EFFECT OF THE ESTROUS CYCLE AND PREGNANCY ON THE WATER HOLDING CAPACITY AND RELATED PROPERTIES OF PORCINE MUSCLE

by

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INTRODUCTION

Water holding capacity is an important muscle characteristic to the meat industry. Processors of meat products such as sausage or canned hams want a meat with a high binding ability. Shrinkage of meat products during processing, storage, and cooking is related to the ability of the muscle proteins to bind water.

Many factors are related to the ability of a muscle to bind water such as muscle pH, color, adenosine triphosphate content, electrolyte content, and location. Animal factors also affect muscle hydration. Some of these are specie, breed, sex, age, and ante-mortem treatment of the animal ("stress" conditions). Much work has been done in the evaluation of these highly interrelated factors as to their effect on muscle water holding capacity.

However, little work has been done on the effect that the estrous cycle, a natural occurring "stress" condition in female mammals, has on muscle water holding capacity. It is known that estrogens cause an increase in uterine weight (smooth muscle) by imbibiting water within the muscle tissue. They also affect almost every other tissue in the body and presumably could cause a related effect on skeletal muscle.

This experiment was undertaken to study the effect of various stages of the estrous cycle and pregnancy on the water holding capacity and related properties of porcine skeletal muscle. Interrelationships between the centrifuge and press methods for determination of water binding ability were studied as were the interrelationships among various *longissimus dorsi* components and quality factors.

LITERATURE REVIEW

Influence of Estrogens and Progestogens on Muscle Quality

Estrogens act directly or synergistically with other hormones to produce a great variety of effects on body chemistry. They affect almost every tissue in the body, but their main action is on the pituitary, the brain, the mammary system, and the female reproductive organs. The estrogens and progestogens under certain conditions can act either synergistically or antagonistically. Promotion of tissue growth, especially uterine growth, is the main action of the estrogens. From four to six hours after the administration of estrogen, uterine weight reached a peak (Turner, 1966). This gain in weight was due mainly to imbibition of water by the tissues. When a graded series of doses of estrogen was given to rats and the uteri weighed six hours after administration, Hisaw (1959) found that as uterine wet weight increased with successive dosages so did the percent of total water. However, dosages of estrogen greater than required for maximum growth at six hours produced less response than the smallest dose. Hawk et al. (1961) working with estrus, luteal-phase, and ovariectomized ewes, showed that in both the estrus and luteal-phase ewes the endometrial and myometrial water content of the uterus was higher than in the ovariectomized ewes. Estrus was a period of estrogen secretion, whereas the luteal-phase of the estrous cycle was dominated by progesterone secretion (Csapo, 1956; Turner, 1966).

A second wave of water imbibition occurred 20 to 24 hours after estrogen injection and was accompanied by cellular proliferation and

accumulation of uterine solids (Turner, 1966). During this time the ribonucleic acid (RNA) and total phosphorylase content of the uterus increased. This increased uterine protein and glycogen concentrations. The actomyosin content of uterine muscle was also increased as was its activity and excitability (Ganong, 1965).

Estrogens affected vascular permeability in ewe uterine endometrium and serosa (Hawk et al., 1963). They intravenously injected trypan blue dye into 83 ewes and measured the intensity of tissue blueing (0-no detectable staining to 9-deep blue-black coloration). Permeability was high in estrus ewes, intermediate in luteal-phase ewes, and low in ovariectomized ewes as indicated by the mean indexes of surface blueness of the endometrium (7.8, 4.5, and 3.2, respectively) and of serosal blueness (7.3, 3.3, and 2.3, respectively).

Ovarian steroids also altered the ionic distribution across the myometrial cell membrane. Csapo (1956) showed that estrogen domination resulted in high potassium and low sodium concentrations inside the cell. Under progesterone domination, the potassium concentration was relatively low and the sodium concentration high inside the cell. Howath (1954) measured the intracellular concentrations of these cations and found a potassium/sodium ratio of 5.3 under estrogen and a ratio of 2.9 under progesterone domination. In the uterine endometrium of estrus and late luteal-phase ewes, Hawk et al. (1961) found a greater proportion of water, sodium, and chloride in the extracellular phase when compared with an ovariectomized control group. Determinations on the myometrium showed extracellular water volume and sodium content per unit weight of

wet tissue to be progressively greater in estrus, luteal-phase, and ovariectomized ewes and intracellular water volume and potassium content to be progressively less.

Wismer-Pedersen and Riemann (1960) reported that the frequency of low pH in the loins of sows was slightly higher than with barrows and boars. They felt this difference was probably due to the estrous cycle which might give rise to increased excitability in the females.

Water Holding Capacity of Meat

Water holding capacity was defined by Hamm (1959) as the ability of meat to hold fast its own or added water during application of any force (pressing, heating, chewing, grinding, etc.). Swelling was defined as the spontaneous uptake of water by meat from any surrounding fluid resulting in an increase of the weight or volume of muscle. The net result of these two effects, caused by the interaction between meat proteins and water, was called meat hydration by Hamm (1959).

The study of the water holding capacity of meat has both scientific and economic importance. Hamm (1960) stated that water holding capacity was closely related to taste, tenderness, color, and other features of meat quality. The affect of muscle water holding capacity is apparent in all processing steps after slaughter (aging, grinding, curing, etc.) and to the consumer during cooking. The sausage processer especially wants muscle with a high water holding capacity (Wismer-Pedersen and Riemann, 1960). Drip losses of stored fresh products and shrinkage of cured products during smoking and cooking are related to the ability

of muscle to bind water.

Muscle proteins (mainly fibrillar proteins) are responsible for water binding in meat. Water in muscle can be tightly or loosely bound to the protein structure with no sharp demarcation between the two. Using adsorption curves to measure water binding, Hamm (1960) found that about 4% of the water was bound very firmly and was given off only at extremely low vapor pressure. This water was bound to certain hydrophilic groups forming between the peptide chains. Another 4-6% was bound if the vapor pressure was increased and probably represented a second water layer formed over the same hydrophilic groups. This water, bound to the proteins by monomolecular and multimolecular adsorption, Hamm (1960) called the "true hydration-water" of muscle and was little influenced by changes in the structure and/or charges of protein. However, the "free" or "immobilized" water in muscle which was retained within the cellular protein membranes and protein filaments was strongly influenced by the spatial structure of the muscle tissue. This "free" water is in close association with the "loose" water in the muscle. "Loose" water was defined as that water which was forced out by application of "very low" pressure (Hamm, 1960).

Muscle hydration is influenced by the net charge and stereo effects (Hamm, 1959). The hydrophilic groups responsible for the binding of water include the polar groups of the side chains of protein, such as the carboxyl-groups and the undissociated carbonyl- and imido-groups of the peptide bonds, in which binding is due to the dipolar nature of water (Hamm, 1960). This means the negative charge of oxygen and the positive

charge of hydrogen do not coincide and, therefore, water is a molecular magnet which is attracted by all kinds of polar groups in the protein. The water bound in the first layer and also the water bound at a distance is affected by this magnetic attraction. Not all charged groups may bind water such as those which compensate their charges by an inter- or intramolecular salt cross-linkage. Hamm (1959) called this the net charge effect. The spatial structure of the muscle also influenced muscle hydration. Tightening the network of the protein molecule decreased immobilized (free) water and increased loose water, whereas loosening the protein structure had the opposite effect. Cleavage of the cross-linkages, which caused the peptide chains to become more flexible allowing water to attach to the polar groups may be causing this loosening effect. Changes of protein charges and attraction or repulsion of charged groups greatly influenced this stereo effect (Hamm, 1959).

Factors Related to the Water Holding Capacity of Meat

pH. The water holding capacity of meat was directly related to muscle pH (Wilson, 1959; Swift and Berman, 1959; Briskey et al., 1959 b, c, 1960; Judge et al., 1960; Wismer-Pedersen, 1960). However, Wilson (1959) found a low relationship between muscle pH and water holding capacity 24 hours post-mortem. The ultimate pH of muscle depends upon type of muscle, species, and physiological condition of the muscle at time of slaughter (Lawrie, 1958). Upon death, the chemical changes that take place in muscle tissue include the disappearance of creatine phosphate, initial resynthesis and ultimate disappearance of adenosine

triphosphate, and production of lactic acid (Briskey and Wismer-Pedersen, 1961: Lawrie, 1966). Post-mortem muscle pH and water holding capacity were highest immediately after slaughter. Within 24 hours, pH and water holding capacity dropped due to rapid anaerobic glycolysis resulting in the formation of lactic acid within the muscle. This was probably due to an abnormally high demand for energy by the cell (Wismer-Pedersen, 1959: Wismer-Pedersen and Riemann, 1960). Accompanying this drop in pH was a decrease in electrical resistance and a loss of adenosine triphosphate (Wilson, 1959; Hamm, 1959, 1960; Swift et al., 1960). Paralleling this, the sarcolema lost some of its semi-permeable characteristics, allowing a freer exchange of electrolytes. This decrease in water holding capacity of muscle might also be due to a denaturation of the contractile protein complex, actomyosin. Denaturation of actomyosin causes it to lose its enzymatic activity (adenosine triphosphatase) and become less soluble in dilute potassium chloride. From this, one would expect a decrease in water holding capacity since protein solubility and the water holding capacity of muscle proteins are intimately associated (Wilson, 1959; Wismer-Pedersen, 1959, 1960; Wismer-Pedersen and Riemann, 1960).

Water holding capacity of muscle is primarily influenced by the net charge of the muscle proteins. In other words, as the pH of meat approached, the isoelectic point of the muscle proteins, their ability to bind water was decreased (Briskey et al., 1959b; Hamm, 1959, 1960; Wismer-Pedersen, 1962). Thus, the lower the ultimate pH the lower the water holding capacity of the meat. The isoelectric point of meat is

about pH 5.0 and at this point hydration will be at a minimum (Hamm, 1959, 1960).

Hamm (1959, 1960) found that pH variation only accounted for one-third of the water holding capacity decrease post-mortem. The other two-thirds was due primarily to the loss of adenosine triphosphate. Wilson (1959) also found that the direct relationship between pH and water holding capacity post-mortem was due to the loss of adenosine triphosphate and myosin solubility rather than the direct effect of pH.

Color. As porcine muscle color varied from light to greyish-pink to dark the expressible juice lost upon heating and centrifuging decreased and muscle pH increased (Judge et al., 1958, 1960). Light penetration had more affect on the change in color of muscle with pH change than did the concentration of myoglobin (Bate-Smith, 1948; Lawrie, 1958; Briskey et al., 1959c; Wilson, 1959). Muscle at a high pH has a "closed" structure which permits the light to pass through a deep layer of pigment resulting in a dark red color. If the superficial muscle layers scatter the light, the muscle appears ligher in color. This occurs with an "open" structure and a low pH.

