# INVESTIGATION OF IN-VITRO DIGESTIVE BEHAVIOR OF BEEF AND POULTRY WHICH ARE TENDERIZED BY ENZYMATIC AND ACIDIC MARINATION

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# INVESTIGATION OF IN-VITRO DIGESTIVE BEHAVIOR OF BEEF AND POULTRY WHICH ARE TENDERIZED BY ENZYMATIC AND ACIDIC MARINATION

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#### **ABSTRACT**

# INVESTIGATION OF *in-vitro* DIGESTIVE BEHAVIOR OF BEEF AND POULTRY WHICH ARE TENDERIZED BY ENZYMATIC AND ACIDIC MARINATION

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Meat contains valuable nutrients, exceptionally high-quality proteins that include all essential amino acids and vitamins and minerals for the human diet. Meat is mostly tenderized to make it more tasteful and palatable. The effects of tenderization on the physical, chemical, and microbiological changes in meat have been investigated in many studies. In this study, acetic acid, citric acid, lactic acid solutions, and a commercial meat tenderization solution were used to tenderize the red and white meat (beef and chicken breast, respectively). The objective of the study was to investigate the effect of different tenderization solutions on the physicochemical properties and the digestibility of meat samples. The meat samples that were kept in acidic and enzyme solutions were analyzed in terms of textural properties, color, water holding capacity (WHC), marinate uptake, cooking loss, TD-NMR relaxation times (T<sub>2</sub>), morphological properties (Scanning Electron Microscope-SEM) both before and after *in vitro* digestion experiments. To understand the extent of digestion, total soluble protein content, and free amino group experiments were also performed. The textural properties gave information about how effective the different solutions

used for tenderization were, and the value obtained was reported as Warner-Bratzler shear (WBS) force. The acidic tenderization showed that the WBS force values decreased for chicken and beef meat in both uncooked and cooked samples compared to the control samples. Besides, acidic tenderization increased the WHC of the meat samples and reduced the cooking loss due to the altering of meat protein by low pH of the solutions. Color change was also observed in the muscle due to tenderization. TD-NMR relaxometry was used to determine the effect of the tenderization on the *in vitro* digestion process. The NMR results showed that the tenderization type changed the state of water in the meat sample and free water amount increased by tenderization. In addition, the total soluble protein content and the free amino group provided an idea about the extent of protein digestion. The digestibility of both beef and chicken meat improved with the help of tenderization. Since meat digestibility is linked to amino acid bioavailability, the results indicated that the best protein digestibility was obtained using the commercial meat tenderization solution.

The study provided valuable results to understand the effect of different tenderization methods both on the physico-chemical properties and on digestion.

**Keywords**: Meat, acidic tenderization, enzymatic tenderization, *in vitro* digestion, TD-NMR

#### ENZİMATİK VE ASİDİK MARİNASYON İLE GEVREKLEŞTİRİLEN KIRMIZI VE BEYAZ ETLERİN *in vitro* SİNDİRİM DAVRANIŞLARININ İNCELENMESİ

Baştürk, Bilge Yüksek Lisans, Gıda Mühendisliği Tez Yöneticisi: Doç. Dr. Mecit Halil Öztop Ortak Tez Yöneticisi: Doç. Dr. Emin Burçin Özvural

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Et, insan beslenmesi için gerekli tüm elzem amino asitleri barındıran yüksek kaliteli proteinleri, vitaminleri ve mineralleri içeren önemli bir gıdadır. Etin daha lezzetli ve kabul edilebilir duyusal özelliklere sahip olması için farklı gevrekleştirme uygulamaları üzerine birçok çalışma yapılmış ve bu süreçlerin fiziksel, kimyasal ve mikrobiyolojik değişimler üzerindeki etkileri birçok çalışmada araştırılmıştır. Bu tez çalışmasında kırmızı ve beyaz eti (sırasıyla dana eti ve tavuk göğsü) gevrekleştirmek için asetik asit, sitrik asit, laktik asit çözeltileri ve ticari bir enzim çözeltisi kullanılmıştır. Tez çalışmasının amacı, farklı gevrekleştirme çözeltilerin etlerin fizikokimyasal özellikleri ve sindirilebilirliği üzerindeki etkilerini araştırmaktır. Çözeltilerde tutulan et örnekleri tekstürel özellikler, renk, su tutma kapasitesi (WHC), TD-NMR relaksasyon süreleri (T2), morfolojik özellikler (Taramalı Elektron Mikroskobu-SEM), toplam çözünür protein içeriği ve serbest amino grupları miktarı yönlerinden incelenmiştir. Tekstürel özellikler, gevrekleştirme için kullanılan farklı çözeltilerin ne kadar etkili olduğu hakkında bilgi vermiş ve elde edilen değerler Warner-Bratzler kesme (WBS) kuvveti olarak belirtilmiştir. Asidik

gevreklestirme, kontrol numunelerine kıyasla hem pişmemiş hem de pişmiş örneklerde tavuk ve sığır eti için WBS kuvvetinin azaldığını göstermiştir. Ayrıca, asidik çözeltilerin düşük pH'sı ile et proteinlerin değişikliğe uğramasından kaynaklı olarak örneklerin su tutma kapasitesi artmış ve pişirme kayıpları azalmıştır. Gevrekleştirme işlemi et dokusunda renk değişikliğine de neden olmuştur. TD-NMR relaksasyon ölçümleri, gevrekleşmenin in vitro sindirim süreci üzerindeki etkisini belirlemek için kullanılmıştır. Sonuçlar, gevrekleştirme tipinin et örneğindeki suyun durumunu değiştirdiği ve gevrekleştirme ile serbest suyun arttığını göstermiştir. Ek olarak, toplam çözünür protein içeriği ve serbest amino grubu, protein sindiriminin gerçekleşme düzeyi hakkında bilgi sağlamıştır. Hem sığır eti hem de tavuk etlerinin sindirilebilirliği, gevrekleştirme ile iyileştirilmiştir. Etin sindirilebilirliği, amino asit biyoyararlanımı ile bağlantılı olduğundan, sonuçlar, ticari enzim çözeltisi kullanılarak en iyi protein sindirilebilirliğinin elde edildiğini göstermiştir. Tezden elde edilen sonuçlar; farklı gevrekleştirme tiplerinin gerek etlerin fizikokimyasal özelliklerine gerekse sindirim davranışına etkisinin anlaşılmasında oldukça faydalı bilgiler sağlamıştır.

**Anahtar Kelimeler**: Et, asidik gevrekleştirme, enzimatik gevrekleştirme, *in vitro* sindirim

Dedicated to all who support and believe me, especially to my family

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#### LIST OF ABBREVIATIONS

AA Acetic acid

ANOVA Analysis of variance

ATP Adenosine triphosphate

BSA Bovine serum albumin

C Control

CA Citric acid

CHO Carbohydrate

CI Confidence interval

CPMG Carl-Purcell-Meiboom-Gill

DFD Dark-firm-dry

DW Distilled water

eV Electron volt

HDP Hydrodynamic pressure

HHP High hydrostatic pressure

LA Lactic acid

M Magnitude

M<sub>0</sub> Maximum magnitude

MTS Meat tenderization solution

N Force

NIR Near-infrared

NMR Nuclear magnetic resonance

OPA o-phthalaldehyde

PAGE Polyacrylamide gel electrophoresis

PSE Pale-soft-exudate

RF Radiofrequency

SDS Sodium dodecyl sulfate

SEM Scanning electron microscopy

t Time

T<sub>1</sub> Spin-lattice relaxation time

T<sub>2</sub> Spin-spin relaxation time

TD Time-domain

UV-VIS Ultraviolet-visible

WBS Warner-Bratzler shear

WHC Water holding capacity

#### **CHAPTER 1**

#### INTRODUCTION

#### 1.1 Meat

Meat is a good source in the human diet in terms of protein and other nutrients. The consumption of meat by humans was dated to prehistoric times (Gehring, 2017). Since prehistoric times, meat has had an inevitable role in the human diet. When consumed with vegetables and carbohydrates sources, it supplies a balanced diet (Wood, 2017).

What makes 'meat' is initially the 'muscle' tissue. The composition of the muscle determines the characteristics of the meat. The nutritional value of meat depends on its composition in terms of protein, carbohydrates, fat, etc. The biochemical composition of the different meat cuts is given in Table1.1. The physical and biochemical changes result in the conversion of the muscle to meat. These processes begin right after the slaughtering. Besides, these postmortem changes determine the quality characteristic of meat, such as tenderness, water holding capacity, color, etc.

Table 1.1 Biochemical composition values of different meat cuts

Type of meat	Water (%)	Protein (%)	CHO (%)	Fat (%)	Ash (%)	Reference
Beef	71.38	16.01	3.89	7.93	0.79	(Homaida, 2019)
Lamb	77.45	19.03	0.75	2.00	0.77	(Hernández et al., 2013)
Pork	72.42	21.53	1.98	2.97	1.10	(Chu et al., 2012)
Chicken	75.03	17.35	1.64	5.12	0.86	(Homaida, 2019)
Fish	76.42	19.64	1.75	0.79	1.40	(Nogueira, 2013)

#### 1.1.1 Conversion of Muscle to Meat

The state of the muscle before slaughtering (perimortem period) determines the changes occurring in postmortem muscle. The main reason for this is that the 'energy' status of the muscle before slaughtering governs the production of lactic acid scale, which determines the beginning of *rigor mortis*. Rigor mortis is a process that is the noticeable sign of death in consequence of chemical changes in the muscle, resulting in the muscle tissue losing its ability to extend and stiffen (Warner, 2016). It is caused by the depletion of the energy (particularly ATP) in the postmortem period of muscle, which develops permanent actomyosin cross-bridges and reduces extensibility (Lonergan et al., 2019).

The most critical factor in the conversion of muscle to meat is the glycogen status of the tissue storage. In anaerobic respiration, glycogen is transformed to lactic acid, which significantly affects the charges of the proteins due to changes in pH. This pH change can result in protein unfolding and will have a direct effect on the water holding capacity and final properties of the meat. pH change observed on the muscle tissue could be either desirable or in an undesirable way (Henckel et al., 2002). If the proper pH decrease is achieved, the resulting meat is in the most desirable state.

The process of conversion of muscle to meat consists of many different steps. However, they can be classified into three main phases: *pre-rigor mortis*, *rigor mortis*, and *tenderization*.

After slaughtering, the pre-rigor mortis phase takes place. In this phase, the vital function of the animal terminates, and the availability of oxygen to the tissues decreases. Even though the availability of oxygen is minor, still the respiration processes are driven aerobically. Besides, the actomyosin and myosin start to dissociate. The average duration of the pre-rigor mortis period is about 3 to 6 hours and can be effected significantly by the type of the animal tissue (Madhusankha & Thilakarathna, 2020).

When the oxygen in the tissue becomes not enough for aerobic respiration, the cells are forced to respirate anaerobically (Matarneh et al., 2017). This marks the transition from *pre-rigor* to *rigor mortis* phase. In the Table 1.2, the onset of rigor mortis process time is given for different types of animals. With anaerobic respiration, the ATP production rate decreases, and lactic acid is produced and pH decreases. Due to this reduced pH, the actomyosin and myosin relaxation process slows down, and they lock down, and the meat reaches to the highest toughness. This effect can be observed by the shortened and contracted muscle fibers.

Table 1.2 Different times to activate *rigor mortis* to reach pH 5.5.-5.7 (Honikel, 2014)

Species	Time (h)
Beef	6 to 12
Lamb	6 to 12
Pork	0.25 to 3
Turkey	<1
Chicken	<1
Fish	<1

After the rigor mortis is completed, there comes the tenderization phase. This is the phase where rigor state is resolved. As time passes, the proteolytic enzymes are activated in the tissue. This activation provides the proteolysis of myofibrillar proteins in the tissue to smaller proteins. The proteolytic enzymes that are activated in the tenderization phase are *calpains*, *cathepsins*, *caspases*, *and proteosomes*. The proteolysis of the myofibrillar proteins results in more soluble proteins, which provides more availability to the protein during digestion.

#### 1.1.2 Quality Attributes of Meat

Meat is consumed in a variety of forms and originates from a variety of animal species. There is a distinction between *fresh and processed meat*. Processed meat has been given additional treatments such as sausages, smoked or salted meat, while

fresh meat is not treated. In the same way, red and white meats have a distinction between each other: red meat is composed of pork, beef, veal, and mutton/lamb, whereas white meat consists of poultry (chicken, turkey, goose, duck, etc.) (Linseisen et al., 2002; Wood, 2017).

Depending on the processes that occurred both on perimortem and postmortem period of the muscle tissue, the characteristic of the meat varies. The main reason for that change is mainly because of the pH of the muscle tissue. Depending on the final pH of the muscle tissue the meat is classified as: normal, pale-soft-exudate (PSE), and dark-firm-dry (DFD) (Lonergan et al., 2019).

