.M.) were selected from carcasses in which the fibre optic probe was used at two hr P.M. to preselect for PSE (values > 130); pH was used at 24 hr P.M. to varify DFD; and finally, visual appraisal was used to confirm the preliminary selection procedures. Since it was impossible to test all methods for each of the 28 loins in one day, equal numbers of the three muscle types were selected and tested in two consecutive days. All loins were stored at 0 °C, 55 % relative humidity until the longissimus, extending from the cranial tip of the ilium to the 10th costi was dissected. After removal of the epimysium, each longissimus was divided into three equal-length sections. Each section was subsequently divided with an electric slicing machine (to maintain uniform thickness) into six, two centimeter-thick transverse slices, giving a total of 18 individual slices for distribution among fifteen methods to predict WHC.
The methods selected were divided into three major categories including 1) a weight loss category, 2) a standard laboratory technique category and 3) a filter paper press and other (rapid) methods category. For each of the 28 muscles, each of the three main method categories was randomly assigned to one of the three muscle sections. Within each major category, each individual method was randomly assigned to either one or two of the six existing slices, depending on quantity needed for the method. For each method, duplicate observations were made on the same or adjacent slice. In some instances, more than one method was assigned to one slice because the size of sample required was negligible to the size of the slice. This rigorous random design was essential because it has been demonstrated (Lundström and Malmfors, 1985) that the longissimus may vary considerably in it's WHC.
In the weight loss category, both chilled muscle drip loss after 48 hr. and cooking loss were used. For each of these measures, three different approaches were used. The first included a procedure described by Honikel (1985), in which the slice was suspended in an air-inflated polyester bag and stored for 48 hr. at 0 °C, 55 % relative humidity. The second method was exactly the same as the first except that the slice was standardized in size (not weight) by using a 4 cm diameter round coring device to remove the centre of the slice which had been firmly supported by a leather loop to prevent spreading (especially PSE muscles) when cutting the core. The third method involved placing the slice on an absorption pad in a styrofoam tray that was subsequently covered tightly with polyester packaging film to simulate retail display conditions. The storage environment was identical for all three methods. Cooking loss of the muscles used for drip loss, was completed by sealing the raw muscle in a partially vacuumed polyester bag and placing it in a 75 °C water bath for one hour. The sample was cooled with tap water, separated from the bag, blotted free of surface fluids, and weighed. The difference between 48 hr. chilled weight and cooked weight was expressed as the percentage cooking loss of the 48 hr. weight.
In the laboratory category of methods, the following were included:
- The swelling test measures water uptake during low speed centrifugation and was described by Wierbicki et al. (1962). This method was modified to use 25 g muscle in 75 ml water.
- The high speed centrifugation (~ 39,000 x G) removes muscle fluids and was described by Bouton et al. (1971).

- Percentage transmission measures the protein solubility as affected by denaturation (high for PSE, low for DFD) and was described by Hart (1962).
- Permitivity in which electrical capacitance ratios calculated at two energy frequences (100 and 700) were determined. Both capacitance and a combination of conductivity and dielectric loss were measured by scanning a frequency range from 5 to 1000 mhz. This was described in detail by Grant et al. (1978).

For the filter paper press and other rapid methods category, the following were included:

- Grau-Hamm (1953) filter paper press using precise sample weight and pressure to determine expressible fluid.
- "Braunschweiger-gerät" (Roemmele et al., 1961), using the commercially available instrument (Julius Schmid GMBH, Tuttlingen, W.Germany) and an unweighd sample (~ 300 ± 50 mg). However, we recorded the weight.

For evaluation of the pressed areas of the filters derived from both methods, the following approaches were used to determine WHC:

- Using the planimeter to measure fluid area, and then expressing results either as percentage of total water, or as ratio of muscle area to muscle + fluid area.
- Hofmann's (1982) width times length measurements of muscle and fluid + muscle areas expressed either as percentage of total water or as ratio of muscle area to muscle + fluid area.
- The "Schablon"-method (Reuter, 1982) in which the areas are assessed with a relatively fast template evaluation system, is expressed as ratio of the muscle area to muscle + fluid area.
- Kapillar volumeter was described by Hoffman (1975).
- The imbibition test to measure absorption of fluids on pH paper within a three-minute period of exposure to a muscle surface, immediately after cutting, was described by Monin et al. (1981).
- The filter paper absorption of excess fluids as determined by either weight increase or subjective visual score was described by Kauffman et al. (1986).