Judge et al. (1958, 1960) and Sayre et al. (1964) found that firmer pork loins had a higher water holding capacity and pH and were darker in color than softer muscled loins. Darker loins were more prevalent from swine slaughtered during cool weather than from those slaughtered during the warm summer months (Judge et al., 1959; Wismer-Pedersen, 1959; Forest et al., 1963). It has also been shown that swine subjected to exercise (3 miles at the rate of 1 mile per hour) immediately before

slaughter had darker muscles with higher myoglobin and ph values than non-exercised swine (Lawrie, 1950, Briskey et al., 1959a).

Porcine muscle showed variation in the distribution of total myoglobin pigment within individual muscle. Muscles with the highest pH and myoglobin concentrations were the darkest in color (Scaife, 1955; Briskey et al., 1960; Topel, 1962).

Electrolyte Content. Sodium and potassium are naturally present in muscle in high concentration, but their influence on muscle water holding capacity appeared to be low (Hamm, 1960). This was because the alkali ions are bound rather loosely to the muscle proteins, whereas the bivalent ions are bound tightly. However, a low correlation was found between water holding capacity of muscle and the concentration of alkali ions in the expressible juice from muscle. Swift and Berman (1959) and Webb et al. (1967) found the potassium content of beef muscle to be negatively correlated and the sodium content to be positively correlated with water retention of beef muscle. In the pH range of about 5.0 to 5.5 (isoelectric point of muscle) added sodium ions caused a greater hydration of muscle than added potassium ions (Hamm, 1960; Lawrie, 1966).

Although the bivalent metals; magnesium, calcium, and zinc; are present in muscle in much smaller amounts than the alkali ions they affect the water holding capacity of muscle greatly despite their relatively low concentration (Bozler, 1955; Hamm, 1959, 1960). Their effect on water holding capacity was decreased as pH decreased because bivalent cation binding occurred by means of the carboxyl groups of protein side chains and this binding decreased as pH decreased (Hamm, 1960). Removal

of calcium from muscle with the use of a cation exchanger caused an increase in water holding capacity. This removal of bivalent ions is a typical case of a stereo effect (Hamm, 1959).

The polyvalent cations may also affect muscle water holding capacity by forming cross linkages between the peptide chains. This causes a tightening of the protein structure and decreased muscle hydration (Hamm, 1960).

Swift and Berman (1959) found direct significant correlations between water holding capacity and pH, zinc content of beef muscle, fat content of beef muscle, and ratio of moisture to protein. Since zinc paralleled pH in its affect on water holding capacity it appeared that zinc may participate as a component or an activator of an enzyme system (Berman, 1961).

Adenosine Triphosphate. Living muscle contains a high concentration of adenosine triphosphate. Immediately after death, the creatine phosphate content of muscle drops rapidly, adenosine triphosphate concentration remains nearly constant for a time and then drops rapidly. Accompanying this is a parallel decrease in pH (Lawrie, 1953). This decrease within 24 hours after slaughter is brought about by decomposition of adenosine triphosphate to adenosine diphosphate, then adenosine monophosphate and finally, to inosine monophosphate (Hamm, 1959). The decrease of adenosine triphosphate after slaughter is proportional to the decrease of muscle water holding capacity post-mortem. Marsh (1952a,b) reported that even if pH remained almost unchanged, the post-mortem decrease of muscle water holding capacity

still occurred. Hamm (1960) showed that by adding adenosine triphosphate to muscle, water holding capacity of that muscle was increased. Adenosine triphosphate apparently formed strong complexes with alkaline earth metals and thereby tied up a part of the bivalent ions. Upon decomposition to adenosine diphosphate and inosine monophosphate the bound cations were released. These then became a part of the protein structure causing a decreased muscle water holding capacity (Hamm, 1959). This decrease of adenosine triphosphate post-mortem may account for two-thirds of the loss of muscle hydration upon slaughter (Hamm, 1959, 1960).

Location of Muscle. Muscle location and function influenced water holding capacity. Different muscles developed characteristic amounts of lactic acid and thus had different ultimate pH values. Muscles which contained high amounts of lactic acid, i.e., low ultimate pH had a low water binding ability (Briskey et al., 1959c, 1960). Water holding capacity of muscle was increased with increased amounts of connective tissue, especially collagen (Hamm, 1960).

Comparison of Methods for Determination of Water Holding Capacity

Determination of water holding capacity is highly empirical. Either the press or centrifuge method was commonly used for the determination of muscle water holding capacity. Deatherage (1957) showed excellent agreement between the press and centrifuge methods for the determination of water holding capacity of fresh meat. The methods did not agree when cooked meat was used. Moe (1964) found that the centrifuge method was

a better indicator of eating quality of beef than the press method. The advantages of the filter paper press method are as follows: 1) the technique is applicable for ground and unground tissue, with or without added water, and heat denatured meat can be used; 2) only 0.3 gram of tissue or homogenate are necessary; 3) the technique requires only a few minutes; 4) the results are fixed on the filter paper, permitting evaluation at any time; and 5) the apparatus, technique, and evaluation are very simple (Hamm, 1960). The centrifuge tube method can be used to study changes in water holding capacity from heating muscle (Hamm, 1960). Another advantage is that two useable fractions are obtained for further study (Deatherage, 1957).

Factors that Influence Myoglobin Concentration

Blood Supply and Availability of Oxygen. Myoglobin and hemoglobin are similar in function in that they both serve to complex with the oxygen required for metabolic activity of the animal. However, their roles are different. Hemoglobin acts as an oxygen carrier in the blood stream, whereas myoglobin is essentially a storage mechanism for oxygen in the cells. Because of the heavy oxygen demand by the heart, myoglobin is present in larger quantities in this organ than in any other tissues. Another contributing factor to the hearts dark color is its high post-mortem pH (Lawrie, 1950). The wing muscles of birds are lower in myoglobin content than the rest of their skeletal muscles because the increased oxygen demand of these tissues is supplied by a highly efficient blood supply (American Meat Institute Foundation, 1960, p. 88).

Specie. Whales have the highest skeletal muscle myoglobin concentration. In the domestic animals, beef has the highest concentration and pork and veal the lowest concentration, while lamb is intermediate (American Meat Institute Foundation, 1960, p. 88).

Exercise and Age. The principal factor causing an increase in myoglobin concentration in muscle is the increased demand for oxygen by the tissue (Lawrie, 1950). This is brought about by maturation or by exercise. Lawrie (1953) showed that enforced activity of muscle caused an increase in myoglobin content provided it continued over time. Even severe exercise for a short time produced no such response. Briskey et al. (1959a) reported that swine adjusted rapidly to repeated exercise. One lot of four hogs was walked one mile/day and another lot 2 to 3 miles/day for four days before being slaughtered. The hams from these hogs were similar in color to non-exercised controls. However, a single severe exercise (3 miles prior to slaughter) produced dark colored, high pH hams in both fasted and full-fed swine. Increased color intensity of the severely exercised lots was not due solely to increased pigment concentration. It was postulated, however, that this increase in color intensity was due to a change in muscle consistency as a result of its higher pH.

Chilling and Freezing. Since color changes seemed to occur during the chilling process of muscle, Briskey et al. (1959b) determined muscle myoglobin concentrations before and after chilling. Their data showed that color changes occurred, but there was no evidence of myoglobin destruction. deDuve (1948) indicated that preliminary freezing of meat

seemed to facilitate myoglobin extraction, but it also rendered the myoglobin more susceptible to subsequent denaturing *in situ*. However, even after freezing, careful grinding of the cell particles was needed for complete extraction. Denaturation of myoglobin may occur if thawed muscle samples are held for long periods of time at room temperature.

pH. Various workers have shown that muscle color was directly related to pH (Scaife, 1955; Lawrie, 1958; Judge et al., 1959; Briskey et al., 1959a, 1960). Lawrie (1950) and Briskey *et al.* (1959a) reported that muscles with the same myoglobin content could appear much darker at pH 7.0 than at pH 6.3.

Factors Influencing Adenosine Triphosphate Concentration

Type of Rigor Mortis. Bendall *et al.* (1963) found that development of rigor mortis markedly affected the rate of adenosine triphosphate disappearance. Porcine muscle characterized by a slow decrease in extensibility, i.e., slow rate of rigor mortis, had a slow rate of adenosine triphosphate degradation. Adenosine triphosphate concentration remained high and nearly constant for the first 30 minutes, and then decreased at a constant rate until it was completely degraded at approximately 280 minutes post-mortem. In carcasses that had a rapid onset of rigor mortis, the adenosine triphosphate concentration decreased rapidly until degradation was complete at about 150 minutes post-mortem. Samples from the carcasses were evaluated for quality 24 hours post-mortem. All samples from the carcasses having a slow decrease in adenosine triphosphate were satisfactory. Those from carcasses

exhibiting a fast rate of adenosine triphosphate degradation were generally of a pale and watery appearance.

Carcasses having a rapid decline in muscle adenosine triphosphate concentration were also characterized by a faster rate of pH decline, a greater rate of lactic acid formation, and a more rapid rate of creatine phosphate breakdown (Bendall et al., 1963). The ultimate pHs and lactic acid concentrations were similar, however, with both types of rigor mortis.

Freezing and Thawing. Freezing muscle tissue immediately post-mortem, decreased the rate of glycolysis. It was completely inhibited, as was adenosine triphosphate degradation, at low temperatures of -30°C. (Partmann, 1963). Storage at -8°C. for 10 to 25 days resulted in the splitting of energy-rich nucleotides in beef, chicken, rainbow trout, and carp muscle, whereas at -24°C. 70 to 100% of the initial adenosine triphosphate was still present after 6 months storage. During thawing of pre-rigor frozen muscle the adenosine triphosphate is degraded rapidly producing thaw rigor (deFremery and Pool, 1960; Partmann, 1963).

deFremery and Pool (1960) demonstrated that adenosine triphosphate breakdown was increased by increasing the thawing temperature of muscle. The time required for a 50% reduction in initial adenosine triphosphate concentration at 40°, 43°, and 52°C. was one and one-half, one, and one-fourth hours, respectively.

Relationship Between Muscle Quality and Cooking Loss

The amount of external fat cover on pork loin roasts greatly affected their total cooking loss. Weir (1960) found that as external fat cover increased from a negligible amount to 0.4 to 0.5 inches, yield of cooked meat decreased 5.5% and drip loss increased from 5.5% to 12%. Total cooking loss was not affected by the amount of external finish, but drip loss increased with increasing amounts of finish and volatile losses decreased (Saffle and Bratzler, 1959; Murphy and Carlin, 1961; Onate and Carlin, 1963).

Braising one-half inch pork chops resulted in an average loss of 33% of the raw weight (Murphy and Carlin, 1961). Drip loss accounted for 3.5 to 7% and volatile loss 23 to 28% of the total cooking loss in this study. Working with pork loin roasts (rib section) Onate and Carlin (1963) observed a 21% total cooking loss (6% drip and 15% volatile). A relationship may exist between total cooking loss and loin eye area, i.e., larger area, more surface exposure, longer cooking time, and more evaporation loss (Weir, 1960).