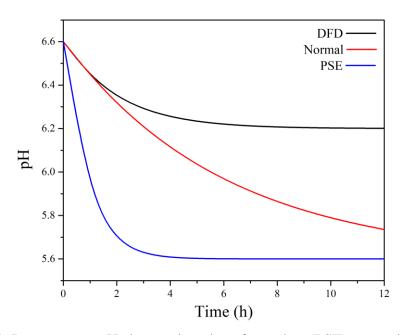


Figure 1.1. Post-mortem pH changes in pale-soft-exudate (PSE), normal and dark-firm-dry (DFD) meats (Lonergan et al., 2019)

As seen in Fig. 1.1, the pH of the muscle determines the status of the meat. In regular meat, it is expected to have a slow pH decrease down to about 5.7 values. The slower decrease provides flavor and texture development. On the other hand, the sudden drop or 'not enough decrease of pH' can lead undesirable final meat. These undesirable ones are PSE and DFD meats.

PSE meat carries a quality problem characterized by the undesirable pale color mostly seen on pork meats. In addition to the typical pork case, it is also possible to observe this problem on beef and poultry (Lonergan et al., 2019). The sudden drop in pH is because of the excessive metabolic activities occurring in the muscle tissue following slaughtering. The abnormal glycolysis in the muscle tissue having PSE problem results in the accumulation of lactic acid. The depressed pH denatures the myofibrillar proteins (Honikel, 2014) and affects the distance between the fibers which in turn effect the light scattering. This is the reason why pale color is formed in PSE meat. The ability to hold water inside the cells decreases due to the shrinking of sarcomeres during this denaturation process. The soft term in the PSE may seem a positive attribute for meat. Still, the softness in raw meat does not show the same behavior during cooking due to the less water holding capacity and high drip loss with denatured sarcomeres resulting from lower pH.

In contrast to PSE meat, the slower or even 'not decrease' of pH in the muscle causes DFD meat. The pH does not drop enough due to the lack of substrate for the glycolysis (Lawrie, 2006). In other words, if the animal undergoes physical stress before slaughtering, the glycogen stored in the muscle tissue is depleted while the animal is alive. As a result, there would be no or less glycogen in the muscle tissue to continue with the anaerobic respiration process, of which result is the formation of lactic acid. From the aspect of physical attributes of the meat, the color of the meat becomes dark, which makes the consumer think that the meat is not fresh. There are some reasons for dark color; (1) too few myoglobin is denatured at this pH; (2) at high pH, myofibrils have ability to bind more water, resulting in meat absorbing more light (Lonergan et al., 2019). Besides this, high pH causes high water holding capacity, which results in quite transparent meat having sticky behavior. The undesirability of PSE and DFD meat restrict their direct consumption. These meats are blended with other meats and used in the processed meat products production.

#### 1.1.3 Meat Parts of the Animal Bodies

There are different parts of the animal body whose characteristic of meat varies due to the mobility of the muscle tissue. Also, the mobility of the muscle affects the final tenderization status of the meat. The age of the animal is directly related with the mobility. This means that when the animal gets older, the muscle fibers get thicker due to more exercise, resulting in tougher meat cuts (McGee, 1984). The contribution of connective tissue to the toughness of the meat is inevitable. It is known that tendons or other connective tissues are responsible for the hardness while chewing the meat. The amount of those tendons or other connective tissue depends on the position of the meat cut obtained. In other words, the part of the body differs in terms of the meat quality attributes. The presence of fat in the muscle tissue result in a weakened muscle tissue, so more tender pieces of meat (McGee, 1984).

When the parts in the body are considered, the neck, shoulder, chest, and front limbs parts of the body give tougher meat when compared to the back part of the animal because the more mobile parts of the animal are not that much relaxed so include more number of muscles and connective tissue parts (McGee, 1984). Nomenclature of different parts of the beef and chicken meats can be seen in Fig. 1.2 and Fig. 1.3, respectively.

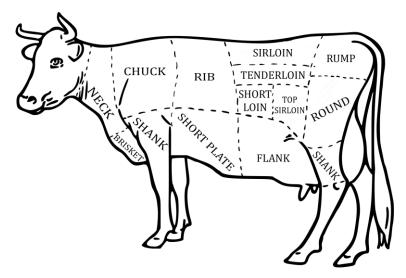


Figure 1.2. Meat parts of the cattle

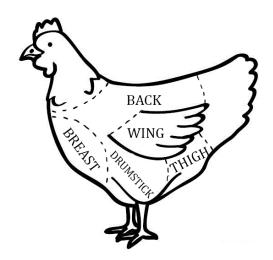


Figure 1.3. Meat parts of the chicken

As stated in Table 1.2, natural tenderization may take longer time to serve the meat right after slaughtering. It is also likely that the consumer may prefer more tenderized meat when compared to the naturally occurring reactions. At this point, external processes are required for the more tender meat. There are different methods to tenderize meat.

#### 1.2 Meat Proteins

It is known that the nutritious source of meat is due to its proteins, so the digestibility of the protein affects the bioavailability of the amino acids. In industry, there are several methods to functionalize the proteins in the meat to increase bioavailability. Zhang et al. (2018) studied the effects of high-pressure processing on the myofibrillar fragmentation. Chian et al. (2019) investigated the use of pulsed electrical fields on the protein digestibility of meat and Peña-Gonzalez et al. (2019) studied the potential use of ultrasound technology as a tenderization tool for meat. These all studies were conducted on the focus of altering the protein structure of the meat. Meat proteins could be classified into three main categories: myofibrillar proteins, sarcoplasmic proteins, and stromal proteins (Seonmin Lee et al., 2021).

# 1.2.1 Myofibrillar Proteins

Total muscle proteins comprise 55-60 % of myofibrillar proteins, and actin and myosin are their major proteins (Sun & Holley, 2011). While myosin is a fibrous protein is a fibrous protein with extended rod form in thick filaments, actin is a globular-shaped molecule (Kang & Singh, 2014). Since the structure of the myofibrillar proteins is not simple, they may interfere with the other proteins during digestion and affect the overall digestibility of the meat proteins (Sante-Lhoutellier et al., 2007).

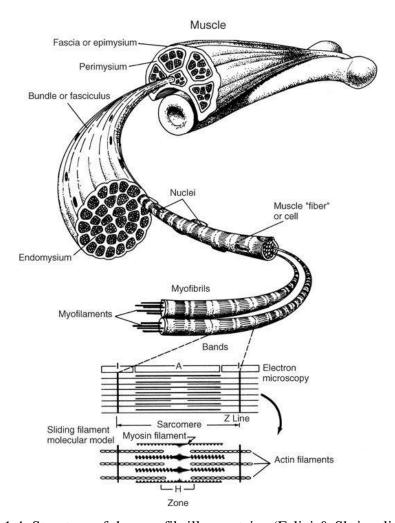


Figure 1.4. Structure of the myofibrillar proteins (Felici & Sbriccoli, 2006)

#### 1.2.2 Sarcoplasmic Proteins

Sarcoplasmic proteins in the meat found in the sarcoplasm or in the fluid surrounding myofibrils are mainly composed of myoglobin, and these proteins have a role in muscle metabolism since these protein includes glycolytic enzymes to control aerobic or anaerobic glycolysis (Bax et al., 2013; Kang & Singh, 2014). It is known that the rigidity of the sarcoplasmic proteins is comparably more diminutive than the myofibrillar ones. The digestion of these proteins begins without any resistance to the gastric fluid (Sayd et al., 2016). Thermal treatment has been shown to increase the susceptibility of myoglobin to digestion.



Figure 1.5. Structure Myoglobin (Wilson & Reeder, 2006)

#### 1.2.3 Stromal Proteins

Collagen is the major stromal proteins, and it contributes to about 25% of total proteins in the meat (Zhang et al., 2020). Connective tissue proteins consist of collagen, elastic and reticulum. Collagen also has unique property of intramolecular cross-links with age, causing increased toughness in meat from older animals (Kang

& Singh, 2014). The purpose of tenderization is mainly because of the collagen tissue in the meat that is undesirable by the consumers due to its tough nature. The digestive resistance of the meat mostly comes from presence of collagen. The hydroxy proline presenting the collagen is the most stable against the digestive activity of the proteolytic enzymes (Zhang et al., 2020). Another reason for digestive resistance is the triple helix nature of the meat collagen (see Fig. 1.6).



Figure 1.6. Triple helix structure of collagen (Shoulders & Raines, 2009)

#### 1.3 Tenderization Methods

Meat toughness mostly relies on, nature, structure and the quantity of connective tissue of as elastin and collagen (Lepetit, 2008). The meat toughness is not an unacceptable quality for consumers (Kemp et al., 2010). Toughness can be divided into the 'background (due to myofibrillar protein)' and 'actomyosin (due to stromal and connective tissue protein)' toughness (Qihe et al., 2006). The myofibrillar proteins were affected by postmortem processing and handling. Whereas the connective tissue proteins are improved by cooking style and temperature (Bekhit et al., 2014).

Tenderization methods are applied to decrease the meat toughness. Tendering fresh meat of post-mortem interventions can be classified into three main categories according to their mode of action: mechanical (or physical), chemical, and enzymatic (Bekhit et al., 2014) and the tenderization methods are represented in Fig 1.4. It is suggested that the mechanical tenderization must occur during the rigor mortis phase while the chemical and enzymatic tenderization must be in the natural tenderization phase.

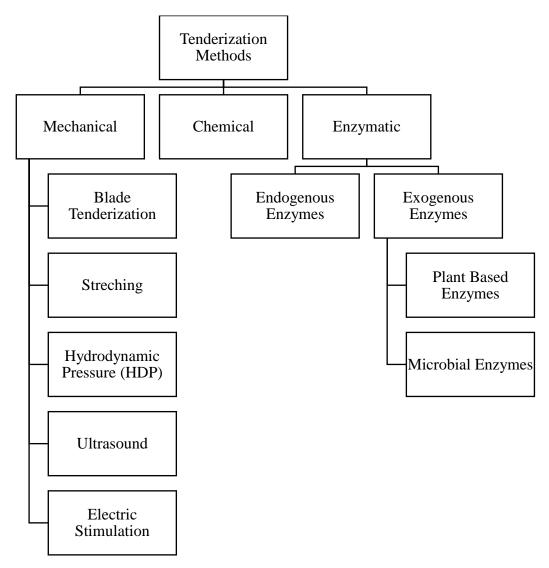


Figure 1.7. Different tenderization methods (Bekhit et al., 2014; Bolumar et al., 2013; Singh et al., 2018)

#### 1.3.1 Mechanical Tenderization

This method depends on applying force on the meat surface to achieve better texture due to the deformation and destruction of the network of the meat structural proteins. In other words, this method alters the connective tissues and myofibrillar proteins by activating the proteolysis, which is achieved by direct interactions between

endogenous enzymes, cofactors, and the substrate (Bekhit et al., 2014). Nevertheless, this method has limited tenderization property for intact meat.

#### 1.3.1.1 Blade Tenderization

Tenderization is performed by blades or some needles, and the technique is also used at commercial scale. Depending on the meat size, the needle's or blade's distance is spaced, and with the penetration of the needles the protein network is weakened by dividing the myofibrillar protein network and the connective tissues. The advantage of this process is that the tenderization takes place without long holding time, high temperatures, or addition of any components. It is helpful for meat that is comprised of high-grade collagen connective tissue. In addition, blade tenderization increases the juices of the blade eye and top sirloin, but there is no effect on the cooking loss. Thomas et al. (2019) studied the quality and sensory characteristics of blade tenderized beef strips. They concluded that the tenderization process did not cause undesirable lipid oxidation due to higher surface area. Also, they found out that the color, sensory and cooking characteristics did not change. In addition, Pietrasik & Shand (2004) reported that blade tenderization accompanied with tumbling provided decreased shear force and hardness of the beef samples up to 50-60 %. In the study of Tasoniero et al. (2019), blade tenderization on the wooden breast chicken fillets was investigated. They concluded that the blade tenderization treatment improved the texture of the wooden breast.

#### **1.3.1.2** Hydrodynamic Pressure (HDP)

Hydrodynamic pressure, known as hydrodyne, includes a small amount of explosive to produce a hydrodynamic shockwave in a fluid medium (generally water) in which vacuum packaged meat is immersed. The mechanism of the tenderization is forming pressure in the range of 70 and 100 MPa for a millisecond in water medium. The abrupt increase in pressure results in the breaking of muscle and connective tissues.

Claus et al. (2001) studied the hydrodynamic shockwave treatment on chicken broiler breasts for tenderization. They measured the efficiency of the process by comparing the shear force of the meats by Warner-Bratzler experimentation. They concluded that there is a 58% reduction in shear force in the treated meats compared to the untreated ones. In another study (Meek et al., 2000), the amount of explosive material and the distance between the material and the meat samples were the main factors of the experimentation. They found the most significant reduction in the Warner-Bratzler shear force when treatment was applied with 350 g at a 20 cm distance.

#### 1.3.2 Chemical Tenderization

For the chemical tenderization, salts, organic acids, and phosphates can be infused into meat by immersion, injection, or marination, and their effect in terms of tenderization is documented (Berge et al., 2001). Tenderness of meat is observed by influencing the muscle structure by changing the solubility of protein or mediating it through the proteases (Bhat et al., 2018). The tenderization mechanism of the chemical methods has been explained by several physicochemical tools. The effect of acid solutions on meat texture is determined by the pH decrease that occurs after treatment. The linked three combination mechanisms explain the acid tenderization: (1) swelling of myofibers, (2) connective tissue weakening, (3) acceleration of postmortem tenderization proteases that have an optimum activity at low pH (Berge et al., 2001). Yoon et al. (2013) tenderized the ground beef patties by using acetic acid. The goal of the study is to evaluate the ability of tenderization on the inactivation of E. coli O157:H7. They found out that the acidic marination reduced the number of E. coli O157:H7 compared to non-acidic tenderization methods. Another study by Yoon et al. (2009) also concluded that the presence of acid provides both tenderness and microbial inactivation. Lawrence & Lawrence (2021) studied the blade tenderization accompanying acid treatment and used lime juice as the acid source.