To describe the physical and biochemical properties of the loin population, the following were included: Japanese Color Standards (Nakai et al., 1975) and Cielab L*, a* and b* values measured with a Hunter Labscan with light source D65; ultimate pH (24 hr. postmortem) was measured with a combination glass probe electrode, and an objective assessment of muscle softness. A 235 g tapered weight attached to a penetrometer instrument was electrically positioned to be in contact with the muscle. The perimeter of the muscle was supported by a leather loop and the temperature of the muscle was maintained at 7 °C. The distance traveled upon release of weight was considered a direct estimate of softness.

Some existing techniques were not included in this experiment. The centrifuge method described by Wierbicki et al. (1957) was not used because special centrifuge tubes could not be obtained. A copper sulfate capillary tube technique was excluded because the test proved ineffective when investigated during the pre-experimental training and procedural planning period. Conversely, some methods were included, not because of their known effectiveness to assess WHC, but because materials were readily available and simple to apply. Each technician involved in the study had been previously trained to measure WHC using a specific method so that technician-variation within methods would be minimized. All methods for each of the 28 longissimus muscles were applied as simultaneously as possible.

The data was analysed with an analysis of variance model. This model contained two random effects representing variation between loins and variation between duplicate observations within loins, with components of variance σ_b^2 and σ_w^2 respectively.

The fixed effects in the model were the factors "days" (representing the two days in which the experiment was performed), "muscle-condition" (PSE, normal, DFD) and "location" (representing the three main locations cranial, middle and caudal of a muscle). Main effects for these factors and the interaction "muscle-condition" x "location" were entered in the model.

Estimated for σ_b^2 and σ_w^2 were obtained from analyses of sums and differences of duplicates. Significance tests (F-tests) for interaction and main effects were obtained from an analysis of sums of duplicate observations. Significance tests (two tailed t-tests) for differences between muscle types were also determined.

For each method the repeatability $\int$ was determined. The repeatability was defined as the correlation coefficient between duplicate observations within muscle type and was determined as $\int = \sigma_b^2/(\sigma_w^2 + \sigma_b^2)$. When duplicate observations are very similar $\int$ should approach 1, and when duplicate observations are dissimilar, $\int$ should approach 0.

When the interaction "muscle-condition" x "location" was not significant and/or relatively unimportant, the F-ratio for "muscle-condition" main effect was used as a measure of discrimination between muscle-types.

In a preliminary analasis, interactions with the factor "days" were studied to examine the reproducibility of the results.

RESULTS AND DISCUSSION

Table 1 identifies the physical and biochemical properties of the 28 longissimus muscles used. This information varifies that three distinctly different muscle quality types existed (PSE, N and DFD) as a result of the selection by visual inspection, use of the fibre optic probe and pH one hr. post mortem. In addition, as the collective results of all the methods shown in Table 2 clearly indicate, the muscle groups represented three distinct levels of WHC as determined indirectly through such expressions as drip loss over time, fluids removed by pressure, uptake of added water, and presence of surface fluids. Therefore, the population would be suitable to examine the quantitative effectiveness that each method possessed in distinguishing variations in WHC between muscle quality types as well as to identify the degree of agreeement between duplicate analyses within muscle type.

The data presented in Table 2 indicates the effectiveness or lack of effectiveness of each method to distinguish among the three muscle types. Most methods separated the groups with considerable ease. However, the cooking loss measurements failed to distinguish differences between PSE and N. For Transmission and Imbibition methods, the mean differences were not statistically significant between N and DFD, whereas permitivity ratios did not clearly separate any muscle types. It should be noted that the frequency range used to measure permitivity is different from Grant's (1978). Table 3 provides the statistical verification for significance in distinguishing among muscle types as well as repeatability of duplicates. The F values for the effect of muscle quality type identify the methods that distinguish muscle types. All methods except for the dielectric constant ratio for permitivity are significant. The t values (corrected when there was a muscle-type x location interaction) more specifically identify those methods which could distinguish between PSE and N, and between DFD and normal. The tests that were non-significant in one or both comparisons included all three cooking loss comparisons between PSE and N; and Transmission test, the Grau-Hamm % fluids, and the imbibition test between DFD and N.

The values measuring the repeatability of duplicates ($\int$) ranged from 0 to 1. Of the 25 values for $\int$, 15 were significantly different from 0 ($p < .05$). Those with especially high values (with correspondingly high and narrow confidence intervals) included the three drip loss methods, the packaged cook loss, the three standard laboratory methods, the two filter paper absorption methods, and the permitivity capacitance ratio. Some filter press methods yielded unexpectedly low values for $\int$.