Wierbicki *et al.* (1957) demonstrated that moisture lost from ground beef increased with increasing cooking temperatures, except between 55° to 65°C., when it decreased somewhat. This variation in fluid retention on heating can be explained in terms of the colloidal transformations that occurred in the soluble meat proteins (Sherman, 1961). At approximately 50°C., coagulation of the soluble nitrogen extracts began. This coagulation within the meat caused the proteins

to interlink and form a barrier to prevent fluid loss (Sherman, 1961). At higher temperatures this structure weakened and fluid retention decreased. Wierbicki et al. (1957) felt that in the 40° to 70°C. range (commencement and completion of protein denaturation) dynamic shifts take place in ionic concentration (potassium, calcium, and magnesium) in such a manner as to promote meat hydration and to counteract the dehydration usually associated with heat denaturation. This tendency was promoted by the addition of sodium chloride which increased the absorption of both potassium and magnesium ions by muscle tissue, but had almost no effect on calcium.

Muscle structure significantly affected cooking loss, i.e., pale, soft, and exudative muscle had a higher cooking loss than dark, firm, and dry muscle (Meyer et al., 1963). Sayre et al. (1964) reported that if rigor onset occurred at pH values below 5.9 and at temperatures above 35°C., the longissimus dorsi was pale and exudative, 40 to 50% of the sample weight was lost during cooking due to evaporation, and cooking rate was slow. Less than 20% of the sample weight was lost by evaporation during cooking when the longissimus dorsi was dark and firm. Wismer-Pedersen (1960) showed that watery muscle absorbed more curing pickle than normal muscle. However, the curing pickle was not firmly bound in the watery muscle and the water holding capacity of cured watery muscle was less than that of normal cured muscle.

Factors Influencing Tenderness

Adenosine triphosphate. deFremery and Pool (1960), working with chicken muscle, reported a positive correlation between adenosine triphosphate disappearance and muscle toughness. The rate of adenosine triphosphate breakdown was greatest at 40° and least at 10°C. Tenderness paralleled this degradation. Muscles were tender when stored at 10° to 20°C. and less tender when stored at 30° to 40°C. This correlation between tenderness and adenosine triphosphate disappearance was due to the fact that decreases in extensibility of muscle and disappearance of adenosine triphosphate are related almost linearly (Bate-Smith and Bendall, 1949). When the muscle adenosine triphosphate level fell below 85% of its initial value, extensibility decreased rapidly and was virtually completed when the level dropped below 15%.

Muscle Water Holding Capacity. Most processes that caused a loosening of protein structure of muscle increased water holding capacity and also caused changes in the tenderness of that muscle (Hamm, 1960). Wierbicki et al. (1956) found that the amount of juice expressed from muscle at 3 and 13 days post-mortem was inversely related to tenderness. Thus, muscles with a high water holding capacity were dark and firm and more tender than low water binding muscles (Lewis et al., 1962; Sayre et al., 1964). Judge et al. (1958, 1960) reported the opposite, i.e., that dark, firm muscles were less tender than light, soft muscles.

Urbin et al. (1962) showed significant differences in free moisture area values (square inches of juice expressed per 500 mg. of sample) between 8 positions within cross-sectional samples of pork longissimus dorsi taken from a 3-rib roast cut anterior to the 12th rib. The medial portion of the longissimus dorsi had lower free moisture values than the lateral portion. Tenderness paralleled these findings. Shear values were lower in the lateral portion where free moisture values were highest.

Marbling. Batcher and Dawson (1960) working with porcine muscle indicated that the amount of intramuscular fat was significantly related to tenderness scores. This is in agreement with the results of Husaini et al. (1950) and Murphy and Carlin (1961). In contrast, Batcher et al. (1962) found tenderness and juiciness scores to be related to intramuscular fat content in only a few cases and the relationship varied with the muscle studied. Tuomy et al. (1966) showed that percent fat was not related to either flavor or texture score of porcine longissimus dorsi muscle.

Various workers have also shown marbling score or intramuscular fat content not to be related to tenderness, juiciness, or flavor scores in beef muscles (Tuma et al., 1962, 1963; Gilpin et al., 1965; Goll et al. 1965; Walter et al., 1965).

Position Affect. The results of Weir (1953) showed that the anterior and posterior parts of the longissimus dorsi were more tender than the middle part. Batcher and Dawson (1960) found the anterior part of the raw longissimus dorsi to be more tender than the posterior part. However,

there was no difference in tenderness when the cuts were cooked.

In 1961, Murphy and Carlin reported that shear values on raw pork loin chops indicated that the proximal (nearest the backbone) portion of the chop to be more tender than the distal portion. At a later date this was found to be true for both raw and cooked pork chops (Onate and Carlin, 1963). Conflicting results have been reported by Urbin et al. (1962) and Alsmeyer et al. (1965a, b) who found the lateral portion of the porcine longissimus dorsi to be more tender than the medial portion.

EXPERIMENTAL PROCEDURE

Sample Selection

The 40 pork loins used in this experiment were purchased from the Maurer-Neuer Division of the John Morrell Packing Company in Arkansas City, Kansas. All the loins were from female swine. Carcasses were selected on the slaughter line and divided into five treatment groups based on stage in the estrous cycle. Stage of estrous cycle was determined by the physical appearance of the ovaries. These selections were made by a graduate student in reproductive physiology. The treatments and selection criteria are given in Table 1. Carcasses were identified and allowed to chill 24 hours. After chilling, the carcasses were cut into wholesale cuts, the right loins were obtained, and brought back to the Kansas State University meats laboratory for further processing.

Table 1. Treatments and sample selection criteria.

Treatment	Approximate Time of Estrous Cycle	Physical Appearance of Ovaries
Pre-puberal	Prior to first estrus	No corpus luteum, no corpus albicans; follicles present; small, underdeveloped ovary
Early luteal	1 to 4 day after ovulation	Corpus luteum formative stage, corpus albicans present; large number of small follicles
Late luteal	5 to 16 day after ovulation	Fully formed, morphologically functional corpus luteum; corpus albicans present; intermediate size follicles
Follicular	20 to 0[a] day after ovulation	No corpus luteum, corpus albicans present; small number of large follicles
Pregnant	Day 20 until next ovulation	Conceptis

[a]Day 0 is first day of estrus.

Cutting Procedure

The longissimus dorsi was divided into 3 sections as follows: A-6th through the 9th thoracic vertebrae, B-2 one inch chops cut on the power band saw from the 10th through the 13th thoracic vertebrae, and C from the 14th thoracic vertebrae through the 2nd lumbar vertebrae.

Subjective Evaluation

Color and firmness were scored on the longissimus dorsi using the Wisconsin Pork Quality standards (1963). Both the anterior and the

posterior edges of sections B and C were evaluated and the average was used as an indication of firmness or color, respectively throughout the section. Marbling was scored to the nearest one-third of a degree using the USDA beef marbling standards. The anterior and the posterior edge of section A was scored and an average was used as an indication of the amount of marbling in the longissimus dorsi. Color, firmness, and marbling standards are presented in Appendix A.

Sample Preparation

The chops from section B were wrapped in polyethylene coated freezer paper, quick frozen at -25°C., and stored at -20°C. for further analysis. The longissimus dorsi was dissected from sections A and C and the muscle was ground 3 times through the fine plate of an Oster food grinder. The muscle from section C was placed in whirl-pak bags, quick frozen (-25°C.), and stored at -20°C. until myoglobin, sodium, and potassium determinations could be made. Water holding capacity (press and centrifuge), percent total moisture, and percent ether extract were run on fresh samples of section A. Section A samples were then frozen until water holding capacity determinations by the centrifuge method could be run on the frozen samples.

Fat Extraction and Moisture Determination

Total moisture and fat content were determined using a modified AOAC (1960) procedure. Extraction thimbles were dried at 120°C., cooled in a desiccator, and weighed. A 3-4 gram sample was immediately weighed into the thimble to the nearest 0.1 mg. on a Gram-atic automatic balance.

Samples were run in triplicate, dried for 12 hours in a vacuum oven at 98°C. and 25 pounds pressure, cooled in a desiccator, weighed, and total water calculated as loss in weight. Samples were then extracted in a Soxhlet extractor with petroleum ether for 12 hours, redried in a vacuum oven at 98°C., cooled, and reweighed. The additional weight loss was expressed as per cent ether extract. Both total moisture and ether extract were expressed as per cent of the original sample weight.

Water Holding Capacity

Water holding capacity was determined on fresh muscle by a modification of the press method of Grau and Hamm (1956) and by a modification of the Wierbicki et al. (1957) centrifuge method. In addition, the water holding capacity of frozen muscle was determined by the Wierbicki et al. (1957) method. The procedure followed is given in Appendix B.

The procedure for the determination of water holding capacity by the press method was as follows: Whatman number 1 filter paper was kept in a desiccator over a saturated potassium chloride solution for one to two days to maintain a constant humidity. When the sample was prepared for measurement, a single sheet of filter paper was removed from the desiccator and placed on a plexiglass plate. The sample (300 mg. $\pm$ 20 mg.) was placed on the midportion of the sheet and immediately another plexiglass plate was placed on the sample. This was repeated twice more for each sample so that there were three meat samples on three pieces of filter paper between four plexiglass plates, i.e., each sample was done in triplicate.

The plexiglass plates were then subjected to 10,000 pounds pressure for five minutes on a Carver Laboratory Press. After pressing, the muscle boundary was outlined with pencil and the muscle sample removed from the filter paper. Later the muscle and juice area and the muscle area were measured with a compensating polar planimeter. Results were expressed as muscle plus juice area, muscle area, juice area, muscle area per gram of sample, and juice area per gram of sample.

Modification of the Wierbicki centrifuge method consisted of weighing a 9 to 14 gram sample of muscle into a polyethylene centrifuge tube, stoppering with a rubber stopper fitted with a capillary tube, and placing the tube in a 70°C. water bath for 30 minutes. Then the sample and juice were transferred to a fritted glass crucible. The crucible was taped to the top of a polyethylene centrifuge tube and centrifuged at 3000 rpm. for 5 minutes. The crucibles were kept in a desiccator over a saturated potassium chloride solution two to three hours before use. Volume of expressed juice was measured to the nearest 0.1 mg. Water content of the juice was determined and the results were expressed as ml. of juice per 100 gram and as % total moisture lost.