They concluded that the addition of lime juice to the blade tenderization system had good results in terms of the final tenderization of the meat.

Besides, the various salts were used for tenderization of meat, such as calcium salts (Whipple & Koohmaraie, 1993), sodium chloride (Goli et al., 2014), phosphates (Vote et al., 2000), ammonium hydroxide (Naveena et al., 2011). The using of salt reduces pH of muscle tissues by diffusion, which results in the swelling and weakening of the muscle and connective tissues (Goli et al., 2014). Sodium chloride, calcium salt, and phosphates promote the tenderness of meat and improves the water holding capacity (Baublits et al., 2005; H. W. Kim et al., 2013). By acceleration of calpain activity the sodium pyrophosphates and sodium chloride injection also increase tenderness of meat in pre-rigor. The mechanisms of the tenderization in postmortem stage can be explained by changing of glycolysis, rate or state of contraction, and rate of proteolysis (Lee et al., 2000). Calcium chloride that is one of the most effective tenderizing agents accelerate meat tenderization by mediating through the activation of calpain. Besides, chloride salt and calcium improve the protein solubility by increasing the electrostatic interaction between meat proteins and ionic solution (Gerelt et al., 2005; Polidori et al., 2000).

#### 1.3.3 Enzymatic Tenderization

The proteolytic activity of the enzymes mediates the degradation of muscle proteins. Proteases (peptidase or proteinases) degrade the proteins by hydrolyzing the peptide linkages (Fernández-Lucas et al., 2017). These enzymes can be classified into two subsections as endogenous and exogenous enzymes.

#### 1.3.3.1 Endogenous Enzymes

The endogenous enzymes are the enzymes secreted by the meat body itself, following the animal's death. The enzymes known as proteases on myofibrillar proteins are divided into cathepsin and calpains (Whipple & Koohmaraie, 1991).

#### 1.3.3.1.1 Cathepsin

Early research was focused on the mechanism of cathepsins which is responsible for improving the tenderness while aging of the meat. The cathepsin, known as an endogenous protease, is present in the lysosome in the living muscle (Huff-Lonergan, 2014). Several types of cathepsins (B, D, L, and H) were primarily investigated for meat tenderness. At a low pH level, most cathepsins are activated at around a pH of 5-6 (Hopkins & Huff-Lonergan, 2004).

Cathepsins can be divided into three categories: cystine (cathepsin B, H, L, and X), serine (cathepsin G), and aspartic (cathepsin D and E) peptidase groups (Sentandreu et al., 2002). The cathepsin enzymes affect myosin and actin degradation. However, they are not the enzyme primarily in charge of tenderization of meat during aging. Cystatins, which are the inhibitor of the cathepsins, obstruct these enzymes in the extracellular space (Kerth, 2013).

#### **1.3.3.1.2** Calpains

Calpains (EC 3.4.22.17) found in muscle are neutral cysteine endopeptidase triggered by calcium ions and thionyl compounds. They are the primary enzymes responsible for the degradation of meat proteins within the first 24 h after death (Huff-Lonergan et al., 1996; Huff-Lonergan & Lonergan, 1999; Koohmaraie, 1992; Moudilou et al., 2010). The activity of the calpains depends on some parameters such as pH, temperature, and calcium ions concentration. The calcium ions concentration is important because the calpains are calcium-activated enzymes (Goll et al., 2003; Koohmaraie & Geesink, 2006). The calpain system is comprised of three proteases in the skeletal muscle: μ-calpain (calpain-1), m-calpain (calpain-2), and p4 (or calpain-3) (Kemp et al., 2010). For the calpain-1 and calpian-2 enzyme activation, the micromolar and millimolar concentration of Ca<sup>2+</sup> is required, respectively (Goll et al., 2003). There is a specific inhibitor of calpain-1,2, which is called calpastatin. By inhibition of these enzymes, their tenderization ability is significantly limited.

For example, *Bos taurus* cattle muscle was more tender than *Bos indicus* cattle meat as the calpasin level was high in the *Bos indicus* (Ferguson et al., 2000; Geesink et al., 1993; Koohmaraie & Shackelford, 1991; Whipple et al., 1990). The target protein of the calpain-1 is specific myofibrillar and costameric proteins (titin, nebulin, troponin, etc.) and with increasing calcium concentration, the activity of calpain-1 increases. Besides, for the postmortem proteolysis, the calpasin-1 is regarded as the main protease (Matarneh et al., 2017). In other words, calpain is a key proteolytic system involved in meat tenderization and some studies showed that the most changes in the postmortem period were explained by this system (Ouali et al., 2006).

#### 1.3.3.2 Exogenous Enzymes

The exogenous enzymes are the enzymes that are intentionally added to the meat to tenderize before cooking. For the plant-based protease enzymes, papain, ficin, bromelain, zingibain, and actinidin are generally employed (Madhusankha & Thilakarathna, 2020). Besides, microorganism-based protease enzymes are used.

#### 1.3.3.2.1 Papain

Papain (EC 3.4.22.2), a known Papaya proteinase from the papaya plant (*Carica papaya*), is typical for commercial use because it has potent proteolytic activity against a wide range of protein types. It is durable to different operational conditions. It is active at high temperature (~65 °C) and a wide pH range (5-8) (Scaman, 2003). These characteristic properties give advantages to papain over other plant-derived protease enzymes such as ficin and bromelain (Fernández-Lucas et al., 2017; Roman et al., 2012). Papain results in the degradation of myofibrillar protein as collagen (Ashie et al., 2002). In the study of Jun-hui et al. (2020), tenderization of jumbo squid muscle with bromelain and papain was examined in terms of the effects on the WHC, muscle hardness, myofibrillar stability, and microstructural alterations. They

concluded that these enzymes had significant effects on these qualities, and they improved muscle tenderness.

## 1.3.3.2.2 Ficin

Ficin (EC3.4.22.3.) is obtained from *Ficus glabra* or *Ficus anthelmintica* latex (Gaughran, 1976), but it is mainly obtained from the fig fruit. It is also reported that it has a tenderizing effect on the meat. The enzyme's optimal activity is between 60-70 °C, and it is found that the enzyme is activated approximately at pH of 7 and 5 for collagen and myofibrillar proteins, respectively. (Allen Foegeding & Larick, 1986; El-Gharbawi & Whitaker, 1963). Besides, the effect of ficin tenderization of meat on the water holding capacity is investigated, and it is concluded that ficin increases the meat protein solubility (Ramezani et al., 2003).

#### **1.3.3.2.3** Bromelain

Bromelain (EC3.4.22.32) is found in the pineapple (*Ananas comosus*), or its stem that contains cysteine proteases. The fruit bromelain has higher proteolytic activity and extensive specificity (Grzonka et al., 2007). Bromelain was examined to enhance meat texture. For this purpose, Chang & Han (2020) investigated the synergic impact of injection and *sous-vide* on pork tenderization at various temperatures and duration using pineapple and kiwifruit. This study showed that both enzymes had substantial softening properties. However, it was found that actinidin was more capable than the bromelain. In addition, the study of Ketnawa & Rawdkuen (2011) reported that the tenderization with bromelain decreased the firmness of chicken, beef, and squid. On the other hand, the use of the bromelain had some drawbacks; it caused over tenderization, mushy texture, and creating off-flavors (Gagaoua et al., 2021).

## **1.3.3.2.4** Zingibain

Zingibain is a robust proteolytic enzyme found in ginger, and it is used as a tenderizing ingredient (Lee et al., 1986; Mansour & Khalil, 2000). Ginger rhizomes provide zingibain enzymes, the proteolytic activity of the zingibain was shown to increase by heating (Naveena & Mendiratta, 2001). It is expensive and easy to use for tenderization of meat, and the study indicated that the higher concentration of the enzyme resulted in the degradation of myofibrillar proteins extensively (Lee et al., 1986; Naveena et al., 2004). The degradation appeared to start at the I band of the sarcomere and continued through the M line. In addition, it was reported that using bromelain and papain in the camel meat burger patties improved the meat physicochemical properties as tenderness, juiciness connective tissue reduction, and collagen solubility increased in the study of Abdel-Naeem & Mohamed (2016).

## 1.3.3.2.5 Actinidin

Actinidin is a proteolytic enzyme, and it is found in gooseberry or kiwi fruit (*Actinidia chinensis*) (Arcus, 1959). The target protein of the actinidin is the myofibrillar proteins of meat(Ha et al., 2014). Some studies showed that the high concentration of actinidin enzyme caused mild tenderization because of the mushy surface. Tenderization with this enzyme prevented overcooking of meat due to its low inactivation temperature (60 °C) (Eshamah et al., 2014; Tarte, 2008). Christensen et al. (2009) conducted a study to see the effect of actinidin tenderization on pork samples. The study showed that the Warner-Bratzler shear decreased, and the tenderness raised even the process did not produce off-flavor and lost the juiciness of the meat.

Moreover, the significant alterations that have taken place in myofibril fragmentation index, particle size, viscosity, and microstructure were proven in the study of Chen et al. (2012). The actinidin enzymes' hydrolysis mechanism decreased the peptide chains length, resulting in increased lightness (Kakash et al., 2019). They also

observed that the hardness of the chicken meat sample decreased by tenderizing with actinidin.

#### 1.3.3.2.6 Microbial Proteases

Protease enzymes originated from the microorganisms that act on connective tissues and myofibrillar protein, have shown to result in myofibrillar fragmentation and degradation of structural proteins (Bekhit et al., 2014).

For an example of microbial enzymes, fungal enzymes are used for meat tenderization. There are applications of meat tenderization by the fungi that are *Rhizomucor miehei* (Sun et al., 2018), *Aspergillus oryzae* (Ashie et al., 2002), *Penicillium chrysogenum* (Benito et al., 2003), and *Aspergillus sojae* (Gerelt et al., 2000). These all examined the effect of the proteolytic activity of extracted enzymes from the fungi. Besides fungal enzymes, bacteria are frequently used for biotechnological enzyme production. These studies mainly focused on *Bacillus subtilis* (McConn et al., 1964; Qihe et al., 2006; Yeh et al., 2002), *Clostridium histolyticum* (Allen Foegeding & Larick, 1986; Takagi et al., 1992), and *Vibrio* spp. (Miller et al., 1989). They primarily evaluated the effect of the proteases on the collagen tissue of the meat.

## 1.4 In Vitro Digestion

In this thesis, effect of different tenderization methods on *in vitro* digestion behavior of meat samples have been studied. It is important to have a look at the details of the digestion and how it has been studied on meat samples in the literature.

The food path after the mouth is followed as the esophagus, the stomach, the small and large intestine, and the rectum is the final place before excretion from the body (Sensoy, 2021). Throughout this pathway, food material does not just move by itself. There are gastric fluids that are accompanying to the food material with this

movement. These fluids are mainly saliva from the mouth, gastric juice from the stomach, and intestinal secretions from the pancreas, liver, and intestinal glands. All fluids have a different activity on the digestion of various components of the material. As stated in Table 1.1, the significant contribution of the digestible biochemical element belongs to the proteins. The digestion of the proteins begins in the stomach with the help of gastric juice and continues in the small intestine with the intestinal fluid.

In vitro studies are conducted in test tubes or test media by mimicking the existing systems (Abbirami et al., 2013). The most advantageous feature of the use of *in vitro* system is to have control over the system. In vivo studies do not provide this ease even though they are natural systems. In *in vitro* studies, there are different approaches. The most basic one is putting the food material and the gastric fluids in the same glass beaker with additional time importance. By shaking, the imitated muscle contraction of the stomach is provided.

In the literature, several *in vitro* digestion procedures have been applied for different types of food. INFOGEST protocol has been developed to prevent the unsuitable endpoint of the static *in vitro* digestion models due to complexity and variability of food structure (Brodkorb et al., 2019). The digestion of casein and whey protein using the INFOGEST protocol showed high correlation of protein degradation with protein digestion after human jejunum (Sanchón et al., 2018). INFOGEST was also used for the assessment of lipid oxidation inhibitory activity of black, green and pink paper *in vitro* digestion of meat (Martini et al., 2021). In the study of Van Hecke et al. (2021), lean meat *in vitro* digestion was performed according to the protocol described by (Versantvoort et al., 2005) to effect of the nitrite salt and ascorbate on oxidation of meat. The other study was performed to determine the effect of cooking methods on the structural change, moisture uptake in to sweet potatoes (Mennah-Govela & Bornhorst, 2016). In this study the classical static digestion protocol has been used as according to protocol of Ozvural & Bornhorst (2018).

## 1.5 Objective of the Study

It is vital to consume red and white meat for the human diet in terms of nutritional value. Meat contains many proteins and essential amino acids that humans must take with their diet. Tenderization processes improve the chemical, physical and biochemical properties, and tenderness is one of the most important properties for the consumer's acceptability. Tenderization causes changes on the connective tissues and myofibrillar proteins, it is also important to understand how different tenderization processes affect the digestion behavior of meat.