Based on these results and in concert with the other considerations (time, financial investment of equipment, adaptability to field conditions and utilization for processing meats) associated with the methods, we concluded that the drip loss methods, especially when size is standardized, are quite appropriate if time required to obtain results and field application are not important. Swelling and centrifugation methods serve as reliable laboratory procedures but require relatively high initial investments of equipment. For rapid, inexpensive and accurate field tests, the filter paper absorption tests proved worthy of consideration and are comparable with the other tests identified above. The imbibition, cook loss and filter paper press methods were less acceptable, and the permitivity method requires more experimentation before it can be given further consideration.

There were a few significant interactions among muscle type, location and day, none of which can be clearly explained other than to recognize (as did Lundström and Malmfors, 1985) the variations in WHC which may exist within the longissimus. It was interesting to observe that effect of location was significant in only three tests. One would expect some interactions to be significant by chance alone, but in this experiment, seven significant values existed which can not be left entirely to chance. One other explanation is a technician effect between the two days, but this was not measured, and efforts were made to minimize it.

The filter paper press methods proved to be quite disappointing, especially since they have been used longer in meat research than most other techniques used in this experiment. This may be explained by such possible errors as excessive evaporation while standardizing sample weight, smallness of sample size, difficulty in applying uniform and constant pressure, and difficulty in assessing a reliable area measurement when using the planimeter.

The imbibition and kapillar volumeter methods were too variable among both muscle types and for duplicates within muscle type. Cooking losses failed to distinguish between N and PSE samples and this may be partially explained by the fact that the samples were tested after the 48 hr. drip loss had been assessed. Although a greater difference may have prevailed if 24 hr. post mortem samples would have been used, we do not beleive this to be valid because, unless the fluids are bound very tightly (as in DFD), it is likely that the fluids will be removed during severe heating. Even though transmission duplicates were reproduced perfectly, it failed to differentiate between DFD and N; however this was not surprising since this method was designed to measure protein solubility as affected by denaturation due to the PSE condition. Since N and DFD samples should be similar in this respect, then the values should not be different. It is apparant that there is more to WHC than the degree of protein denaturation, even though this in itself must be a contributing factor.

As to which method to choose ultimately for quantitating WHC, this must also depend on availability of equipment, the urgency for results and purpose to which the method is applied. If practicality is of concern, than the drip loss methods should always be considered - especially when size of sample is controlled. It already had been explained by others (Honikel, 1985) that larger samples have proportionately smaller surface areas and consequently would lose less fluid during storage. If we would presume that muscles possessing the PSE condition usually originate from carcasses possessing larger loin muscle areas, than the difference between PSE and N should be reduced. The data in this experiment imply this in that the samples standardized to a smaller size produced higher drip loss percentages among all muscle types and the differences between types were greater and the consistancy of duplicate results was more acceptable. Both the swelling and centrifuge methods meet all statistical requirements, but the availability of equipment and time required may be a limiting factor. The swelling method is of interest since it is the only one that measures uptake of added water instead of fluid loss. This principle is especially related to processing conditions. The filter paper absorption method in which either a wetness score is established or the absorbed fluids are weighed, appears to be an attractive procedure. As reported earlier (Kauffman et al., 1986), the approach is not only reasonably accurate, but is also rapid, inexpensive and easy to apply under field conditions. The wetness score is more adaptable because it does not require an analytical balance. However, the fluid weight approach is easier to standardize.

In summary, we conclude from this study that there are several rather effective methods to assess WHC and the decision as to which to use depends on the circumstances. Most of the methods tested could separate the three muscle quality types, but the preciseness varied as did the reproducibility of duplicate analyses. In addition to becoming more familiar with all the existing methods, we either confirmed or uncovered several obscure principles that contributed to our more complete understanding of WHC and its complexities.

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TABLE 1. Quantitative description of muscle types.