Cooking Loss

A modified broiling method was used for determination of cooking loss (Harrison, 1966). Samples were thawed, weighed to the nearest 0.1 mg. on a Gram-atic automatic balance, and placed on a 3-4 inch high wire rack set in a shallow aluminum pan. A glass thermometer was inserted into the center of each chop to determine end point cooking

temperature. The wire rack, aluminum pan, and thermometer were weighed to the nearest 0.1 mg. before placing the sample on them. Cooking was done in a rotary oven at 205°C. to an internal chop temperature of 76°C. It was not necessary to turn the chops during cooking because the heat reached them uniformly from all sides and produced an evenly browned product. After cooking the chops were turned and allowed to cool to 40°C. before reweighing. Total cooking loss was calculated as loss in weight during cooking. The wire rack, aluminum pan, and thermometer were reweighed and the increase in weight was recorded as drip loss. The difference between total cooking loss and drip loss was recorded as volatile loss. Results were reported as per cent total cooking loss, drip loss, and volatile loss. Samples were run in duplicate.

Electrolyte Determination

Sodium and potassium were analyzed on a Beckman DU-2 spectrophotometer adapted for flame analysis by a modification of the method of Kirton and Pearson (1963). A 1.5-3.5 gram muscle sample was homogenized with a Vir Tis homogenizer for 4-5 minutes in 75 ml. of 2% trichloracetic acid (TCA). The flask was rinsed with 2% TCA and the mixture diluted to 100 ml. with 2% TCA. This was stored overnight at 5°C. The homogenized samples were then centrifuged at 3000 rpm for 15 minutes. Before determinations were run the supernatant was diluted 1:3 with deionized water using an automatic diluter. All solutions were made up with distilled deionized water. A standard curve was used to determine the sodium and potassium content of the muscle samples. The standards contained 100,

200, 400, 600, and 800 mg.% potassium and 10, 25, 50, 75, and 100 mg.% sodium in a deionized water solution. Sodium was determined at a wavelength of 590 mu and potassium at a wavelength of 768 mμ. Samples were run in duplicate and the results were reported in grams per kilogram of muscle.

Shear Measurement

Shear determinations were made by a Warner-Bratzler shearing device on one-half inch cores from the medial (nearest the spinous process) and lateral portions of 1-inch chops after analysis for cooking loss was completed. Thus, the chops were cooled uniformly for 30 minutes before shear determinations were made. Samples were run in duplicate and one shear per core was taken.

Myoglobin Analysis

Fifty grams of sample from section C was used for pigment analysis. Pigments were extracted using a cold 0.1 N acetate buffer solution. The procedure followed is listed in Appendix C.

Myoglobin determination was done by the carbon monoxide conversion method as described in Appendix D. The method consisted of first buffering the filtrate with N/15 Na_2HPO_4, centrifugation, and refiltration. Then carbon monoxide was slowly bubbled through the filtrate in Thunberg tubes for 10 minutes. A reducing agent (Na_2S2O_4) was added and carbon monoxide was bubbled through the filtrate for an additional 10 minutes. Cuvettes were quickly filled with the solution and read at 538 mμ and

568 mμ on a Beckman DU-2 spectrophotometer. Myoglobin concentrations were calculated using the extinction coefficients and equations reported by Poel (1949).

The procedures listed in Appendixes C and D are modified from those used by Fleming et al. (1960) and Romans (1964).

Statistical Analysis

A simple one way analysis of variance was run on each of the 31 variables studied. Simple correlation coefficients were determined between all 31 variables by the statistical laboratory. Correlations of .312 and .403 were required at the .05 and .01 levels with 40 observations for significance (Snedecor, 1956, p. 174). T-tests were also run to determine differences between tenderness values between the medial and lateral portions of the chop and differences between ml. of juice expressed and percent moisture lost by heating and centrifugation before and after freezing. A value of 3.96 was required for significance at the .05% level (Snedecor, 1956, p. 249).

RESULTS AND DISCUSSION

No significant differences were found between the 5 treatments studied. Treatment means and "F" values for the variables studied are given in Tables 2 and 3. Apparently stage of estrous cycle and pregnancy had little or no effect on porcine longissimus dorsi composition and quality. A major limitation of the procedure followed was that no background information could be obtained about the porcine carcasses selected such as

Table 2. Treatment means and F values for the 31 variables studied.

Variables Studied	Treatment means (average of 8 observations)					
	Pre-puberal	Early-luteal	Late-luteal	Follicular	Pregnant	F test
Marbling Score[a]	18.12	20.38	15.69	17.44	15.75	1.35[e]
Color Score[a]	2.81	2.91	2.97	2.81	2.69	0.57
Firmness Score[a]	2.78	2.84	2.66	2.91	2.62	1.11
% Total Moisture	75.12	74.73	75.84	75.36	75.14	0.34
% Ether Extract	5.03	6.18	4.56	5.56	5.33	0.88
Myoglobin mg/g	0.71	0.71	0.70	0.78	0.65	0.95
Sodium g/kg	0.44	0.44	0.42	0.47	0.41	1.65
Potassium g/kg	3.88	3.79	3.92	4.03	3.96	0.71
% Total Moisture (FF)[b]	79.09	79.66	79.46	79.80	79.36	0.52
% Ether Extract (MF)[c]	20.01	24.08	18.49	22.22	21.25	1.06
Myoglobin mg/g (MF)[c]	2.90	2.84	2.91	3.20	2.64	0.75
Myoglobin mg/g (MFF)[d]	3.05	3.02	3.04	3.38	2.79	0.81
Sodium g/kg (MF)[c]	1.79	1.74	1.72	1.91	1.67	1.24
Sodium g/kg (MFF)[d]	1.89	1.85	1.80	2.02	1.76	1.40
Potassium g/kg (MF)[c]	15.68	15.08	16.35	16.46	16.02	0.70
Potassium g/kg (MFF)[d]	16.50	16.06	17.11	17.41	16.91	0.69

[a]Marbling: 2 = devoid, 36 = extremely abundant +
Color: 1 = extremely pale, 5 = dark
Firmness: 1 = soft and watery, 5 = very firm and very dry

[b]Fat free

[c]Moisture free

[d]Moisture and fat free

[e]All F tests were nonsignificant

Table 3. Treatment means and F values for the 31 variables studied.

Variables Studied	Treatment means (average of 8 observations) Pre-puberal	Early-luteal	Late-luteal	Follic-ular	Preg-nant	F test
% Cooking Loss						
Total Loss	28.09	27.24	28.41	28.94	30.12	1.66[c]
Drip Loss	2.33	2.19	2.13	2.50	2.79	1.04
Volatile Loss	25.70	25.05	26.28	26.45	27.33	1.01
Shear Value[a]						
Medial	5.46	5.45	5.46	5.08	5.36	0.23
Lateral	5.79	6.56	6.11	5.02	5.39	1.89
Centrifuge Method WHC[b]						
Fresh Muscle						
% Moisture Expressed	35.90	34.96	38.11	38.01	39.80	1.45
Ml. of Juice Expressed/100 g	28.61	27.76	30.60	30.60	32.08	1.84
Frozen Muscle						
% Moisture Expressed	34.85	31.81	35.44	35.06	36.18	0.94
Ml. of Juice Expressed/100 g	27.92	25.30	28.68	28.35	29.18	1.07
Difference[d]	98.95	96.85	97.32	97.05	96.38	0.45
Press Method WHC[b]						
Meat + Juice Area	32.26	34.26	33.63	32.05	32.16	1.72
Meat Area	13.11	13.54	12.98	13.50	12.88	0.18
Juice Area	19.16	20.66	20.66	18.55	19.82	1.04
Meat Area/g	43.64	44.84	43.15	45.11	42.31	0.22
Juice Area/g	63.98	68.32	68.56	61.95	63.60	1.00

[a]Lbs. shear force for 1/2 inch cooked cores

[b]Water holding capacity

[c]All F tests were nonsignificant

[d]% moisture expressed from a frozen sample minus % moisture expressed from a fresh sample plus 100

breed, chronological age, feeding regime, management practices, or antemortem treatment. All the swine used should have come out of the same herd and been at least half-sib related. Also different end points could have been determined, i.e., taste panel (tenderness and juiciness scores), intercellular and extracellular potassium and sodium content, adenosine triphosphate content, and pH. These factors were probably confounded in the treatments and might have caused a reduction in the effect of the stages studied.

Since no significant differences were found, all possible correlations were run between the 23 variables studied plus percent total moisture on a fat free basis, percent ether extract on a moisture free basis and myoglobin, sodium, and potassium content on a moisture free and moisture plus fat free basis using all 40 observations. In addition, T-tests were run to determine differences between shear force values between the medial and lateral portions of the chop and differences in water binding ability due to freezing as determined by the centrifuge method, i.e., differences in percent moisture lost and ml. of juice expressed per 100 grams.

No significant difference was found in shear value due to position. However, the medial position in the majority of the samples tended to be more tender. Over all average shear values were 5.36 for the medial position and 5.78 for the lateral position.

A significant difference in percent water lost and ml. of juice expressed due to freezing was shown. Fresh samples had a greater moisture loss than frozen samples (37.36% vs. 34.67%). This was reported as percent difference in the results (frozen minus fresh plus 100). A greater amount

of juice was also expressed in the fresh state. Greater amounts of moisture loss would be expected after freezing due to rupture of the sarcolemma by ice crystals. Error in sampling the frozen muscle might account for some of the difference in results. There was a large amount of drip loss in the sample bags and this might not have been thoroughly mixed with the muscle before determinations were run.

Means, ranges, and standard deviations for the 31 variables used are presented in Tables 4 and 5. Myoglobin values are within the lower ranges reported by Ginger et al. (1954) and Briskey et al. (1959a). Gillett et al. (1965) found the potassium content of pork longissimus dorsi muscle to range from 3.54 to 3.96 g/kg. and the sodium content to range from .41 to .51 g/kg. of wet tissue. These values are in agreement with those reported in this paper. Drip cooking loss values are lower than those shown by Murphy and Carlin (1961) and Onate and Carlin (1963), but total cooking loss and volatile loss values are essentially in agreement. Lower drip loss values were probably due to the fact that the chops in this study were completely free of external fat, whereas in the other studies external fat was left on the chops or roasts.

Since it is almost impossible to obtain the same amount of fat cover on each sample, error is introduced into the cooking loss values. Also by leaving some external fat on the sample a true muscle cooking loss value is not obtained.

Table 4. Ranges, means, and standard deviations of the 31 variables studied.

Character Studied	Range	Mean	Standard Deviation
Marbling Score[a]	8.50 - 26.50	17.48	4.80
Color Score[a]	2.25 - 4.00	2.84	0.39
Firmness Score[a]	2.25 - 3.00	2.76	0.32
% Total Moisture	71.56 - 77.80	75.24	1.90
% Ether Extract	1.91 - 9.83	5.33	1.81
Myoglobin mg/g	0.45 - 1.07	0.71	0.13
Sodium g/kg	0.36 - 0.56	0.44	0.05
Potassium g/kg	3.13 - 4.60	3.91	0.30
% Total Moisture (FF)[b]		79.47	1.09
% Ether Extract (MF)[c]		21.21	5.85
Myoglobin mg/g (MF)[c]		2.90	0.64
Myoglobin mg/g (MFF)[d]		3.06	0.65
Sodium g/kg (MF)[c]		1.77	0.24
Sodium g/kg (MFF)[d]		1.86	0.25
Potassium g/kg (MF)		15.92	1.86
Potassium g/kg (MFF)		16.80	1.77

[a]Marbling: 2 = devoid, 36 = extremely abundant +
Color: 1 = extremely pale, 5 = dark
Firmness: 1 = soft and watery, 5 = very firm and very dry

[b]Fat free

[c]Moisture free

[d]Moisture and fat free

Table 5. Ranges, means, and standard deviations of the 31 variables studied.