The objective of this study is to investigate the effect of different tenderization methods on beef and chicken samples before and after *in vitro* digestion. As the tenderization methods, acidic solutions (acetic acid, lactic acid, and citric acid) and a commercial enzymatic solution was used. The effect of tenderization was assessed through different physico-chemical measurements. The physical examination of the meat before and after treatments were performed by Warner-Bratzler shear experiments. The morphological changes were examined by scanning electron microscopy experiments (SEM). Water holding capacity, color experiments were performed as further quality tests. To understand the interaction of proteins between tenderization and digestion solutions a non-destructive method; TD-NMR relaxometry was used. Extent of hydrolysis was further analyzed by free amino group and soluble protein content analysis.

The specific objectives of the study can be listed as;

- to compare acidic and enzymatic tenderization in terms of the physical attributes;
- to find out which tenderization method will ease the digestion more
- to see if TD-NMR can be used to explain the changes occurring at microstructural level

#### **CHAPTER 2**

### MATERIALS AND METHODS

## 2.1 Materials

Since the meat used in the study could have caused significant variation on the results, it was assured that meat was provided from the same supplier all the time. The same section of the animal was used in all studies. It was also assured that rigor state of the animals had passed. Initial quality analysis (*pH*, hardness, color, water holding capacity) was performed for the initially bought samples. If significant deviation was observed in the values, that batch was not used for the experiments. Extensor carpi radialis beef muscle and musculus pectoralis thoracica chicken breast muscles were supplied from a local market in Ankara, Turkey. The muscle samples were cut in cube-shaped in 15 mm length, and they were stored at -18 °C until the experiment was conducted.

In addition, a commercial meat tenderization solution (MTS) known to be 'natural protease' solution was purchased from Alfasol (İstanbul, Turkey). Since the source of the protease was not known; a further characterization was performed using SDS-PAGE gel electrophoresis and activity of the enzyme mix was also determined as will be explained later.

## 2.2 Tenderization and Cooking

Before tenderization, the frozen meat samples were thawed at 4 °C for one night. Later, the samples were immersed in the tenderization solutions of 1 % (w/v) acetic acid (AA), citric acid (CA) and lactic acid (LA) for 24 hours. The percent of the acidic solutions usage were based on literature studies (Aktaş et al., 2003). Besides, the MTS was used for enzymatic tenderization, and the MTS was used without

dilution. To determine the effect of the time on tenderization, the meat sample was exposed to meat tenderization solutions at different times (10, 20, 30, 40, 50, 60, and 1440 min). In addition, meat was tenderized with distilled water to observe its effect on the samples in acid treatment. The ratio of the meat to solutions is 1:5, and the samples in closed petri dishes were tenderized for 24 hours at 4 °C. After tenderization, the tenderized meat and control were cooked at 105 °C for 1 hour in an oven.

## 2.3 Marinade Uptake and Cooking Loss

Marinate uptake was measured with before and after the tenderization of meat weight. After tenderization, the excess surface moisture of the meat was removed slightly with paper towels. It was expressed in % as:

% marinated uptake = 
$$\frac{marinated\ weight-raw\ meat}{raw\ weight} \times 100$$
 (Eqn. 3)
(Burke & Monahan, 2003)

Before and after cooking, the excess surface moisture of meat samples was discharged using paper towels, and the weights of the samples were recorded. Cooking loss was calculated as follows:

% cooking loss = 
$$\frac{marinated\ weight-cooked\ weight}{marinated\ weight} \times 100$$
 (Eqn. 4)
(Yusop et al., 2010)

## 2.4 pH and Color

The pH meat samples was measured according to method of Burke & Monahan (2003) with slight modifications. The sample taken before and after cooking was homogenized with distilled water at a ratio of 1 to 9 using a homogenizer (IKA; T18 Digital Ultra-Turrax or WiseTis Homogenizer, Witeg Labortechnik GmbH,

Germany) until a homogenous mixture is obtained. The measurements were performed using a portable pH meter in quaternary replications.

The color of the uncooked and cooked meat surface was measured using a benchtop spectrophotometer (Datacolor 110<sup>TM</sup>, Lawrenceville, NJ, USA). Before the measurements, the instrument was calibrated, and the measurement was performed against a white plate. The color was represented by using L\* (lightness), a\* (redness), and b\* (yellowness) color system.

## 2.5 Water Holding Capacity (WHC)

The meat samples' water holding capacity (WHC) was determined according to the method described by Wardlaw et al. (1973). Before and after cooking, 0.6 M, 7.5 ml NaCl solution was added to 5 grams of meat sample, and it was stirred for 1 min using vortex. After holding it for 15 min at 4 °C, it was centrifugated for 25 min at 2263 ×g (MF-80, Hanil Science Industrial Co. Ltd., South Korea). The supernatant's volume was measured after centrifugation, and WHC in percentage was expressed as:

WHC (%) = 
$$\frac{\textit{Volume of NaCl after centrifuge-Volume of the supernatant}}{\textit{Volume of NaCl after centrifuge}} \times 100$$

(**Eqn. 5**) (Maqsood et al., 2018)

## 2.6 Warner-Bratzler Shear (WBS) Force

Shear force of un/cooked and digested samples were measured by a texture analyzer (Brookfield, Ametek, CT3, Middleboro, MA, USA). Each sample was measured with a TA-CKA blade probe compression test with a trigger load of 0.01 N and 0.05 mm/s probe speed. The samples were cut perpendicular to the fiber direction. The test parameter was 10.0 mm compression with two cycles. The maximum shear force

(N) was obtained from the deformation curve. Each sample was measured with 8 replicates.

## 2.7 In Vitro Digestion

As the digestion protocol, studies of Mennah-Govela & Bornhorst (2016) and Roman et al. (2012) were followed with some modifications. Only, the cooked samples were subjected to in vitro digestion. The simulated saliva was prepared by mixing α-amylase (2g/L), mucin (1g/L), NaCl (0.117g/L), KCl (0.149g/L), and NaHCO<sub>3</sub> (2.1g/L) in deionized water. Then, the pH of the mixture was adjusted to 7 by adding 0.01 N NaOH. Besides, the simulated gastric juice was prepared by the following component in deionized water: pepsin (1.0 g/L), mucin (1.5 g/L), NaCl (8.78 g/L), and pH was adjusted to around 2.0 with 0.1 N HCl. Also, for the simulated intestinal juice preparation, NaHCO<sub>3</sub> (33.6 g/L), bile extraction (20 g/L), pancreatin (4.8 g/L) in deionized water were mixed. The cooked meat sample in cube-shaped (20 g) was placed in an Erlenmeyer flask, and the simulated saliva (0.2 mL saliva/ g sample) was added to it and shaken gently for 30 seconds. After 100 mL simulated gastric solution (pre-warmed to 37 °C) was put in the flask immediately, the flask was covered and placed in a shaking water bath (at 37 °C, 100 rpm) for 120 minutes. After gastric digestion, 125 ml of intestinal solution was added, and the pH of the solution was adjusted to 6.5 with 5M NaOH, and the sample was put in the shaking bath for 120 minutes at 37 °C and 100 rpm. After 2 hours, the sample was put into an ice bath to stop digestion by inactivating the enzymes.

## 2.8 Free Amino Group Determination by OPA Method

To understand the extend of protein hydrolysis with digestion, OPA method was used first (Batista et al., 2010; Duque-Estrada et al., 2019; Faber et al., 2010; Rutherfurd, 2010; Schasteen et al., 2002; Yi et al., 2016). The digestion juice was centrifuged at 1500g for 20 min (4 °C), and the supernatant of the digestion juice

was collected and filtered. The experiment was performed according to Nielsen et al. (2001) with minor modifications. To begin, 80 mg of o-phthalaldehyde (OPA) was dissolved in 2 mL 95 % (v/v) ethanol solution. After decomposing OPA, a 100 ml volumetric flask was filled with 50 mL 100 mM borax buffer at pH 9.75, OPA solution, 200  $\mu$ l  $\beta$ -mercaptoethanol, and 5 ml 20 % (w/v) sodium dodecyl sulfate (SDS). The mixture's volume was increased to 100 mL. The 0.5 mL of sample solution and 1.5 mL reagent solution were combined and maintained at room temperature for 2 minutes. The absorbance values of the samples were measured at 340 nm and the amount of the free amino groups in the digestion juice using the calibration curve within an interval between 1 g/L and 10 g/L. The obtained calibration curve was found by concentration (g/100 mL) vs. absorbance graph in Appendix A.

## 2.9 Soluble Protein Content Determination by Lowry Method

In addition to OPA, as a second method to investigate the digestion; the amount of the soluble protein was determined using the Lowry method (Lowry et al., 1951). At the beginning of the experiment, the reagent of the Lowry solutions given in Table 2.1 was prepared. Lowry solution was prepared by blending reagent of A: 1:2 at the ratio of 100:1:1 volumetrically, respectively. 0.5 mL processed digestion juice sample and 2.5 mL Lowry reagent were mixed and incubated at room temperature for 10 min. After 0.25 mL Folin-Ciocalteu's phenol reagent (diluted with 2N stock solution in the ratio of 1:1 added to tubes, the mixture was incubated for 30 min in the dark environment. The absorbance values of samples were measured at 680 nm by a UV/VIS Spectrophotometer (Optizen Nano-Bio, Mecasys Co. LTD, Korea). For the calibration curve, bovine serum albumin (BSA) is used as the standard in a concentration range of 0.03125-0.5 g/L, and the calibration curve was given the Appendix A.

Table 2.1 The formulation of Lowry reagents

Reagent 1	2% CuSO4.5H2O
Reagent 2	2% C <sub>4</sub> H <sub>4</sub> KNaO <sub>6</sub> .4H <sub>2</sub> O
Reagent A	2% Na <sub>2</sub> CO <sub>3</sub> in 0.1 NaOH

# 2.10 Relaxation Times Measurement through Time Domain Nuclear Magnetic Resonance (TD-NMR) Relaxometry

To understand the solvent-protein interactions with tenderization and digestion, TD-NMR experiments were performed via 0.48 Tesla ( $^{1}$ H frequency of 20.34 MHz) system (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany) with a 10 mm RF coil. To measure the relaxation times, samples were put into the sample tubes at a height of 1.5 cm. Samples were measured before and after tenderization, also before and after cooking.  $T_2$  relaxation times of the samples were measured with Carl-Purcell-Meiboom-Gill (CPMG) pulse sequences. For  $T_2$  experiments, 400 echoes were used with an echo time of 2000  $\mu$ s, a repetition delay of 30  $\mu$ s, and 32 scans. 90° pulse duration was 3.40  $\mu$ s for the coil.  $T_2$  data were analyzed both mono exponentially and bi exponentially using Relax8 software (Resonance Systems GmbH, Kirchheim/Teck, Germany).

## 2.11 Enzyme Activity

The commercial meat tenderization solution's enzyme activity was measured according to Shin et al. (2008) with some modifications. The incubation mixture (1 mL of 2 mmol/L CaCl<sub>2</sub> and 0.2 mol/L Tris-HCl buffer, pH 7.5) containing 10 mg casein and 1 mL of meat tenderization solution was incubated at 35°C for 60 min. The reaction was terminated by adding 2 mL of 5 g/L chilled trichloroacetic acids and centrifuged at 2263 g for 15 min. The number of proteolytic products in the resulting supernatant was measured by absorbance at 280 nm. One unit of caseinolytic activity was defined as the amount of enzyme that caused an increase of 0.1 absorbance units at 280 nm after 60 min incubation at 35°C.

The absorbance value measured at the elevation from blank (whose enzyme activity was directly terminated before substrate addition) from this experimentation is 0.106±0.006. Therefore, the enzyme activity was determined as 0.106 unit/mL MTS.

## 2.12 Protease Enzyme Mixture Analysis by Gel Electrophoresis

Firstly, the polyacrylamide gel concentration was decided according to the expected size of the protease enzyme mixture to be investigated by preliminary experiments. Two different gels were prepared, the stacking gel (10 %) and the resolving gel (18 %). They had different concentrations to align the protein samples first and then separate them at the same time. In this way, only the size was going to be the parameter that separates the proteins. Protein samples were treated by SDS and βmercaptoethanol. The presence of  $\beta$ -mercaptoethanol causes protein denaturation by the breakage of the disulfide bonds. Sodium dodecyl sulfate (SDS), which is the detergent, binds to the hydrophobic region of the denatured protein. The SDS-Protein complex carries a net negative charge; hence the complex moves towards a positive electrode (anode), and the separation is achieved based on the size of the proteins. Buffers having different pH values were used as running buffer (6.8) and gel buffer (8.8). Samples were denatured at 95 °C for 5 minutes after the chemical treatments while the gel was formed. Then samples were loaded in the gel, and by applying an electrical potential, negatively charged proteins migrated to the positive electrode. In the end, the proteins were separated depending on their size. Larger molecules migrated slower and smaller ones migrated faster (Laemmli, 1970).