Trait	Number of loins	Muscle type: PSE 9	Normal 10	DFD 9
Ultimate pH		5.45$^{v}_{v}$ ± 0.13[a]	5.58$^{v}_{w}$ ± 0.19	6.30$^{w}_{x}$ ± 0.27
Hunter L*		60.5 ± 3.2	55.4 ± 2.7	47.8 ± 4.7
Hunter a*		6.6 ± 1.3	5.4 ± 1.6	5.2 ± 1.7
Hunter b*		15.8 ^{v} ± 0.8	14.0 ^{w} ± 0.7	11.8 ^{x} ± 0.8
Japanese Color Score[b]		2.0 ^{v} ± 0.8	3.2 ^{w} ± 0.5	5.0 ^{x} ± 0.6
Marbling Score[c]		1.1 ± 0.3	1.5 ± 0.3	2.2 ± 0.4
Firmness[d], mm		21.0 ± 2.3	21.4 ± 2.5	19.9 ± 2.3

a) $\bar{X}$ ± sd. Means of mean differences within row having a different superscript are significantly different $P < .05$).
b) 1 = pale, 6 = dark (Nakai et al., 1975).
c) 1 = devoid, 5 = excessive (NPPC, 1976).
d) distance traveled by plunger; a larger value should reflect greater softness.

TABLE 2. All methods compared to muscle types.

Method	Number of loins	Muscle type PSE 9	Normal 10	DFD 9
Suspended, drip loss, %		$8.8^{V} \pm 2.0$[a]	$4.3^{W} \pm 1.6$	$1.0^{X} \pm 0.2$
Suspended, cook loss, %		$29.3^{V} \pm 3.2$	$29.9^{V} \pm 3.0$	$19.4^{W} \pm 3.7$
Suspended, sized, drip loss, %		$11.0^{V} \pm 3.3$	$4.6^{W} \pm 1.6$	$1.2^{X} \pm 0.3$
Suspended, sized, cook loss, %		$30.5^{V} \pm 2.2$	$30.8^{V} \pm 2.5$	$22.1^{W} \pm 4.6$
Packaged, drip loss, %		$7.8^{V} \pm 1.6$	$3.3^{W} \pm 1.2$	$1.5^{X} \pm 0.3$
Packaged, cook loss, %		$30.6^{V} \pm 2.1$	$28.5^{V} \pm 2.5$	$20.8^{W} \pm 3.1$
Swelling, %		$20.4^{V} \pm 6.0$	$38.0^{W} \pm 6.3$	$129.4^{X} \pm 35.4$
Centrifugation, %		$36.1^{V} \pm 4.2$	$23.8^{W} \pm 5.2$	$11.9^{X} \pm 4.6$
Transmission, %		$49.6^{V} \pm 23.4$[a]	$25.5^{W} \pm 5.3$	$17.9^{W} \pm 6.2$
Permitivity, A. capacitance[b]		$1.210^{V} \pm .010$	$1.200^{W} \pm .012$	$1.192^{W} \pm .011$
B. dielectric constant[c]		$4.349 \pm .147$	$4.326 \pm .153$	$4.272 \pm .135$
Grau-Hamm A. Planimeter cm², % Fl. of total		$37.1^{V} \pm 4.3$[a]	$28.9^{W} \pm 2.5$	$26.1^{W} \pm 4.4$
B. Planimeter M/F[d] ratio		$.24^{V} \pm .04$	$.36^{W} \pm .04$	$.44^{X} \pm .07$
C. Width x height cm², % Fl. of total		$49.1^{V} \pm 5.7$	$38.3^{W} \pm 4.2$	$34.5^{W} \pm 6.0$
D. Width x height M/F ratio		$.29^{V} \pm .05$	$.41^{W} \pm .05$	$.48^{X} \pm .07$
E. Schablon M/F ratio		$.26^{V} \pm .04$	$.38^{W} \pm .04$	$.44^{X} \pm .06$
Braunschweiger A. Planimeter cm², % Fl. of total		$34.7^{V} \pm 5.2$	$29.2^{W} \pm 4.5$	$23.2^{X} \pm 4.8$
B. Planimeter M/F ratio		$.25^{V} \pm .05$	$.34^{W} \pm .06$	$.45^{X} \pm .07$
C. Width x height cm², % Fl. of total		$45.1^{V} \pm 8.0$	$36.8^{W} \pm 7.6$	$29.7^{X} \pm 6.5$
D. Width x height M/F ratio		$.30^{V} \pm .06$	$.40^{W} \pm .07$	$.50^{X} \pm .07$
E. Schablon M/F ratio		$.28^{V} \pm .05$	$.39^{W} \pm .06$	$.48^{X} \pm .07$
Hofmann Kapillar volumeter, µl		$53.3^{V} \pm 18.9$	$23.3^{W} \pm 8.8$	$8.3^{X} \pm 5.3$
Imbibition, time, sec.		$52.0^{V} \pm 50.6$	$150.3^{W} \pm 46.2$	$180.0^{W} \pm 0.0$
Filter paper wetness A. fluid wt., mg		$128.9^{V} \pm 14.2$	$60.1^{W} \pm 24.4$	$26.9^{X} \pm 9.6$
B. visual score, %[e]		$88.4^{V} \pm 10.4$	$16.8^{W} \pm 18.1$	$0.4^{X} \pm 0.7$