Character Studied	Range	Mean	Standard Deviation
% Cooking Loss			
Total Loss	23.52 - 32.70	28.56	2.43
Drip Loss	1.00 - 4.12	2.39	0.74
Volatile Loss	21.33 - 31.04	26.16	2.40
Shear Value[a]			
Medial	3.40 - 8.00	5.36	0.93
Lateral	3.20 - 9.00	5.78	1.29
Centrifuge Method WHC[b]			
Fresh Muscle			
% Moisture Expressed	24.10 - 46.00	37.36	4.62
Ml. of Juice Expressed/100 g	19.40 - 38.10	29.93	3.75
Frozen Muscle			
% Moisture Expressed	25.10 - 47.40	34.67	4.85
Ml. of Juice Expressed/100 g	19.40 - 39.40	27.88	4.15
Difference[c]	89.10 -106.30	97.31	4.02
Press Method WHC[b]			
Meat + Juice Area	27.89 - 37.79	32.87	2.25
Meat Area	9.91 - 18.03	13.20	1.92
Juice Area	12.35 - 24.42	19.77	2.58
Meat Area/g	31.53 - 60.24	43.81	6.68
Juice Area/g	41.17 - 78.93	65.28	8.44

[a] Lbs. shear force for 1/2 inch cooked cores

[b] Water holding capacity

[c] % moisture expressed from a frozen sample minus % moisture expressed from a fresh sample plus 100

Factors Related to Water Holding Capacity

One of the purposes of this experiment was to study the effects of level of muscle components on muscle water holding capacity. Samples for chemical analysis were obtained from the 6th thoracic vertebrae through the 2nd lumbar vertebrae excluding the 10th through the 13th thoracic vertebrae. Muscle components studied were percent total moisture, percent ether extract, and myoglobin and electrolyte content, namely sodium and potassium. In addition, these variables were compared on a moisture free, fat free, and combined moisture fat free basis. The relationships between subjective measures and muscle water holding capacity were also studied. The results are summarized in Table 6.

Marbling score was significantly correlated to ml. of juice expressed per 100 g. of both fresh (-.387*) and frozen (-.312*) sample as determined by the centrifuge method. Press meat area and meat area per gram were positively correlated with marbling score (.397* and .381*). Thus, samples with a high amount of intramuscular fat had large pressed meat areas. Negative nonsignificant correlations were found between marbling score and juice area (-.124) and juice area per gram (-.143) obtained by the press method. These data indicated as marbling score increased, water holding capacity as determined by both centrifuge and press methods tended to increase (or expressible moisture decreased). Judge et al. (1958) and Hamm (1960) concluded similarly that meat with a high intramuscular fat content should have a higher water holding capacity than meat with a low fat content, whereas Goll et al. (1965) found marbling

Table 6. Simple correlations between longissimus dorsi components plus subjective measures and water holding capacity measurements.

Characteristic studied vs.	WHC[a] - Centrifuge Method				WHC[a] - Press Method					
	Fresh Muscle		Frozen Muscle							
	% Moisture expressed	Ml. of juice expressed/ 100 g	% Moisture expressed	Ml. of juice expressed/ 100 g	Difference[b]	Meat + juice area	Meat area	Juice area	Meat area/g	Juice area/g
Marbling Score[c]	-.287	-.387*	-.248	-.312*	.030	.178	.397*	-.124	.381*	-.143
Color Score[c]	-.322*	-.290	-.387*	-.338*	-.097	-.126	.329*	-.368*	.370*	-.309*
Firmness Score[c]	-.567**	-.582**	-.565**	-.552**	-.030	-.109	.516**	-.458**	.538**	-.449**
% Total Moisture	-.118	.081	.216	.371*	.397*	.097	-.195	.233	-.185	.243
% Ether Extract	.080	-.089	-.091	-.225	-.202	-.090	.208	-.238	.189	-.239
Myoglobin mg/g	.052	.118	.171	.220	.147	.044	-.037	.028	-.014	.095
Sodium g/kg	-.120	-.140	.031	.022	.183	-.012	.139	-.141	.157	-.080
Potassium g/kg	.089	.144	.031	.074	-.065	-.115	-.224	.090	-.215	.083
% Total Moisture (FF)[d]	-.105	.027	.269	.368*	.446**	.053	-.071	.099	-.080	.114
% Ether Extract (MF)[e]	.069	-.080	-.031	-.152	-.117	-.070	.184	-.201	.162	-.203
Myoglobin mg/g (MF)[e]	.015	.137	.226	.319*	.256	-.062	-.101	.096	-.076	.158
Myoglobin mg/g (MFF)[f]	.020	.131	.226	.310	.250	.059	-.087	.082	-.064	.146
Sodium g/kg (MF)[e]	-.184	-.090	.157	.230	.402*	.048	.024	.000	.043	.060
Sodium g/kg (MFF)[f]	-.175	-.103	.147	.202	.379*	.031	.052	-.036	.069	.026
Potassium g/kg (MF)[e]	-.022	.138	.151	.277	.208	-.020	-.266	.096	-.256	.199
Potassium g/kg (MFF)[f]	-.010	.138	.154	.270	.198	-.037	-.259	.177	-.250	.180

[a]Water holding capacity

[b]% moisture expressed from a frozen sample minus % moisture expressed from a fresh sample plus 100

[c]Marbling: 2 = devoid, 36 = extremely abundant +; Color: 1 = extremely pale 5 = dark: Firmness: 1 = soft and watery, 5 = very firm and very dry

[d]Fat free

[e]Moisture free

[f]Moisture and fat free

*$P < .05$

**$P < .01$

score and muscle water holding capacity (centrifuge) to be unrelated.

Correlations of -.322*, -.387*, -.338*, .329*, -.368*, .370*, and -.309 were reported between color score and percent moisture lost from fresh and frozen tissue as determined by the centrifugation method, ml. of juice expressed from frozen muscle by heating and centrifugation and meat area, juice area, meat area per gram, and juice area per gram values obtained by pressing. All samples in this study were reasonably desirable in color so the range in color scores was narrow (2.25 to 4.00).

Firmness scores were directly related to centrifuge water holding capacity measurements for both fresh and frozen muscle at the .01% level of significance. Correlations of .516**, -.458**, .538**, and -.499** were also observed between firmness scores and press meat area, juice area, meat area per gram, and juice area per gram. Values for firmness exhibited a range of 2.25 to 3.00 with a mean of 2.76. Thus, a high firmness score indicated a muscle with a high water binding ability. Judge et al. (1958, 1961) and Sayre et al. (1964) have shown that firmer muscles have a higher water binding ability than soft muscles.

Only two significant relationships were observed between percent total moisture and water holding capacity. Total moisture percentage was related to ml. of juice expressed per 100 g. of frozen sample (.371*) and percent difference between fresh and frozen muscle samples (.397*). When these correlations were expressed on a fat free basis they were .368* and .446** respectively. Moe (1964) showed a significant correlation between water holding capacity (centrifuge method) and percent moisture, but not between press determination and percent moisture.

Water holding capacity has previously been shown not to be correlated with total muscle moisture (Hamm, 1960).

Water holding capacity determined by the methods studied was not strongly related to percent ether extract on a wet or dry tissue basis. Intramuscular fat content seems to influence the water binding ability of that muscle (Hamm, 1960). No significant correlation was found by Saffle and Bratzler (1959) between fat content and defrosting drip in certain swine muscles.

A correlation of .319* was observed between myoglobin concentration on a moisture free basis and ml. of juice lost per 100 g. of frozen sample by the centrifuge method. On a wet tissue basis no significant relationships were found between myoglobin content and factors measuring water holding capacity. Muscles dark in color usually have a high water holding capacity (Judge et al., 1958, 1960; Sayre et al., 1964), pH, and myoglobin concentration (Scaife, 1955; Briskey et al., 1960; Topel, 1962).

No significant correlations were found between total muscle sodium and potassium content and water holding capacity on a wet tissue basis. Percent difference between fresh and frozen muscle was correlated with muscle sodium content on a dry basis (.402*) and on a moisture plus fat free basis (.379*). Other relationships were very weak, but the correlation signs are of interest. Sodium content of muscle was negatively related to expressible moisture (percent moisture lost in fresh sample -.120 and juice area -.141) and potassium content positively related (percent moisture lost in fresh sample .089 and juice area .090). Water holding capacity of beef muscle was shown to be positively correlated

with the sodium content and negatively correlated with the potassium content by Swift and Berman (1959). Results reported by Webb et al. (1967) indicated a similar trend, but their correlations were weak and non-significant.

The results of this experiment showed that no differences existed between the methods used for determination of water holding capacity in their ability to predict the effect of muscle composition on muscle water binding ability.

Correlations Between Methods of Water Holding Capacity Determinations

In this experiment water holding capacity of muscle was determined by both the press and centrifuge methods. Centrifuge results were expressed as percent total moisture lost and ml. of juice lost per 100 g. of sample on both fresh and frozen samples; press results as meat plus juice area, meat area, juice area, meat area per gram, and juice area per gram. The results are reported in Table 7.

Interrelationships between fresh and frozen sample water binding ability (centrifuge and press methods) were highly significant. Percent moisture lost in the fresh sample was highly correlated to percent moisture lost after freezing (.641**) and to ml. of juice expressed per 100 g. of frozen sample (.594**). Correlations of .685** and .670** were found between ml. of juice lost per 100 g. of fresh tissue and percent moisture lost and ml. of juice lost after freezing, respectively. Meat plus juice area was not correlated with any of the centrifuge water holding capacity measurements. Meat area was highly correlated with

Table 7. Correlations between two methods of water holding capacity determination.

Characteristic Studied vs.	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
WHC[a] Centrifuge Method								
Fresh Muscle								
1) % Moisture Expressed	.979**	.641**	.594**	-.177	-.569**	.310	-.544**	.273
2) Ml. of Juice Expressed/ 100 g.		.685**	.670**	-.164	-.603**	.348*	-.576**	.310
Frozen Muscle								
3) % Moisture Expressed			.986**	-.127	-.589**	.341*	-.565**	.338*
4) Ml. of Juice Expressed/ 100 g.				-.111	-.589**	.356*	-.565**	.353*
WHC[a] Press Method								
5) Meat + Juice Area					.170	.683**	.113	.681**
6) Meat Area						-.565**	.989**	-.595**
7) Juice Area							-.597**	.967**
8) Meat Area/g								-.618**
9) Juice Area/g								

[a]Water holding capacity

*$P < .05$

**$P < .91$

percent moisture lost in the fresh (-.569**) and frozen sample (-.589**) and ml. of juice lost in the fresh (-.603**) and frozen (-.589**) samples. Meat area per gram of muscle was also highly related to centrifuge method results. Significant correlations of .348*, .341*, and .356* were determined between juice area and ml. of juice lost per 100 g. of fresh tissue, percent moisture lost (frozen) and ml. of juice lost from the frozen sample. Juice area per gram was only related to percent moisture lost (.338*) and ml. of juice lost (.353*) from frozen muscle tissue.