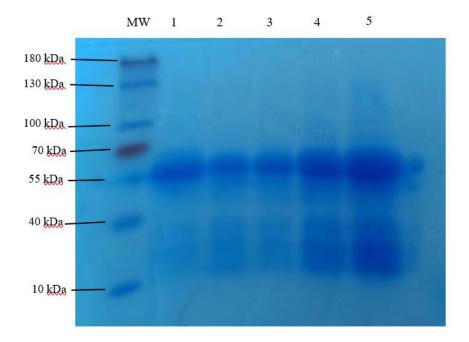


Figure 2.1. Gel result of SDS-PAGE with β-mercaptoethanol of protease mixture using different enzyme concentrations, (MW) Molecular Weight Standard, (1) 0.5% (w/v) concentration of enzyme mixture, (2) 1% (w/v) concentration of enzyme mixture, (3) 2 % (w/v) concentration of enzyme mixture, (4) 5 % (w/v) concentration of enzyme mixture, (5) 10 % (w/v) concentration of enzyme mixture.

According to SDS-PAGE results, intense bands show higher ratios of the components/protein subunits. The strongest protein bands were obtained from samples 4 and 5 due to their high concentration in the solution.

Gel results showed that molecular weights of all protein fractions were below 70 kDa. The most intense bands were found around the 40-55 kDa region. These proteases might be identified under the category of 'serine proteases' and 'cysteine proteases' (Dacheux et al., 2002; David et al., 2007). These digestive enzymes are vital since they can cut peptides in various proteins (Yousef et al., 2003).

The smear formation observed in the gel picture, especially between 10-40 kDa molecular weight region, could have 2 possible explanations. The first reason might be the no use of protease inhibitors to prevent the proteolytic activity before electrophoresis, which resulted in the proteases digesting each other (Claeys et al.,

1995). There was no specific separation of the proteins in this region. This situation might be clarified by the presence of various low molecular weight unfolded protein fractions. Another explanation would be the high concentration and solubility.

# 2.13 Statistical Analysis

Analysis of Variance (ANOVA) was used to determine the differences (Minitab Inc., Coventry, UK). Tukey's comparison test at a 95 % confidence interval was used for pairwise comparisons. To check the relation between the outputs of the Lowry method and OPA method, Pearson correlation analysis was conducted between 18 results, then the correlation coefficients and p values were reported at a significance level of 5%.

## 2.14 Experimental Design

The parameters and the testing levels for each factor and the measurements are summarized in Table 2.2 and Table 2.3.

Table 2.2 Experimental design table for beef meat tenderization

Tenderization Material	Tenderization Duration (min)	Cooking	Digestion	Measurements
AA CA LA	0, 1440	Uncooked,	Before digestion,	pH WBS Force Marinate uptake Cooking loss WHC
Enzyme (MTS)	0, 10, 20, 30, 40, 50, 60, 1440	Cooked	After digestion	Color TD-NMR: T <sub>2</sub> OPA Lowry

Table 2.3 Experimental design table for chicken meat tenderization

Tenderization Material	Tenderization Duration (min)	Cooking	Digestion	Measurements
AA  Enzyme (MTS)	- 0, 1440	Uncooked, Cooked	Before digestion, After digestion	pH WBS force Marinate uptake Cooking loss WHC Color TD-NMR: T <sub>2</sub> OPA Lowry

#### **CHAPTER 3**

#### RESULTS AND DISCUSSION

## 3.1 Beef Tenderization

## 3.1.1 Acidic Solution Tenderization

## **3.1.1.1 pH** Values

The pH values of the meat samples that are tenderized by different methods before and after cooking are given in Table 3.1. Acid-tenderization decreased the pH values significantly as expected (p<0.05). The pH values of the samples tenderized with acidic solutions were lower than the pH values of C and DW after the cooking process (p<0.05). It was an expected result since the pH values of the samples are supposed to decrease as acid solutions penetrate meat muscle (Goli et al., 2011). Moreover, Narayan et al. (2013) showed that acid marination lowered the pH of goat meat curry. Significant differences were also detected with respect to the acid type as well (p<0.05). The studies showed that the pH of the meat samples had a relationship with the pKa of the acid used (Aktaş et al., 2003; Burke & Monahan, 2003). CA and LA samples were found to be indifferent which was consistent with their pKa values. pKa of CA (3.06) and LA (3.86) are lower than the AA (4.75) (Ref). Therefore, the pH of AA samples was higher than other acidic treated samples (Ouellette & Rawn, 2015).

pH values showed an increase for C and DW samples after cooking (p<0.05). This has been explained by the decrease in the available acidic groups in the meat proteins (Bagarinao et al., 2020). The availability of the acidic groups could have been affected from the denaturation of the meat proteins.

Table 3.1 pH values of beef meat samples

Tenderization Type	Uncooked	Cooked
$\mathbf{C}$	$6.09^{bA} \pm 0.05$	$6.56^{aA}\pm0.04$
$\mathbf{DW}$	$5.84^{bA} \pm 0.12$	$6.47^{aA} \pm 0.07$
$\mathbf{A}\mathbf{A}$	$3.82^{aB}\pm0.02$	$3.81^{aB} \pm 0.01$
CA	$3.29^{aC} \pm 0.16$	$3.12^{aC} \pm 0.15$
LA	$3.40^{aC}\pm0.03$	$3.29^{bC} \pm 0.06$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

## 3.1.1.2 Water Holding Capacity

The ability of proteins to keep water from being released or remove from their structure is known as water holding capacity (WHC) (Haque et al., 2016). The WHC of the sample treated with acid solutions before and after cooking is given in Table 3.2. The WHC values of the C and DW samples could not be determined since these samples did not release any water during the experiments. In the experiment protocol as stated before 0.6 M NaCl was used. It is probable that, this concentration was not sufficient to release the water out from the C and DW samples. However, to be consistent with the protocol that value was not changed.

The WHC values of the samples tenderized with AA was found to be higher than the samples tenderized with other acids before cooking (p<0.05). The reason of difference WHC between acidic treatment can be explained by the degradation of integrin which are a family of adhesion receptors because the degradation of integrin protein had an effect on decreasing the WHC (Huff-Lonergan, 2009). It is probable that degradation was more in CA and LA samples resulting in lower WHC values due to lower pH. That was also consistent with the pH results.

Cooking decreased the WHC of sample significantly as expected (p<0.05). Meat loses water during cooking since water is moved to the extracellular region of muscle

due to shrinkage. The denaturation of myofibrillar proteins triggers this loss due to aggregation (Botinestean et al., 2018; Kondjoyan et al., 2013; Whitehurst & van Oort, 2009).

Table 3.2 Water Holding Capacity (WHC) (%) of tenderized beef meat

Tenderization Type	Uncooked	Cooked
$\mathbf{C}$	-	-
$\mathbf{DW}$	-	-
$\mathbf{A}\mathbf{A}$	$28.33^{aA}\pm2.36$	$14.22^{\text{bA}} \pm 5.55$
CA	$16.89^{aB} \pm 1.39$	$9.00^{bA} \pm 0.42$
LA	$22.33^{aAB} \pm 1.41$	$14.00^{bA} \pm 1.89$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-B), are significantly different each sample (p<0.05)

## 3.1.1.3 Warner-Bratzler Shear Force Analysis

The Warner-Bratzler shear (WBS) force values of C, DW, AA, CA and LA samples are given in Table 3.3. When the WBS (N) values of the uncooked samples after tenderization were compared to the control sample, it was found that tenderization with AA, CA, and LA decreased the WBS values significantly (p<0.05). The samples increasing WBS force refers to increasing hardness and reduced tenderness of the meat sample. In other words, it was obvious that acidic treatment worked as a marination technique and increased the tenderness. The reason for low the WBS values of acid tenderized samples can be explained by muscle swelling. The low pH below the isoelectric point of meat proteins (pI~5.3) causes the protonation of the carboxyl group and amino groups of the amino acids, which results in repulsion between protein chains and lead to a more discontinuous network (Haque et al., 2016; Swatland, 2002). This disruption in the network resulted in lower WBS values. Increase in WHC with acidic tenderization was also consistent with this finding. With repulsion, swelling occurred and WHC increased. It is important to point out that acid types did not show any difference on the WBS values (p>0.05).

On the other hand, WBS force values of the DW sample were higher than all samples in uncooked samples (p<0.05). As stated before, the tenderness of the meat depends significantly on the pH, and the isoelectric point of meat proteins is around pH 5.3. At pHs close to pI, electrostatic interactions are minimum and fibrous proteins in the tissue are expected to be closer to each other as will be confirmed later by SEM experiments. Distilled water had a pH of 6-6.5, and meat samples tenderized in DW had a final pH around 5.84. The proteins forming a more continuous network could have resulted in higher WBS values.

Table 3.3 The Warner-Bratzler Shear force (N) of uncooked and cooked beef meat

Tenderization Type	Uncooked	Cooked
C	$21.02^{\text{bB}} \pm 2.77$	$51.81^{aA} \pm 7.23$
$\mathbf{DW}$	$50.94^{aA} \pm 2.69$	$49.81^{aA} \pm 6.56$
$\mathbf{A}\mathbf{A}$	$6.88^{bC} \pm 0.67$	$19.27^{aB} \pm 2.51$
CA	$5.23^{bC} \pm 0.73$	$21.97^{aB} \pm 2.27$
$\mathbf{L}\mathbf{A}$	$5.94^{bC} \pm 0.75$	$9.20^{aC} \pm 1.22$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

When the effect of the cooking on the WBS value of the samples was examined, it was found that the WBS values of the DW sample did not change after cooking (p>0.05). Nevertheless, the WBS force value of the C and acidic tenderized meat samples increased (p<0.05). It was hypothesized that this could have occurred due to the decrease of water in the samples with the cooking process. Besides, the denaturation of connective tissues and myofibrillar proteins could have caused changes in meat tenderness during cooking (Christensen et al., 2000). The difference in WBS force values between uncooked and cooked samples was not unusual since the shear force for the raw meat relates to the background (or collagen) toughness while shear force for cooked meat depends on myofibrillar toughness and myofibrillar toughness increases with cooking (Gök et al., 2019). Besides, the difference between the acidic treatment was observed, and lactic acid treatment showed that lower WBS force in cooked sample. The reason of this difference may

be that lactic acid affected collogen tissues more than other acid because acid treatment effects the perimysium connective tissues and collagen, reducing the thermal stability by decreasing the denaturation temperature (Hinkle, 2010; Hosseini & Esfahani mehr, 2015)

#### 3.1.1.4 Color Measurement

L\*, a\*, and b\* values of the acidic tenderized meat samples are given in Table 3.4. Since L\* and a\* values are more related with red meat quality, they are discussed in detail. As stated before calibration was done with respect to 'white 'sample That is why some values are reported as negative.

The results showed that there were significant differences between cooked and uncooked samples and with acidic tenderization (p<0.05). For L\* values, samples of CA-LA and uncooked C and LA samples were found similar (p>0.05). In contrast, the other samples were significantly different (p<0.05). Besides, the L\* value of the DW sample was different from C, LA, and CA. The lightest samples were control and LA samples. Color of the meat depends on the presence of the heme pigment that is myoglobin. Loss of myoglobin can lead to pale color (Zhuang & Bowker, 2016). However it is important to mention that pH is also an important factor that could affect the color (Shikh Zahari et al., 2021). Distance between myofibrils changes with pH and that has a direct effect on the light scattering behavior. Thus, color is affected. So, it may not be possible to explain the color change just on a single factor. Lightness decreased with AA and CA whereas on LA samples no change was observed. DW samples had the lowest lightness values. That might have been affected from the aggregated myofibrils.

The results showed that control and DW had the highest  $a^*$  and  $b^*$  values in both uncooked and cooked samples (p<0.05), so that it can be said that these samples were redder than the acid-treated samples. The dramatic change observed in the  $a^*$  values of uncooked and cooked samples tenderized in acidic solutions could also have been

occurred due to the denaturation of myoglobin due to acidic conditions. Myoglobin is also affected from cooking and converted to metmyoglobin (Sen et al., 2014). This also causes significant changes in color.

Table 3.4 The L\*, a\*, b\* values of tenderized beef meat samples before and after cooking

	T	*_	*c	×.	q	p*
Tenderization Type	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
C	$-56.98^{aC}\pm1.36$	-49.27bBC±3.25	$8.67^{aA}\pm 2.34$	$5.81^{aA}\pm031$	$8.40^{aB}\pm0.71$	$9.81^{aB}\pm0.83$
DW	$-42.08^{aA}\pm0.84$	$-36.79^{aA}\pm4.05$	$7.39^{aAB}\pm1.26$	$6.01^{\mathrm{aA}}\pm1.01$	$12.13^{\text{bA}}\pm1.07$	$16.16^{\mathrm{aA}}\pm1.52$
$\mathbf{A}\mathbf{A}$	$-48.21^{aB}\pm0.95$	$-45.31^{aB}\pm1.73$	$0.86^{\mathrm{aBC}} \pm 0.05$	$-0.62^{\mathrm{bC}}\pm0.04$	$6.05^{ m aBC} \pm 0.85$	$5.00^{\mathrm{aC}}\pm0.60$
CA	$-49.44^{aB}\pm2.31$	$-50.95^{\mathrm{aBC}} \pm 1.18$	$3.24^{\mathrm{aBC}}\pm0.08$	$2.22^{aB}\pm0.74$	$7.18^{aB}\pm0.33$	$6.59^{aC}\pm0.23$
LA	$-55.93^{aC}\pm1.19$	$-54.97^{aC}\pm 3.86$	$2.30^{\mathrm{aC}}\pm0.46$	$1.57^{\mathrm{aB}}\pm0.20$	$4.11^{aC}\pm0.50$	$3.40^{{ m aC}}{\pm}0.48$

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05) Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

## 3.1.1.5 Monoexponential Analysis of TD-NMR Relaxometry Measurements

TD-NMR relaxometry through measurement of  $T_2$  relaxation times provides direct information on the interaction between water protons and exchangeable protons in proteins (Bertham & Ersen, 2004) and it could be expressed as either a mono exponential or multiexponential behavior:

Mono exponential Model: 
$$M = M_0 e^{-t/T_2}$$
 (Eqn. 6)

Multiexponential model: 
$$M = \sum_{i}^{n} M_{0_i} e^{-t/T_{2_i}}$$
 (Eqn. 7)

Multiexponential behavior is observed when water is distributed in different proton pools. However, it is always a good idea to examine the mono exponential values to obtain the general trend of the changes observed on the relaxation times.