a) $\bar{X} \pm$ sd of all duplicates within muscle type. Means of mean differences within row having a different superscript are significantly different ($P < .05$).
b) Ratio for values of frequencies at 100 and 700 on upper curve
c) Ratio for values of frequences of 100 and 700 on lower curve
d) Muscle area ÷ fluid + muscle area.
e) Score range = 1 to 100, filter paper diameter = 45 mm.

TABLE 3. Statistical Comparisons of Methods to Quantitate WHC in Porcine Muculature.

	Method[a]	Analysis of variance F values[b] Muscle Type[c]	t values[b] PSE vs. normal	DFD vs. normal	Comparison of duplicates ∫[b] sd	.05 Confidence interval for ∫
1.	Suspended drip loss	82.1[x]	8.0	6.0	.61 ± .13	0.29 - 0.82
2.	Suspended cook loss	64.5[x]	0.5	10.2	.41 ± .18	0.01 - 0.71
3.	Suspended, sized, drip loss	81.2	7.6	3.8	.82 ± .07	0.70 - 0.94
4.	Suspended, sized, cook loss	30.8	0.1	6.2	.50 ± .16	0.13 - 0.77
5.	Packaged drip loss	76.9	8.8	3.5	.74 ± .10	0.67 - 0.94
6.	Packaged cook loss	38.9[x]	2.0	7.0	.69 ± .11	0.41 - 0.86
7.	Swelling	140.1[x]	2.2	13.2	.86 ± .06	0.71 - 0.94
8.	Centrifugation	64.9	5.9	6.0	.63 ± .13	0.32 - 0.83
9.	Transmission	10.8	3.4	1.0	1.00 ± .00	0.99 - 1.00
10.	Permitivity, A. Capacitance[d]	6.4	2.2	1.4	.78 ± .09	0.56 - 0.90
	B. Dielectric constant[e]	2.8	0.3	0.8	.49 ± .16	0.13 - 0.75
11.	Grau-Hamm Press, A. Planimeter cm²	37.3	5.5	2.0	.15 ± .21	0.01 - 0.54
	B. Planimeter M/F[f] ratio	66.3	5.6	4.1	.13 ± .22	0.01 - 0.52
	C. Width x height cm²	57.4	5.2	2.0	.00 ± .21	0.00 - 0.30
	D. Width x height M/F ratio	78.8	5.7	3.9	.00 ± .22	0.00 - 0.39
	E. Schablon M/F ratio	65.4	6.1	3.7	.17 ± .21	0.01 - 0.57
12.	Braunschweiger, A. Planimeter cm²	22.5	2.8	3.1	.23 ± .21	0.01 - 0.69
	B. Planimeter M/F ratio	41.8[x]	3.8	4.2	.31 ± .20	0.01 - 0.73
	C. Width x height cm²	19.8[x]	3.3	2.9	.17 ± .21	0.01 - 0.66
	D. Width x height M/F ratio	46.4	5.0	4.5	.10 ± .22	0.01 - 0.58
	E. Schablon M/F ratio	46.6	4.6	3.9	.18 ± .21	0.01 - 0.66
13.	Hofmann Kapillar volumeter	40.0	6.0	3.0	.46 ± .17	0.10 - 0.74
14.	Imbibition time	34.8	6.5	1.9	.51 ± .16	0.15 - 0.76
15.	Filter paper wetness, A. Fluid weight	67.8	8.7	4.1	.82 ± .07	0.64 - 0.92
	B. Visual Score	111.1	12.9	2.9	.80 ± .08	0.59 - 0.91

a) N = 27 methods 3-9, 11, 12; and 28 for remainder
b) significant values

	$p < .05$	$p < .01$
F	3.6	6.0
T	2.1	2.5
∫	.33	.45

c) Methods with superscript 'x' have a significant muscle type x location interaction
d) Ratio for values of frequencies at 100 and 700 on upper curve
e) Ratio for values of frequencies at 100 and 700 on lower curve
f) muscle area ÷ fluid + muscle area