The various ways of expressing water binding ability when determined by the press method were also interrelated. Meat plus juice area was correlated with juice area (.683**) and juice area per gram (.681**); meat area with juice area (-.565**), meat area per gram (.989)**), and juice area per gram (-.595**); juice area with meat area per gram (-.597**) and juice area per gram (.967**); and meat area per gram with juice area per gram (-.618**). As pressed meat area and meat area per gram of sample increased, juice area and juice area per gram decreased.

Interpretation of the measurements used for expression of muscle water holding capacity indicate that as ml. of juice expressed per 100 g. of either fresh or frozen muscle tissue by heating and centrifugation increased, percent moisture lost increased, meat plus juice area decreased, meat area decreased, juice area increased, meat area per gram decreased, and juice area per gram increased. In other words, a muscle with a high water holding capacity would have less juice lost per 100 g. due to

centrifugation, a larger pressed meat area, and a smaller pressed juice area than a muscle with low water binding ability.

This data showed that expression of press results as meat area or meat area per gram were more highly correlated with centrifuge method results than juice measurements. It is also interesting to note that significant correlations existed between press measurements and centrifuge measurements on both fresh and frozen samples. Deatherage (1957) demonstrated excellent agreement between the centrifuge and press methods for the determination of water holding capacity of fresh tissue.

Relationships of Water Holding Capacity Measurements with Cooking Losses and Tenderness

Cooking loss of products is a problem for both the packer and consumer since it represents an economic loss to them. Shrinkage is often used synonymously with cooking loss. Most of the loss during cooking is due to evaporation from the surface of the product and should be strongly correlated to muscle water holding capacity. Correlations of .465** and .466** were found between percent total cooking loss and percent water lost and ml. of juice expressed per 100 g. of frozen sample determined by the centrifuge method (Table 8). Highly significant relationships were also shown between percent volatile loss and percent moisture lost (.413**) and ml. of juice expressed (.409**) from frozen samples. Percent drip cooking loss values were not correlated with water holding capacity values of frozen muscle obtained by the centrifuge method or other results. Goll *et al.* (1965) found that

Table 8. Correlation coefficients between water holding capacity measurements and cooking losses plus shear values.

Variable Studied	Percent Cooking Losses			Shear Values[c]	
	Total	Drip	Volatile	Medial	Lateral
WHC[a] Centrifuge Method					
Fresh Muscle					
% Moisture Expressed	.224	-.049	.236	-.022	.050
Ml. of Juice Expressed/100 g.	.259	-.005	.259	.023	.074
Frozen Muscle					
% Moisture Expressed	.456**	.175	.413**	.142	.182
Ml. of Juice Expressed/100 g.	.466**	.198	.409**	.172	.195
Difference[b]	.304	.267	.227	.197	.163
WHC[a] Press Method					
Meat + Juice Area	.062	-.190	.122	.132	.191
Meat Area	.014	-.082	.043	.136	-.238
Juice Area	.072	-.055	.088	.022	.330
Meat Area	-.010	-.114	.028	.133	-.245
Juice Area/g	.012	-.124	.048	.003	.315

[a]Water holding capacity

[b]% moisture expressed from a frozen sample minus % moisture expressed from a fresh sample plus 100

[c]Lbs. shear force for 1/2 inch cooked cores

*P $<$.05

**P $<$.01

water holding capacity of beef muscle was not related to percent drip or volatile cooking loss. A possible explanation is that drip loss is mainly due to fat loss from the chop and the centrifugation method measures expressible moisture. Water holding capacity of the fresh samples was not related to cooking loss. This indicated that freezing had some effect on the ability of the muscle proteins to bind moisture. Expressible moisture determinations were made on samples stored at 2 to 3°C., 96 to 120 hours after processing. Storage time might also have affected muscle water holding capacity. Correlations between water holding capacity determined by the press method and cooking losses were very low and insignificant. Pressing was done on fresh samples only, which may account for the low correlations. However, highly significant correlations were found between water holding capacity of frozen muscle determined by the centrifuge method and water holding capacity of fresh muscle determined by pressing. Also centrifuge method correlations on fresh muscle were much higher than press method correlations. It appears that the moisture lost during cooking may be the same moisture expressed by the centrifugation method, which is essentially a heating method, but not by the press method. Another possible explanation is that no protein denaturation occurs during pressing similar to that occurring during heating of muscle tissue.

In this study no relationship was found between tenderness of cooked chops and any means of expressing water holding capacity. Theoretically a relationship should exist since the more moisture muscle binds the more tender and juicy it should be. Lewis et al. (1962), Moe (1964),

and Sayre et al. (1964) reported that muscles with a higher water binding ability were more tender than muscles with a low water holding capacity.

Cooking Loss Interrelationships

Interactions between cooking loss values showed percent total cooking loss to be highly correlated with percent volatile loss (.955**), but not drip loss (.188). The correlations are reported in Table 9. Volatile cooking loss made up a greater proportion of the total cooking loss than did drip loss. The method of cookery probably affected this proportion as did the fact that the chops were completely free of external fat. A modified broiling method of cookery was used in this experiment, i.e., the chop was placed 3 to 4 inches above a drip catch pan and cooked in an oven at 205°C. to an internal temperature of 76°C. Since drip loss is mainly fat loss the absence of external fat would tend to decrease this part of the total cooking loss.

Table 9. Interrelationships between cooking loss measurements.

Measurement	Drip Cooking Loss	Volatile Cooking Loss
Total cooking loss	.188	.955**
Drip cooking loss		-.110

**$P < .01$

If volatile loss is mostly evaporation loss (moisture loss) and drip is mainly fat, then a high negative correlation should exist between them. However, in this study a weak nonsignificant negative correlation was found ($r = -.110$).

Relationships of Subjective Measurements Plus *Longissimus dorsi* Components to Cooking Losses and Tenderness

Subjective measurements used in this study (marbling score, color score, and firmness score) were not strongly related to cooking loss or tenderness (Table 10). Both color and firmness scores were negatively, but nonsignificantly correlated with all cooking losses. Gilpin *et al.* (1965) and Goll *et al.* (1965) working with beef also found no consistent relationship between marbling score and cooking losses or tenderness.

Nonsignificant correlations were also observed between cooking losses or tenderness and percent total moisture, percent ether extract, myoglobin concentration, and sodium content on a wet, dry, fat free and/or dry and fat free basis. Correlations of $.332^{*}$ and $-.322^{*}$ were found between the total potassium content of fresh muscle tissue and percent drip and percent volatile cooking losses. Since potassium is primarily an intracellular ion and if it is related to the ability of muscle to bind water, a negative relationship was expected with volatile loss. Drip loss contained some moisture so the same relationship to drip loss could be rationalized. Also since drip is mainly fat loss and a muscle with a high fat content tends to have a high water holding capacity, a negative relationship between drip loss and potassium content was expected.

Table 10. Simple correlations between subjective measurements plus longissimus dorsi components and cooking losses plus tenderness.

Variable Studied vs.	Percent Cooking Losses			Shear Values[e]	
	Total	Drip	Volatile	Medial	Lateral
Marbling Score[a]	.030	-.112	.056	-.048	-.000
Color Score[a]	-.188	-.160	-.140	.067	.175
Firmness Score[a]	-.225	-.118	-.186	-.212	-.204
% Total Moisture	.115	.122	.087	.212	.163
% Ether Extract	-.001	-.034	.001	-.174	-.118
Myoglobin mg/g	.117	.201	.065	.107	-.029
Sodium g/kg	-.048	.066	-.069	-.022	-.178
Potassium g/kg	-.218	.332*	-.322*	.088	-.169
% Total Moisture (FF)[b]	.206	.179	.157	.149	.136
% Ether Extract (MF)[c]	.038	.016	.026	-.140	-.089
Myoglobin mg/g (MF)[c]	.140	.196	.091	.164	.042
Myoglobin mg/g (MFF)[d]	.145	.204	.092	.156	.033
Sodium g/kg (MF)[c]	.032	.120	-.000	.095	-.069
Sodium g/kg (MFF)[d]	.029	.119	-.004	.074	-.087
Potassium g/kg (MF)[c]	-.061	.278	-.142	.174	-.013
Potassium g/kg (MFF)[d]	-.069	.306	-.159	.168	-.031

[a]Marbling: 2 = devoid, 36 = extremely abundant +
Color: 1 = extremely pale, 5 = dark
Firmness: 1 = soft and watery, 5 = very firm and very dry

[b]Fat free

[c]Moisture free

[d]Moisture and fat free

[e]Lbs. shear force for 1/2 inch cooked cores

*$P < .05$

Correlations Between Objective and Subjective Measurements

Industry has need of rapid subjective tests that will accurately measure muscle characteristics. Correlation coefficients between objective measurements and subjective measures of the same characteristics are important in developing such tests. Objective evaluations used in this study were percent total moisture and ether extract, myoglobin, sodium, and potassium concentration, percent cooking loss (total, drip, and volatile), Warner-Bratzler shear values, and water holding capacity by the centrifuge and press methods. These relationships are shown in Table 11. Relationships of subjective measures to cooking losses, tenderness, and water holding capacity have been covered in a previous section (Table 10 and Table 6).

Marbling Score. As was expected percent total moisture was negatively correlated with marbling score (-.448**). On a fat free basis the relationship was positive (.124).

Percent ether extract had a fairly strong relationship (.684**) to marbling score of the longissimus dorsi muscle. Even though these should measure the same muscle component, factors that tend to decrease this relationship are the existence of lipid particles too fine to be visible as marbling, lipid deposits along connective tissue seams, and the presence of ether soluble substances other than true fat. Ether extraction removes all these lipids and thus, tends to give a higher muscle fat value than marbling score (Cover et al., 1956). A correlation of .714** was observed between marbling score and percent ether extract (dry weight basis).

Table 11. Simple correlations between objective and subjective measurements.