 $T_2$  relaxation times of samples before and after cooking are given in Table 3.5. The results showed that the  $T_2$  values of samples tenderized with acidic solutions were longer than the C and DW samples both before and after cooking (p<0.05). Short  $T_2$  times indicated immobile water adjacent to the proteins, whereas a longer  $T_2$  indicated water in the protein matrix, within interval cavities, or free water within the meat sample (Cornet et al., 2020). The difference on  $T_2$  values between the acid-treated and control sample was that samples tenderized with acidic solution had absorbed water from the solution during the tenderization. In addition, acidic solutions due to the higher concentrations of  $[H^+]$  tend to have longer relaxation times (McLachlan, 1980). The longer  $T_2$  values of the acid tenderized meat could also be attributed to the pores inside the meat being filled with water due to swelling (Cornet et al., 2020). The solvent uptake with marination was also confirmed by marinate uptake results (Table 3.6). Higher uptake resulted in longer  $T_2$  times.

Table 3.5 The T<sub>2</sub> relaxation time values (ms) of uncooked and cooked beef meat

Tenderization Type	Uncooked	Cooked
$\mathbf{C}$	$41.04^{aB} \pm 4.11$	$30.79^{bC} \pm 2.70$
$\mathbf{DW}$	$55.90^{aB} \pm 8.58$	$35.982^{bC} \pm 0.51$
$\mathbf{A}\mathbf{A}$	$140.66^{\mathrm{aA}} \pm 12.55$	$81.58^{\mathrm{bB}} \pm 12.36$
CA	$125.80^{aA} \pm 17.40$	$117.52^{aA} \pm 10.70$
LA	$161.20^{aA} \pm 21.20$	$121.80^{aA} \pm 18.10$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

When the values with cooking were examined, it was observed that T<sub>2</sub> values of C, DW and AA samples reduced after cooking (p<0.05). It was an expected result compared with the WHC values because the acid treatment caused increased water in the protein structure and decreased after cooking. With cooking, denaturation of myosin and shrinkage of longitudinal of muscle fibers is observed (Micklander et al., 2002) resulting in water loss and that was confirmed with cooking loss results. Water loss from the system is directly reflected as a decrease on the T<sub>2</sub> values. On the other hand, T<sub>2</sub> values of CA and LA tenderized meat samples did not show any statistical differences before and after cooking despite the significant changes on cooking loss (p>0.05). That was a clear indication that tenderizing with CA and LA had resulted in a different interaction with proteins and the acidic solution and should be further examined with biexponential results.

Table 3.6 Marinade uptake (%) and cooking loss (%) of beef meat

Tenderization Type	Marinate Uptake	Cooking Loss
$\mathbf{C}$	-	$43.68^{AB} \pm 1.71$
$\mathbf{DW}$	$-8.195^{\mathrm{B}} \pm 0.58$	$47.69^{A}\pm1.86$
$\mathbf{A}\mathbf{A}$	$72.70^{A} \pm 4.57$	$25.14^{B}\pm3.60$
CA	$70.90^{A} \pm 5.32$	$37.18^{\circ}\pm0.60$
LA	$76.66^{A} \pm 9.57$	22.34 <sup>C</sup> ±6.06

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

# 3.1.1.6 Biexponential Analysis of TD-NMR Relaxometry Measurements

The biexponential model that is used for fitting the data is as follows:

$$M = M_{0_1} e^{-t/T_{21}} + M_{0_2} e^{-t/T_{22}}$$
 (Eqn. 8)

The biexponential function constants ( $T_{21}$  and  $T_{22}$ ) obtained by TD-NMR relaxometry analysis are given in Table 3.7. The shorter component ( $T_{21}$ ) belongs to water that is found in highly organized protein structures with high myofibrillar protein concentrations such as actin and myosin. In contrast, the slower relaxing component ( $T_{22}$ ) corresponds to the water placed between fiber bundles and intermyofibrillar water (Shaarani et al., 2006). When  $T_{21}$  values were examined, it was observed that the  $T_{21}$  values of the sample tenderized with acidic solutions before and after cooking were longer than the C and DW samples (p<0.05).  $T_{21}$  values being longer in acidic tenderization indicated there was more water associated with the myofibrillar matrix as acidic tenderization is expected to result in an increased myofibrillar space (Burke & Monahan, 2003).  $T_{21}$  values decreased after cooking (p<0.05) as  $T_{21}$  was associated to the water within the myofibrillar matrix, and denaturation of myosin protein resulted in reduced myofibrillar lattice space through the expelling water from the fibers (Ling et al., 2020).

Table 3.7 The T<sub>21</sub> and T<sub>22</sub> relaxation times (ms) of uncooked and cooked beef meat

	T <sub>21</sub>		T <sub>22</sub>	
Tenderization Type	I∃ncooked	Cooked	Uncooked	Cooked
C	$37.07^{aB} \pm 2.63$	18.80 <sup>bC</sup> ±1.23	104.00 <sup>aC</sup> ±3.54	72.13 <sup>bB</sup> ±10.51
$\mathbf{DW}$	$42.61^{aB}\pm4.33$	$17.23^{bC} \pm 1.24$	$281.27^{aA} \pm 8.95$	$76.17^{bB} \pm 2.25$
AA	132.27 <sup>aA</sup> ±12.46	$37.08^{bB} \pm 1.56$	132.27 <sup>aBC</sup> ±12.46	89.49 <sup>bB</sup> ±12.56
CA	142.35 <sup>aA</sup> ±1.77	$67.78^{\text{bA}} \pm 5.76$	123.90 <sup>aBC</sup> ±24.30	$167.50^{aA} \pm 17.30$
LA	149.15 <sup>aA</sup> ±13.79	$79.89^{bA} \pm 9.70$	$160.40^{aB} \pm 21.80$	$148.80^{aA} \pm 10.18$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

T<sub>22</sub> values of acid-treated in the uncooked samples were significantly longer than the control sample and shorter than the DW samples (p<0.05). T<sub>22</sub> was attributed to free water within the protein matrix. Therefore, it can be concluded that acid tenderized meat samples had more free water than others which was also confirmed my marinate uptake. The longer relaxation times of acidic solutions with high [H<sup>+</sup>] concentrations could have also contributed to the longer relaxation times. On the other hand, the uncooked DW sample had longer T<sub>22</sub> values despite insignificant marinate loss. This was a clear indication that in DW sample; salts within the matrix leached out and resulted in longer relaxation times (Duflot et al., 2019). The higher WBS force values for DW samples could also be explained with this. With the release of salt ions, the proteins could aggregate more and result in a tougher network (Zheng et al., 2019).

In the cooked samples, the  $T_{22}$  value of AA sample was found to be similar with C sample (p>0.05). When the effects of the cooking process on the samples were examined, it was determined that the  $T_{22}$  values of CA and LA did not change significantly (p>0.05). As explained in monoexponential  $T_2$  results, it was clear that CA and LA had affected the matrix differently and the free water was more dominant in these samples compared to AA samples. This is expected to result in different sensory perceptions on the final samples. pH, WHC values of CA and LA samples were also different than the AA samples in uncooked meats as explained before. Thus, the trend was consistent with the other experiments as well.

## 3.1.2 Use of Meat Tenderization Solution (MTS)

Red meat was tenderized at different times with a commercial meat tenderization solution that is basically a protease enzyme mix. The effects of tenderization time on the samples were determined before and after the cooking process.

## 3.1.2.1 pH

The pH values of the tenderized meat at different times were measured. For uncooked samples, no pH change was observed with time. pH values were around 6.00±0.15. For cooked samples, there was a slight increase in the pH and pH of cooked samples was approximately 6.20±0.09 with no change in time.

# 3.1.2.2 Water Holding Capacity

The methodology used for WHC determination resulted in negative values which was an indication that with the used experimental protocol no water was released after centrifugation both for cooked and uncooked samples. It was probable that 0.6 M NaCl used in the experiments were not sufficient to cause dehydration on the samples (Puolanne & Peltonen, 2013). However, to be consistent with acidic marination experiment the concentration was not changed. Nevertheless, the results were clearly indicating that enzymatic tenderization changed the meat structure significantly as will be discussed in the SEM images. The reason for this sample could be explained with shrinkage of muscle fiber resulting in decrease in the immobile water, and the degradation of meat protein causing a loss in water holding capacity of the proteins (Cheng et al., 2020; Maqsood et al., 2018).

## 3.1.2.3 Warner-Bratzler Shear Force Analysis

The Warner-Bratzler shear force (N) values of the meat sample exposed to enzymatic tenderization before and after cooking are given in Table 3.8. It was concluded that enzymatic tenderization for 20 minutes and longer than 20 minutes decreased WBS force of uncooked samples (p<0.05). The effect of the enzymatic tenderization on WBS force meat has already been proved (Maqsood et al., 2018; Mendiratta et al., 2010; Moon, 2018). Decrease in WBS force with enzymatic tenderization resulted in protein extractability and collagen solubility (Mendiratta et al., 2010).

On the other hand, the WBS values of cooked samples tenderized at different times were lower than the control samples (p<0.05). At the same time, no significant difference was found between the hardness values of the treated samples (p>0.05). The study of Yusop et al. (2010) found similar results in which the toughness of the meat samples did not change significantly with time.

Table 3.8 WBS force (N) of beef meat treated with enzymatic solution at different time

Tenderization Time (min)	Uncooked	Cooked
0 (control)	$56.16^{aA} \pm 6.69$	$49.39^{aA} \pm 5.85$
10	$50.64^{aA} \pm 7.63$	$33.14^{bB}\pm4.67$
20	$29.12^{aB}\pm3.02$	$34.77^{aB} \pm 5.42$
30	$28.83^{aB} \pm 6.14$	$28.10^{aB} \pm 4.53$
40	$24.16^{bB} \pm 1.46$	$35.60^{aB} \pm 3.97$
50	$30.16^{bB} \pm 1.81$	$36.31^{aB}\pm3.79$
60	$29.62^{aB}\pm3.48$	$27.59^{aB} \pm 4.12$
1440 (24 hours)	$28.82^{aB} \pm 3.64$	$29.38^{aB} \pm 1.27$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-B), are significantly different each sample (p<0.05)

#### 3.1.2.4 Color Measurements

The color values of the samples tenderized in the commercial enzyme solution at different times were measured, as seen in Table 3.9. The L\* values of the sample tenderized for 24 hours decreased at the end of 24 hours (p<0.05). On the other hand, for the cooked sample, the L\* values were all similar to each other statistically, (p>0.05). According to these results, it was concluded that prolonged enzymatic tenderization caused an increase in the darkness of the meat. Besides, when a\* values are examined, 24-hours tenderizing decreased the a\* values in the uncooked samples (p<0.05). Significant difference was found between the a\* values of the cooked samples (p<0.05). It is important to highlight that color values were not affected drastically as was observed in acidic tenderization. It was reported that after dipping

5 hours, the enzyme solution caused fading of meat color due to release of myoglobin from meat into enzyme solutions (Gerelt et al., 2000).

Furthermore, it was determined that  $b^*$  values of the sample tenderized enzymatically in uncooked or cooked samples were like the control sample statistically (p>0.05). The  $a^*$  and  $b^*$  values of meat did not change after treating with the enzyme solution.

Table 3.9 L\*, a\* and b\* values of beef meat treated with enzymatic solution at different time

	*			a*	*q	*
Tenderization Time (min)	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	-49.82 <sup>aB</sup> ±0.82	$-48.35^{aA}\pm0.63$	$11.17^{aA}\pm1.53$	7.07 <sup>bA</sup> ±0.30	$10.44^{aA}\pm 1.66$	$11.98^{aAB}\pm0.47$
10	$-51.41^{aB}\pm037$	$-48.49^{aA}\pm4.53$	$11.03^{aA}\pm0.88$	$4.72^{bC}\pm0.68$	$11.34^{aA}\pm0.31$	$12.00^{aAB}\pm1.68$
20	$-50.46^{\mathrm{aB}}\pm0.46$	$-49.64^{\text{Aa}}\pm1.76$	$11.62^{aA}\pm0.16$	$5.06^{\mathrm{bBC}}\pm0.86$	$11.62^{aA}\pm0.34$	$12.08^{\text{aAB}}\pm0.69$
30	$-50.86^{aB}\pm2.77$	$-51.78^{aA}\pm 2.82$	$10.02^{aA}\pm1.16$	$4.24^{\text{bC}}\pm0.14$	$9.37^{aA}\pm0.14$	$10.48^{aB}\pm1.03$
40	$-51.30^{aB}\pm1.15$	$-48.43^{aA}\pm 2.83$	$9.71^{aA}\pm0.83$	$5.66b^{ABC} \pm 0.24$	$9.90^{\text{bA}}\pm0.21$	$13.55^{aA}\pm0.76$
20	$-51.25^{aB}\pm1.98$	$-51.51^{aA}\pm1.19$	$9.10^{\mathrm{aA}}\pm0.18$	$5.68^{\mathrm{bABC}}\pm0.19$	$10.15^{\mathrm{aA}}\pm0.78$	$11.63^{\mathrm{aAB}}\pm0.84$
09	$-51.03^{aB}\pm3.08$	$-51.95^{aA}\pm0.32$	$9.60^{aA}\pm0.73$	$7.05^{\mathrm{bA}}\pm0.45$	$10.14^{aA}\pm0.99$	$10.78^{\text{aAB}}\pm0.33$
1440	$-39.36^{aA}\pm0.29$	$-50.89^{\mathrm{bA}}\pm4.05$	$4.74^{\mathrm{aB}}\pm0.90$	$6.63^{\mathrm{aAB}}\pm1.04$	$9.92^{aA}\pm1.03$	$11.45^{aAB}\pm1.74$

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05) Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

# 3.1.2.5 Monoexponential Analysis of TD-NMR Relaxometry Measurements

 $T_2$  values of the enzymatic tenderized samples are given in Table 3.10. All treated samples at different times in the uncooked and cooked meat were similar to the control sample (p>0.05). Thus, monoexponential  $T_2$  analysis results were not sufficient to explain the water-solvent interactions in the samples.

Table 3.10 T<sub>2</sub> values (ms) of the cooked and uncooked beef meat treated with enzymatic solution at different time

Tenderization Time (min)	Uncooked	Cooked
0 (control)	$44.27^{aAB}\pm2.18$	31.24 <sup>bA</sup> ±2.88
10	$45.39^{aAB} \pm 4.43$	$25.70^{\text{bAB}} \pm 1.94$
20	$40.56^{aB}\pm1.92$	$22.61^{\text{bB}} \pm 1.87$
30	$42.97^{aAB}\pm2.63$	$24.39^{bB} \pm 1.83$
40	$48.23^{aA}\pm1.75$	$26.71^{\text{bAB}} \pm 2.44$
50	$45.23^{aAB}\pm1.21$	$24.45^{\text{bB}} \pm 0.34$
60	$43.06^{aAB}\pm2.30$	$26.11^{\text{bAB}} \pm 2.05$
1440 (24 hours)	$43.09^{aAB} \pm 0.96$	$27.23^{\text{bAB}} \pm 2.04$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-B), are significantly different each sample (p<0.05)

## 3.1.2.6 Biexponential Analysis of TD-NMR Relaxometry Measurements

 $T_{21}$  and  $T_{22}$  values of meat samples before and after cooking are in Table 3.11. The results show that tenderization at different times caused significant differences in  $T_{21}$  values of uncooked and cooked samples (p<0.05). Both relaxation times decreased with cooking as observed in acidic tenderization.  $T_{21}$  has been described as the protons attached closely to the polar groups in protein. Except 10 min soaking time there was not a significant difference on these values (p<0.05). The abrupt decrease at 10 min could be explained by dehydration of the sample. After 10 min soaking time, enzyme became more active, proteins hydrolyzed and protons associated with the peptides become more mobile due to decrease in molecular weight (Deng et al.,

2020). On the other hand, for the slow relaxing component,  $T_{22}$  values increased after 40 minutes in cooked and uncooked samples (p<0.05) which can be explained with the same reasoning.

Table 3.11  $T_{21}$  and  $T_{22}$  values (ms) of the cooked and uncooked beef meat treated with enzymatic solution at different time

	T <sub>21</sub>		T <sub>22</sub>	
Tenderization Time (min)	Uncooked	Cooked	Uncooked	Cooked
0 (Control)	$33.67^{aC} \pm 4.23$	10.29 <sup>bB</sup> ±1.04	$54.00^{aB} \pm 5.76$	39.11 <sup>aBC</sup> ±4.85
10	$22.56^{aD} \pm 0.52$		.,,	$31.15^{bC} \pm 3.62$
20	39.67 <sup>aABC</sup> ±0.17	$11.02^{\text{bB}} \pm 0.54$	$41.96^{aB}\pm3.96$	$26.84^{bC} \pm 3.85$
30	43.34 <sup>aA</sup> ±3.73	$11.08^{\text{bB}} \pm 1.45$	$43.45^{aB} \pm 2.63$	$30.91^{bC} \pm 4.73$
40	$41.63^{aAB} \pm 1.28$	$17.17^{\text{bA}} \pm 0.82$	116.37 <sup>aA</sup> ±8.92	$56.57^{\text{bAB}} \pm 4.11$
	39.24 <sup>aABC</sup> ±0.71			$57.29^{bA} \pm 3.98$
	$38.65^{aABC} \pm 0.99$			$60.98^{bA} \pm 4.59$
1440 (24 hours)	36.38 <sup>aBC</sup> ±0.82	17.33 <sup>bA</sup> ±1.51	124.43 <sup>aA</sup> ±6.29	67.73 <sup>bA</sup> ±10.63

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

## 3.1.3 *In Vitro* Digestion for Beef Meat

## 3.1.3.1 Warner-Bratzler Shear Force Measurement

The effect of the *in vitro* digestion on the meat toughness was investigated, and the results are given in Table 3.12. These experiments were only performed for cooked samples. Besides, it is important to mention that as it is done in most *in vitro* studies; 'chewing action' is not considered. So, the results have been evaluated from that context.

It was observed that there were significant differences in hardness values of the samples following digestion (p<0.05). When the effect of *in vitro* digestion on each sample was examined, it was found that digestion decreased the hardness values of

the C, DW and AA samples (p<0.05). A similar result was stated in the study of Mennah-Govela & Bornhorst (2016) for sweat potatoes after *in-vitro* digestion. They noted that the hardness of the potato decreased after digestion. The reason for this decrease in WBS force values was explained by the degradation of the myofibrillar tissue during *in vitro* digestion (Patel & Welham, 2013).

On the other hand, the WBS force of CA and LA increased after the digestion. For the hydrolysis of meat, the gastric juice must diffuse into the food matrix (Mennahgovela et al., 2020). Therefore, the acidic treatment may have caused changes on the surface of the meat and on meat proteins and could have resulted in a barrier for the enzyme to penetrate and digest. It is also interesting to note that WBS force MTS did not change after digestion (p>0.05). AA samples also had the lowest WBS values following digestion compared to other tenderization treatments.

Table 3.12 The WBS values (N) of beef meat before and after in vitro digestion

Tenderization Type	Undigested	Digested
C	51.81 <sup>aA</sup> ±7.23	$20.32^{\text{bDE}} \pm 2.83$
$\mathbf{DW}$	$49.81^{aA}\pm6.56$	$27.83^{\text{bBC}} \pm 3.29$
$\mathbf{A}\mathbf{A}$	$19.27^{aB} \pm 2.51$	$15.63^{\text{bE}} \pm 2.30$
CA	$21.27^{bB} \pm 2.27$	$35.01^{aA}\pm1.90$
LA	$9.20^{bC} \pm 1.22$	$32.05^{aAB} \pm 3.31$
MTS	$29.38^{aB}\pm1.27$	$25.65^{\text{aCD}} \pm 3.04$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-D), are significantly different each sample (p<0.05)

# 3.1.3.2 Biexponential Analysis of TD-NMR Relaxometry Measurements

The data for the samples exposed to digestion did not fit well for mono exponential model. Therefore, this time just the biexponential results are discussed. Table 3.13 shows the NMR biexponential function ( $T_{21}$  and  $T_{22}$ ) values of red meat samples before and after *in vitro* digestion.

There were significant differences of  $T_{21}$  between the samples before and after digestion for all sample (p<0.05) which was expected due to the pH changes exposed during digestions. For all sample except the enzyme treatment,  $T_{21}$  values decreased. The decrease can be explained by the digestion of myofibrillar protein resulting in the shrinkage and letting water out from the matrix. Therefore, there was no space in the myofibrillar structure for water to be entrapped, and migration of water molecules from myofilament space to exterior of cellular space (Mazaheri Kalahrodi et al., 2021). Since MTS samples are already hydrolyzed samples the further protease activity with digestion enzymes could have resulted an increase in the myofibrillar water and therefore an increase in the relaxation times.

Table 3.13 T<sub>21</sub> and T<sub>22</sub> values (ms) of undigested and digested beef meat

	T <sub>21</sub>		T <sub>22</sub>	
Tenderization Type	Undigested	Digested	Undigested	Digested
C	18.80 <sup>aC</sup> ±1.23	15.20 <sup>bAB</sup> ±0.52	$72.13^{aB} \pm 10.18$	43.29 <sup>bB</sup> ±5.58
$\mathbf{DW}$	$17.23^{aC} \pm 1.97$	$13.03^{bB} \pm 0.80$	$76.17^{aB} \pm 2.25$	$48.62^{\text{bAB}} \pm 7.35$
$\mathbf{A}\mathbf{A}$	$37.08^{aB} \pm 1.56$	$23.20^{bA} \pm 5.98$	$89.49^{aB} \pm 12.56$	$66.45^{aA} \pm 6.75$
CA	67.78 <sup>aA</sup> ±5.76	$14.23^{\text{bAB}} \pm 4.38$	$167.50^{aA} \pm 17.30$	$50.57^{\text{bAB}} \pm 6.94$
LA	$79.89^{aA} \pm 9.70$	$13.34^{bB} \pm 1.55$	$148.80^{aA} \pm 10.18$	$49.77^{\text{bAB}} \pm 1.49$
MTS	$11.61^{bC} \pm 0.28$	$14.11^{aB} \pm 0.62$	$39.26^{bC} \pm 2.14$	$55.30^{aAB} \pm 3.90$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-D), are significantly different each sample (p<0.05)

When T<sub>22</sub> times were examined, it was observed that no distinct difference existed among the samples. Since this relaxation component is more related to free water; it is probable that the digestive juices governed the protein matrix and resulted in a similar relaxation time behavior.

# 3.1.3.3 Total Soluble Protein and Free Amino Group Analysis after Digestion

The quality of meat protein is determined by its content and quantity of essential amino acids, as well as the ability of the body to absorb amino acids and peptides during digestion (Scudero & Iguel, 2010). Therefore, to understand the effect of the tenderization type on the digestibility of meat after *in vitro* digestion, the solubility of protein hydrolyzed and passed to digestive juice was investigated. The total soluble protein contents of the digestive juices (mg/mL) of the sample are given in Table 3.14. After *in vitro* digestion, the highest amount of total soluble protein was determined in the samples tenderized with MTS (p<0.05).

Increase in the total soluble protein content in the enzyme treated samples might have explained by the increased the permeability of myofibrillar proteins (Gokoglu et al., 2017). This is also not surprising result since with the digestive juices; the samples have been exposed to a 2<sup>nd</sup> protease treatment. A similar result was obtained for enzyme treatment of squid meat (Gokoglu et al., 2017); hen meat (Naveena & Mendiratta, 2001). For acidic tenderization, no change on the soluble protein content was observed with respect to control samples.

Table 3.14 Total soluble protein content (mg/mL) of beef meat digestion juice

Tenderization Type	Total soluble protein content
C	$2.835^{BC} \pm 0.110$
$\mathbf{DW}$	$2.958^{\mathrm{B}} \pm 0.085$
$\mathbf{A}\mathbf{A}$	$2.670^{\circ} \pm 0.062$
CA	$2.844^{\mathrm{BC}} \pm 0.051$
$\mathbf{L}\mathbf{A}$	$2.759^{BC} \pm 0.107$
MTS	$4.9018^{A} \pm 0.116$

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

Since the digested proteins could not be all soluble and determined with the Lowry agent, a 2<sup>nd</sup> test was performed to understand the extent of digestion Free amino group can be also used to provide information about the digestibility of meat

tenderized with different solutions (Zhou et al. (2021). The free amino group contents (mg/mL) in the digestion juices are given in Table 3.15. The results showed that CA and MTS treated samples were higher than the control in the free amino group (p<0.05). Besides, the control and DW had the lowest free amino group. Citric acid tenderized samples were followed by acetic acid ones. In overall, results showed that except LA all-tenderization methods increased the digestibility of the meat samples. The reason why LA did not work as good as other acids should further be explored.

Table 3.15 Free amino group (mg/mL) of beef meat digestion juice

Tenderization Type	Free amino group
$\overline{\mathbf{C}}$	1.2968 <sup>CD</sup> ±0.1221
$\mathbf{DW}$	$1.2011^{\text{CD}} \pm 0.0327$
$\mathbf{A}\mathbf{A}$	$1.3243^{\mathrm{C}} \pm 0.0277$
CA	$1.5574^{\mathrm{B}} \pm 0.0530$
LA	$1.1426^{\mathrm{D}} \pm 0.0464$
MTS	$1.8686^{A} \pm 0.0519$

Means within the same column, followed by the different capital letters (A-D), are significantly different each sample (p<0.05)

# 3.1.3.4 Morphological Characterization through Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used for detecting the changes in meat texture caused by acidic and enzymatic tenderizations.