Character Studied vs.	Marbling Score[a]	Color Score[a]	Firmness Score[a]
% Total Moisture	-.448**	.185	-.089
% Ether Extract	.684**	-.102	.087
Myoglobin mg/g	-.156	.364*	.233
Sodium g/kg	.234	-.070	-.008
Potassium g/kg	-.397*	-.214	-.219
% Total Moisture (FF)[b]	.124	.198	-.043
% Ether Extract (MF)[c]	.714**	-.099	.071
Myoglobin mg/g (MF)[c]	-.269	.384*	.163
Myoglobin mg/g (MFF)[d]	-.220	.383*	.176
Sodium g/kg (MF)[c]	-.034	.042	-.050
Sodium g/kg (MFF)[d]	.061	.029	-.038
Potassium g/kg (MF)[c]	-.527**	-.016	-.192
Potassium g/kg (MFF)[d]	-.466**	-.036	-.200

[a]Marbling: 2 = devoid, 36 = extremely abundant +
Color: 1 = extremely pale, 5 = dark
Firmness: 1 = soft and watery, 5 = very firm and very dry

[b]Fat free

[c]Moisture free

[d]Moisture and fat free

*$P < .05$

**$P < .01$

Myoglobin concentration on a wet tissue basis was weakly correlated with marbling score (-.156). Removing the effects of fat and moisture increased the correlation strength somewhat (-.269 and -.220), but not enough for significance.

Correlations of .234, -.034, and .061 were observed between sodium concentration on a fresh, dry, and moisture and fat free basis versus marbling score. Potassium, on the other hand, was significantly related on the same bases at -.397*, -.527**, and -.466** to marbling score. As intramuscular fat content increased, muscle potassium content decreased. It appears that marbling is related to muscle water holding capacity (Hamm, 1960). Thus, these results tend to indicate that muscle water holding capacity and potassium content are negatively related to each other.

Color Score. A correlation of .364* was shown between muscle myoglobin concentration and color score. When the effects of fat, and fat plus moisture were removed the strength of the relationship was .384* and .383*, respectively. According to Winkler (1939), Briskey et al. (1959), and others, light penetration and pH have more effect on color of muscle than myoglobin concentration. Muscles with a high pH and "closed" structure are dark. A low pH "open" structured muscle has a pale color. This could explain partially the low correlation between muscle myoglobin content and color score.

Firmness Score. Firmness score was found to be nonsignificantly correlated with all objective tests except water holding capacity (Table 6).

Interrelationships Between Subjective Measures

Marbling and color scores were observed to be positively correlated with firmness score at .325* and .509**. A nonsignificant correlation of .035 was found between marbling score and color score. Results are

reported in Table 12.

Table 12. Interrelationships between subjective measurements.

Measurements	Color Score[a]	Firmness Score[a]
Marbling Score[a]	.035	.325*
Color Score[a]		.509**

[a]See Table 2

*$P < .05$

**$P < .01$

Relationships Between Wet Sample Values and Dry and/or Fat Free Values

Removing the effects of the variables fat and total moisture is very useful when working with correlations, especially if there is wide variation between samples. After removing the effect of these variables the muscle components can be evaluated on a more equal basis. The correlation coefficients of muscle component values calculated on a wet sample weight basis versus moisture free, fat free, and/or moisture plus fat free sample basis are summarized in Table 13.

Interrelationships Among *Longissimus dorsi* Components

Percent total moisture in muscle was negatively correlated (-.842**) to percent ether extract on a wet tissue basis (Table 14). Judge *et al.* (1958) also found a highly significant negative correlation between

Table 13. Simple correlations between wet sample objective values versus fat free (FF), moisture free (MF), or moisture and fat free (MFF) values and moisture free (MF) versus moisture and fat free (MFF) values.

Variables Studied vs.	Calculated Values	Correlations
% Total Moisture	FF	.666**
% Ether Extract	MF	.986**
Myoglobin mg/g	MF	.946**
Myoglobin mg/g	MFF	.962**
Myoglobin mg/g - MF	MFF	.997**
Sodium g/kg	MF	.829**
Sodium g/kg	MFF	.889**
Sodium g/kg - MF	MFF	.990**
Potassium g/kg	MF	.789**
Potassium g/kg	MFF	.834**
Potassium g/kg - MF	MFF	.992**

**$P < .01$

muscle moisture and fat content. Removing the effect of moisture from the ether extract value a decrease in strength of relationship was noted (-.748**). Correlations of .593**, .542**, .478**, .367*, .784**, and .724** were found between percent total moisture and myoglobin concentration moisture free and moisture plus fat free, and sodium and potassium content on the same basis. On a fat free basis, percent moisture was highly related to the same variables except with percent ether extract calculated on a dried sample weight.

Strong relationships were observed between percent ether extract and myoglobin or potassium concentration moisture free and moisture and fat free. Correlations were -.425**, -.355*, -.680**, and -.580**,

Table 14. Interrelationships among longissimus dorsi components.

Characteristic Studied	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1) % Total Moisture												
2) % Ether Extract	-.842**											
3) Myoglobin mg/g	.307	-.176										
4) Sodium g/kg	-.090	.271	.174									
5) Potassium g/kg	.242	-.245	-.048	.044								
6) % Total Moisture (FF)[a]	.666**	-.158	.317*	.213	.106							
7) % Ether Extract (MF)[b]	-.748**	.985**	-.133	.305	-.219	-.005						
8) Myoglobin mg/g (MF)[b]	.593**	-.425**	.946**	.113	.015	.497**	-.357*					
9) Myoglobin mg/g (MFF)[c]	.542**	-.355*	.962**	.140	.001	.501**	-.285	.997**				
10) Sodium g/kg (MF)[b]	.478**	-.231	.334*	.829**	.166	.559**	-.148	.439**	.436**			
11) Sodium g/kg (MFF)[c]	.367*	-.091	.316*	.889**	.135	.549**	-.009	.387*	.393*	.990**		
12) Potassium g/kg (MF)[b]	.784**	-.680**	.158	-.028	.789**	.496**	-.604**	.381*	.340*	.410**	.321*	
13) Potassium g/kg (MFF)[c]	.724**	-.580**	.147	.016	.834	.523**	-.498**	.351*	.318*	.414**	.340*	.991**

[a]Fat free

[b]Moisture free

[c]Moisture and fat free

*$P < .05$

**$P < .01$

respectively. On a dry sample basis the correlation strengths appeared to be decreased ($r = -.357^{*}$, $-.285$, $-.604^{**}$ and $-.498^{**}$), although this was not tested statistically.

As muscle myoglobin concentration increased, percent total moisture on a fat free basis and sodium content on a moisture free and moisture plus fat free basis increased significantly. Correlations of $.317^{*}$, $.334^{*}$, and $.316^{*}$ were observed. When the effects of moisture and moisture plus fat were removed myoglobin concentration was significantly related to both sodium content ($.439^{**}$, $.387^{*}$, $.436^{**}$, and $.394^{*}$) and potassium content ($.381^{*}$, $.351^{*}$, $.340^{*}$, and $.318^{*}$) on a moisture free and moisture and fat free basis, respectively.

Correlations of $.410^{**}$, $.414^{**}$, $.321^{*}$, and $.340^{*}$ were observed between muscle sodium content on a moisture free and moisture plus fat free basis and muscle potassium content on a moisture and moisture plus fat free basis.

Interpretation of the data showed that as percent total moisture in the muscle sample increased, percent ether extract decreased, percent total moisture on a fat free basis increased, percent ether extract (moisture free) increased, and muscle myoglobin, sodium, and potassium on a moisture free and moisture plus fat free basis all increased. In addition, most of the correlations observed were highly significant indicating that strong interrelationships existed among the longissimus dorsi components presented in this experiment.

SUMMARY

In this experiment, 40 female porcine carcasses were selected on the slaughter line in a commercial packing plant. Selection was based on stage in the estrous cycle as determined by the physical appearance of the ovaries. The stages selected were pre-puberal, early luteal, late luteal, follicular and pregnancy. The right loins from these carcasses were obtained and processed at the university meats laboratory.

All possible correlation coefficients were calculated between the 23 variables presented in this study plus percent total moisture and ether extract and muscle myoglobin, sodium, and potassium content on a fat free, moisture free, and/or moisture plus fat free basis.

No significant differences were found between the 5 treatments studied. Apparently stage of the estrous cycle and pregnancy had little or no effect on porcine longissimus dorsi components and quality.

Relationships of longissimus dorsi components and subjective measures to muscle water holding capacity were studied. Of the three subjective measures used in this study, firmness score had the strongest relationship to muscle water holding capacity. A firm, dark, and highly marbled muscle sample was associated with a high water binding ability.

Few significant relationships were observed between percent total moisture, percent ether extract, muscle myoglobin, sodium, and potassium content and water holding capacity as measured by the centrifuge and press methods.

Highly significant positive correlations were found between fresh and frozen samples in their ability to bind water as determined by the centrifuge method. The interrelationships between the press method determinations were also strongly related, i.e., as pressed meat area and meat area per gram increased, juice area and juice area per gram decreased. Significant correlations were found between almost all press measurements and centrifuge measurements on both fresh and frozen samples. The data indicated that expression of press results as meat area or meat area per gram were more highly correlated with centrifuge method results than press juice area measurements.

Total cooking loss and volatile cooking loss were shown to be strongly related to frozen muscle water holding capacity as determined by the centrifuge method. No significant correlations were found between fresh sample water holding capacity determinations and cooking loss measurements. Also muscle water holding capacity was not related to cooked shear values from either the medial or lateral portion of the pork rib chops. In addition, most of the correlations between subjective measures or longissimus dorsi components and cooking loss or shear values were low and nonsignificant.

A nonsignificant relationship was observed between volatile loss or total cooking loss and drip cooking loss. However, total cooking loss and volatile loss were strongly related.

Marbling score was significantly related to most of the objective determinations used in this study, i.e., percent total moisture (negative), percent ether extract (positive), muscle potassium concentration

(negative), and various measures of muscle water holding capacity. Color score was correlated with muscle myoglobin content and water holding capacity. Firmness score was only related to muscle water binding ability. Interrelationships between the three subjective measures used showed marbling and color score to have a significant positive relationship with firmness score. However, marbling and color score were not significantly related.

Most of the relationships between the longissimus dorsi components (percent total moisture, percent ether extract, muscle myoglobin, sodium, and potassium content) were significant.

More significant correlations might have been obtained if greater variation existed in characteristics studied. The 40 porcine loins studied were fairly similar in physical and chemical composition.

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APPENDICES

APPENDIX A

Color, firmness, and marbling standards

Color	Firmness
1) Extremely pale	Soft and watery
2) Pale	Moderately soft and moderately pale
3) Uniformly grayish-pink	Moderately firm and moderately dry
4) Moderately dark	Firm and dry
5) Dark	Very firm and very dry

Marbling

2) Devoid
5) Practically devoid
7) Traces -
8) Traces
9) Traces +
10) Slight -
11) Slight
12) Slight +
13) Small -
14) Small
15) Small +
16) Modest -
17) Modest
18) Modest +
19) Moderate -
20) Moderate
21) Moderate +
22) Slightly abundant -
23) Slightly abundant
24) Slightly abundant +
25) Moderately abundant -
26) Moderately abundant
27) Moderately abundant +
28) Abundant -
29) Abundant
30) Abundant +
31) Very abundant -
32) Very abundant
33) Very abundant +
34) Extremely abundant -
35) Extremely abundant
36) Extremely abundant +

APPENDIX B

Water Holding Capacity
Centrifuge Method

Procedure

1. Weigh out 25 g. of muscle sample into upper portion of Wierbicki Centrifuge tube with sample resting on a loose fitting fritted glass disk.

2. Close tube with a #6 rubber stopper fitted with a glass capillary tube.

3. Immerse tubes into a 70°C. water bath to a depth of 9/10 of the tube length for exactly 30 minutes.

4. After heating, place tubes into a 20 to 25°C water bath for 10 minutes to cool (solidification of fat should be avoided).

5. Centrifuge for 10 minutes at 1000 r.p.m.

6. Read the volume of juice in the graduated portion of the tube using a magnifying glass.

7. Determine % water in juice by drying the juice in a vacuum oven for 12 hours at 98°C and 25 pounds pressure (% water in juice = F).

Cleaning

Centrifuge Tubes

1. Wash with hot soapy water.

2. Rinse with distilled water and acetone.

3. Wipe dry with a lint free towel.

Fritted Glass Disks

1. Wash with hot soapy water.

2. Rinse with distilled water

3. Soak the disks in dilute sulfuric acid to dissolve substances of meat adsorbed to the filter.

4. Rewash and rinse to remove the acid.

5. Store in a desiccator over saturated KCl for at least 24 hours before reusing.

Calculation

$$\% \text{ moisture expressed} = \frac{\text{Ml. of juice expressed/100 g. x F}}{\% \text{ total moisture in sample}} \times 100$$

Precision

When heating is carried out at 70°C., duplicates usually agree to .1 ml. of juice per 25 g. of sample.

APPENDIX C

Pigment Extraction

References

deDuve, Christian. 1948. A spectrophotometric method for the simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. Acta Chemica Scandinavica. 2:264. (See page 277).

Hawk, Philip B., Bernard L. Oser, and William H. Summerson. 1954. Practical Physiological Chemistry (13th ed.). McGraww-Hill Book Co., New York. p. 871.

Procedure

1. Weigh out to nearest 0.1 gm. approximately 50 grams of ground or homogenated meat in tared, 250 ml. homogenizing beaker.

2. Blend the meat with 120 ml. of cold, .01N acetate buffer solution for 3 minutes in a Vir Tis homogenizer at high speed.

3. Pour slurry into a 250 ml. polyethylene bottle. Rinse homogenizer beaker with buffer and pour into the bottle. Centrifuge at 3000 r.p.m. for 15 minutes.

4. Filter the supernatant through a wad of cotton into a 250 ml. Erlenmeyer flask.

5. Re-extract the residue with 80 ml. of the buffer solution (homogenize for 1 1/2 minutes). Pour slurry into same bottle, rinse homogenizer beaker and then centrifuge for 15 minutes at 3000 r.p.m.

6. Filter supernatant through the cotton and into the 250 ml. flask. Rinse the bottle and pour liquid over the cotton. Rinse the funnel and cotton.

7. Filter the extract through Whatman No. 3 filter paper into a 250 ml. volumetric flask. Rinse the Erlenmeyer flask. Rinse the funnel and filter paper. Fill the flask to volume.

Notes

The buffer solution is prepared as follows:

1. Prepare .1N acetic acid solution.
   11.45 ml. acetic acid filled to 2 liters.

2. Prepare .1N sodium acetate solution.
   16.408 gms. sodium acetate filled to 2 liters.

3. When the .01 buffer solution is needed, mix the above stock solution with distilled water in the following ratio:

10 ml.	.1N acetic acid
10 ml.	.N sodium acetate
180 ml.	distilled water

The pH of the buffer is approximately 4.5.

APPENDIX D

Carbon Monoxide Conversion

References

deDuve, Christian. 1948. A spectrophotometric method for the simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. Acta Chemica Scandinavica. 2:264. (See page 272).

Poel, W. E. 1949. Effect of anoxic anoxia on myoglobin concentration in striated muscle. Am. J. Physiol. 156:44.

Procedure

1. Pipette 50 ml. of the extract into an Erlenmeyer flask containing 2.01 grams of $Na_2HPO_4.7H_2O$ (anhydrous 1.055).

2. Allow the solution to stand until all of the phosphate has dissolved.

3. Pour into a 40 ml. centrifuge tube and centrifuge for 15 minutes at 3000 r.p.m.

4. Filter through Whatman No. 3 filter paper.

5. Rinse a Thunberg tube with the solution and pour in approximately 10 ml. of the solution.

6. Evacuate the tube.

7. Bubble carbon monoxide through the solution for 10 minutes (about 2 bubbles per second). Then add a pinch (100 mg.) of $Na_2S_2O_4$. Bubble the carbon monoxide for 10 additional minutes.

8. Rinse a cuvette with a portion of the solution and quickly pour the solution into the cell. Fill the cell to the top and cover.

9. Immediately read the solution of the spectrophotometer at wave length 538 and 568 mu against a blank of N/15 Na_2HOP_4.

Calculations

Mg. Mb/gm. tissue =

$$\text{MbCo} \quad \frac{17{,}000 \times (0.25 + d) \times 1000}{\text{gm. of sample}}$$

17,000 = equivalent of the pigments.

.25 = volume of the extract in liters.

1000 = conversion from gm. to mg. of pigment

d = volume increase of extract due to the addition of N/15 Na_2HPO_4 (0.005 l.).

MbCo = molar concentrations of carbonyl Mb calculated by use of Poel's (1949) equations:

$$\text{MbCo} = \frac{D_{568} \times E^{Hb}_{538} - D_{538} \times E^{Hb}_{568}}{E^{Mb}_{568} \times E^{Hb}_{538} - E^{Mb}_{538} \times E^{Hb}_{568}}$$

E = molar extinction coefficient

$E^{Hb}_{538} = 14.8 \times 10^3$
$E^{Hb}_{568} = 14.5 \times 10^3$
$E^{Mb}_{568} = 11.8 \times 10^3$
$E^{Mb}_{538} = 14.8 \times 10^3$

$$\text{Mgs. Mb/gm. Meat} = \frac{\text{MbCo} \times 17 \times (250 \text{ mls. volume} + 5 \text{ mls. dilution})}{\text{wt. of sample}}$$

$$= \frac{\text{MbCo} \times 4335}{\text{wt. of sample}}$$

Notes

1. The 2.01 (anhydrous 1.055) gms. of $Na_2HPO_4.7H_2O$ in 50 ml. of solution makes a N/15 solution. This offsets the acidifying effect of $Na_2S_2O_4$.

2. Carbon monoxide is a poisonous gas which cannot be detected by odor. Therefore, it must always be used under a hood. Make sure the exhaust fan is working. Extend the exhaust line from the side arm of the Thunberg tube upward under the hood while working. Always work with the window of the hood about half way down.

3. A slight headache is the first symptom of carbon monoxide poisoning. If this is noticed, turn off the gas and check the system for leaks. Also check the exhaust of the hood. If due caution is taken, there is no danger from the gas: but, carelessness cannot be tolerated.

4. Antidote for carbon monoxide poisoning: Remove to fresh air immediately and call for pulmotor; apply artifical respiration for at least one hour or until the pulmotor arrives. Administration of oxygen containing 5% of carbon dioxide is beneficial; inhalation of ammonia or amyl nitrite is often of value.

5. After operation, turn the valve on the carbon monoxide tank off. Then, with the exhaust fan still running, turn the valve on the pressure gauge until all of the gas is expelled from it. Then close the pressure gauge valve by reversing the turn.

EFFECT OF THE ESTROUS CYCLE AND PREGNANCY ON THE WATER HOLDING CAPACITY AND RELATED PROPERTIES OF PORCINE MUSCLE

by

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AN ABSTRACT OF A MASTER'S THESIS

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The effect of various stages of the estrous cycle (natural estrogen - progestogen variation in female mammals) and pregnancy on the water holding capacity (WHC) and related properties of porcine skeletal muscle was studied.

A group of 40 female porcine carcasses selected on the slaughter line in a commercial packing plant based on stage in the estrous cycle as determined by the physical appearance of the ovaries were used in this study. The longissimus dorsi from the 6th through the 9th thoracic vertebral location was used for WHC, total moisture and ether extract determinations. Cooking loss and shear force determinations were made on samples from region of the 10th through the 13th thoracic vertebrae. Muscle myoglobin, sodium, and potassium content determinations were made on samples from the 14th thoracic vertebrae through the 2nd lumbar vertebrae.

No significant differences were found between the 5 stages studied (pre-puberal, early luteal, late luteal, follicular, and pregnant). Because of this all possible correlations were run between the 31 variables studied using 40 observations.

Little relationship seemed to exist between any longissimus dorsi component studied and WHC determined by centrifuge or press method.

Centrifuge method results showed that fresh and frozen samples were significantly correlated in their ability to bind water. As the ml. of juice expressed from a fresh sample increased, percent water expressed from a fresh ($r = .979^{**}$) and frozen sample ($r = .685^{**}$) increased, and ml. of juice lost from the frozen sample increased ($r = .670^{**}$).

Interrelationships between the press method measurements were also strongly related. Meat area was strongly correlated with meat area per gram ($r = .989^{**}$), juice area ($r = -.565^{**}$), and juice area per gram ($r = -.595^{**}$); juice area with meat area per gram ($r = -.597^{**}$) and juice area per gram ($r = .967^{**}$); and meat area per gram with juice area per gram ($r = -.618^{**}$). Significant correlations were also found between press and centrifuge measurements on both fresh and frozen muscle samples. Press results expressed as meat area measurements were more highly correlated with centrifuge method results than press juice area measurements.

Total cooking loss and volatile cooking loss were strongly related to frozen muscle WHC (centrifuge method), but not to fresh muscle WHC by either centrifuge or press method. Muscle WHC was not found to be related to shear values in this experiment. Also most of the correlations between subjective measures or longissimus dorsi components and cooking loss or shear values were low and nonsignificant. A significant correlation of $.955^{**}$ was found between total cooking loss and volatile cooking loss. However, volatile cooking loss or total cooking loss were not related to drip cooking loss ($r = -.110$ and .188, respectively).

Marbling score was significantly related to most of the objective measurements presented, i.e., percent total moisture ($r = -.448^{**}$), percent ether extract ($r = .684^{**}$), muscle potassium concentration ($r = -.397^{*}$), and various measures of WHC (more marbling - higher WHC); color score with muscle myoglobin content ($r = .364^{*}$) and WHC; and firmness score with only muscle water binding ability. Marbling and color

score were related to firmness score ($r = .325^{*}$ and $.509^{**}$, respectively), but marbling and color score were not related ($r = .035$).

Most of the relationships between the longissimus dorsi components (percent total moisture, percent ether extract, muscle myoglobin, sodium, and potassium content) were significant.

More significant correlations might have been obtained if greater variation between treatments had been present.