The SEM image of C sample is given in Fig 3.1. When the C sample was examined, it is seen that the muscle fibers were arranged in an orderly manner. It is also noteworthy that there are long refraction-like gaps in the image. These voids may have occurred due to the expansion of some components such as oil, water, or air (Salcedo-sandoval et al., 2013). The post-digestion control sample showed a completely different morphology. The sample appears as an elongated plate or fiber. This may be due to digestion enzymes breaking down the proteins.

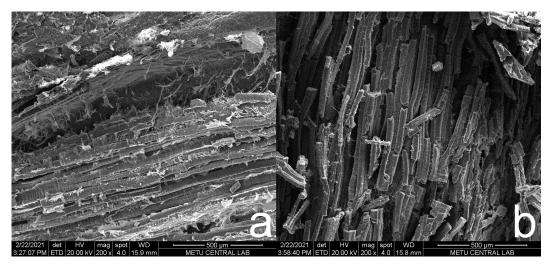


Figure 3.1. SEM images of C samples. Cooked (a) and digested (b)

Besides, when the sample's morphology DW, it is seen in Fig 3.2 that, indentations and protrusions were observed after cooking. It has also void and fractured appearance as C samples. After the digestion, the gaps in the structure increased, and it seemed to gain a larger particle size.

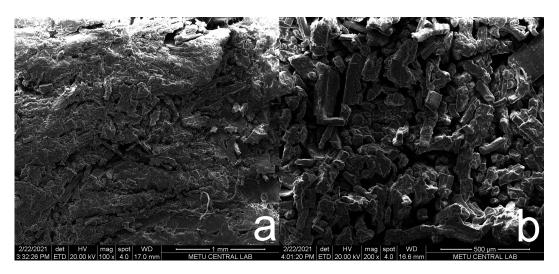


Figure 3.2. SEM images of DW sample. Cooked (a) and digested (b)

In Fig 3.3, the SEM image of the AA sample after cooking and digestion are showed. The image of the AA sample after cooking shows a flat structure in general, although there are various large or small gaps in certain spots. It is obvious that both cooking and acetic acid affected the proteins. AA samples had a less rough and flatter

appearance than the control sample. Following digestion, sample became rougher and more indented.

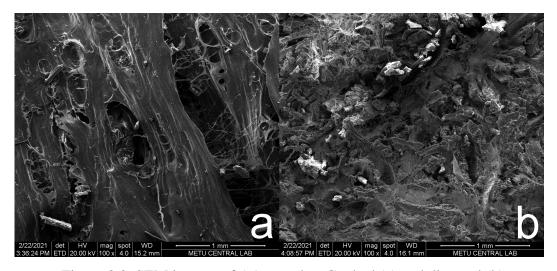


Figure 3.3. SEM images of AA samples. Cooked (a) and digested (b)

The SEM images of CA are given in Fig 3.4 and The CA of cooked sample was similar to AA samples. The structure of the cooked sample tenderized with citric acid is flat and perforated; however, after *in vitro* digestion, the sample turned into a rough structure with lumps and clusters of different sizes and structures. It is seen that there are retiform-shaped structures in some parts of the sample. With all these changes, it was clearly seen that both the type of acid during the tenderization and the enzymes in the digestive juice had tremendous effects on the proteins.

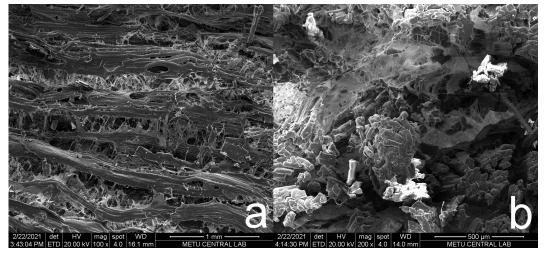


Figure 3.4. SEM images of CA samples. Cooked (a) and digested (b)

From Fig 3.5 it can be said that the surface of the cooked LA samples showed a more complex, uneven, and fragmented presence than the AA and CA samples. Small, dense, and fragmented structures drew attention after *in vitro* digestion of samples with lactic acid treatment.

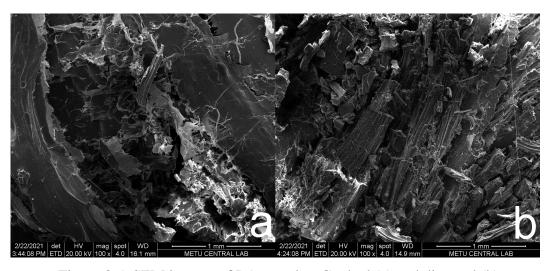


Figure 3.5. SEM images of LA samples. Cooked (a) and digested (b)

Besides, cooked and digested meat of SEM images are given in Fig 3.6. The sample tenderized with an enzyme solution consisted of thin, long, adjacent fiber-like forms. The enzyme solution seemed to result in a much different structure to the red meat sample than the acidic tenderization. Enzyme treatment forced opened and flattened the structures in the sample. It was clear that digestion caused fiber structure to decrease in size and increased the space between the fibers. Qihe et al. (2006) stated that they observed a great deformation and fragmentation in the image of the elastase enzyme treated beef. The morphological image obtained by tenderization using elastase enzyme was very similar to the fibrous image obtained with the enzyme solution in this study.

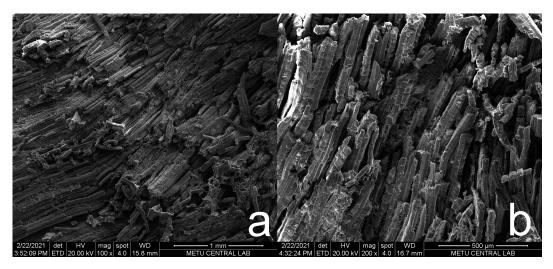


Figure 3.6. SEM images of MTS samples. Cooked (a) and digested (b)

## 3.2 Chicken Meat Tenderization

In addition to red meat tenderization, acidic and enzymatic tenderization was also applied to white meat, and *in vitro digestion* process was applied to the cooked samples. For the enzymatic and acidic tenderization, commercial meat tenderization solution and acetic acid were used, respectively. The chicken breast meat was tenderized with acetic acid and MTS for 24hrs. The reason of the using only acetic acid in this part of experiments was that the lowest WBS force value of cooked samples was achieved with this acid.

The pH values of the AA samples before and after cooking were determined to be lower than other samples (p<0.05). It is an expected result that the pH values of the samples will decrease as acid solutions penetrate the meat. Besides, the cooking affected the pH of the sample, except for AA tenderization. Denaturation of the proteins ad unfolding could have an effect on this change (Pippen et al., 1965). In control, DW and MTS samples no change was observed before and after cooking (p>0.05).

Table 3.16 pH values of chicken meat

Tenderization Type	Uncooked	Cooked
C	$6.2567^{\text{bA}} \pm 0.0451$	$6.5867^{aA} \pm 0.0611$
$\mathbf{DW}$	$6.1667^{\mathrm{bA}} \pm 0.0116$	$6.6567^{aA} \pm 0.1124$
$\mathbf{A}\mathbf{A}$	$4.1933^{aB} \pm 0.0586$	$4.0533^{aB} \pm 0.0737$
MTS	$6.2600^{\text{bA}} \pm 0.1212$	$6.5300^{aA} \pm 0.0100$

Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-B), are significantly different each sample (p<0.05)

## 3.2.1 Water Holding Capacity

When the WHC of the sample was investigated, only WHC of AA sample was detected before and after cooking. On the other hand, the WHC of other samples was negative so they were not reported. The acidic solution enabled the expansion of the cavities between myofibrillar due to electro-statistic repulsion and improved the interaction between myosin and water. Therefore, the ability to hold water was higher than other samples (Roldán et al., 2014). A similar result was found in the study of Ünal et al. (2020). It was found that that acetic acid tenderization increased the WHC of chicken breast meat.

### 3.2.2 Warner-Bratzler Shear Force

The WBS force results of acidic acid and enzymatically marinated chicken meat before and after cooking and after *in vitro* digestion are given in Table 3.17. The WBS force of AA samples were found to be the lowest before and after cooking (p<0.05). A similar result was obtained in the study of Zhang et al. (2020) where they studied the tenderization of chicken breast with acid.

It was also observed that the WBS of the DW and MTS samples were similar and the highest (p>0.05). As the stated in previous parts, the reason of these differences

between sample is that the WBS force depends on the pH and isoelectric point of the chicken meat, the isoelectric point of the chicken breast is around 5.5; therefore pH of meat near this point resulted in an increase on the WBS force values (Cercel et al., 2015).

The most interesting result obtained was the significantly higher WBS values of chicken meat with the enzyme solution compared to beef samples. This increase may be explained similar to DW. In other word, the pH of meat tenderized with enzyme was near the isoelectric point of the chicken meat protein, which caused increasing of the WBS force of the MTS sample. In addition to pH, there is also the buffer of the enzyme solution; therefore, the change has been more.

Acetic acid was observed to be more effective than enzymes in the tenderization of chicken breast since it showed the lowest value.

Digestion effected the WBS as well and following the digestion, the control and acetic acid tenderized samples were found to have the lowest hardness, while the DW was found to be the hardest one (p<0.05). Enzyme treated samples had lower WBS values.

Table 3.17 WBS force (N) values of uncooked, cooked, and digested chicken meat

Tenderization Type	Uncooked	Cooked	Digested
C	$13.61^{bB} \pm 1.93$	$18.58^{aC} \pm 1.72$	$9.86^{cC} \pm 0.91$
$\mathbf{DW}$	$17.09^{cAB} \pm 1.80$	$37.92^{aA}\pm3.61$	$33.75^{bA} \pm 2.95$
$\mathbf{A}\mathbf{A}$	$2.70^{\text{cC}} \pm 0.26$	$4.59^{bD} \pm 0.66$	$8.78^{aC} \pm 1.05$
MTS	$19.15^{\text{bA}} \pm 2.63$	$27.21^{aB}\pm3.39$	$25.82^{aB}\pm3.27$

Means within the same row, followed by the different small letters (a-c) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-D), are significantly different in each sample (p<0.05)

When the effect of cooking and *in vitro* digestion on each sample was investigated, the hardness of both C and DW treated samples increased after cooking, but again decreased after *in vitro* digestion (p<0.05). The reason for this reduction in WBS force was the degradation of the myofibrillar tissue during digestion (Patel &

Welham, 2013). The hardness of the AA increased after cooking but increased significantly after digestion (p<0.05). Although the hardness of the enzyme-treated sample increased after cooking (p<0.05), it did not change significantly after digestion (p>0.05). The increase in WBS was observed after cooking due to the decrease of water in the samples with the cooking since the denaturation of connective tissues and myofibrillar proteins caused changes in meat tenderness during cooking (Christensen et al., 2000).

## 3.2.3 Monoexponential Analysis of TD-NMR Relaxometry Measurements

The results of the cooking and *in vitro* digestion on the TD-NMR T<sub>2</sub> time values of meat samples with different types of tenderizations are given in Table 3.18. It was concluded that the T<sub>2</sub> values of the AA before cooking was higher than the other samples as observed in beef samples (p<0.05). The longer T<sub>2</sub> result was found to be reasonable and consistent with the hardness results as acetic acid solution denatured chicken meat protein at a higher rate and this could have which increased the T<sub>2</sub> of the sample because of the free water increase (p<0.05). The longer T<sub>2</sub> values was an indication that the sample had higher bulk water (Micklander et al., 2002). It is obvious the chicken samples had absorbed the marinade solution and the free water content increased. T<sub>2</sub> values of AA was found to be longer than the other samples after cooking and digestion (p<0.05).

Table 3.18 T<sub>2</sub> values (ms) of uncooked, cooked, and digested chicken meat

Tenderization Type	Uncooked	Cooked	Digested
C	$41.32^{aB} \pm 0.05$	$22.51^{cB} \pm 1.05$	$24.78^{bB} \pm 0.44$
$\mathbf{DW}$	$42.89^{aB} \pm 3.55$	$23.71^{bB} \pm 1.31$	$22.93^{\text{bBC}} \pm 2.14$
$\mathbf{A}\mathbf{A}$	$85.25^{aA} \pm 8.99$	$67.15^{aA}\pm8.80$	$41.86^{bA} \pm 0.88$
MTS	$46.01^{aB}\pm1.84$	$22.91^{bB}\pm0.38$	$20.05^{bC} \pm 1.00$

Means within the same row, followed by the different small letters (a-c) are significantly different each process (p<0.05)

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

The effects of the cooking process on the  $T_2$  of samples were investigated as well. It was observed that  $T_2$  values of all samples reduced dramatically after cooking except AA (p<0.05) since the amount of free water decrease in the sample during the cooking because the denaturation and shrinkage of protein structures (Kondjoyan et al., 2013). The sample treated with acid treatment did not lose bulk water during cooking since it already had higher WHC due water uptake.

In addition to cooking, the digestion also increased the  $T_2$  value of C sample, whereas  $T_2$  of acetic acid tenderized sample decreased (p<0.05). The reason for the change in  $T_2$  values of AA samples was that acid altered the meat proteins by the denaturation of myosin and shrinkage of the longitudinal muscle fibers occurred causing water loss (Micklander et al., 2002). However, there was no significant change in the  $T_2$  values after the digestion sample was kept in distilled water and tenderized with the enzyme solution (p>0.05). Biexponential analysis was not reported for the chicken samples.

Table 3.19 The L\*, a\* and b\* values of chicken meat tenderization of different solution before and after cooking

	Ľ	*	<i>a</i>	a*	7	$\mathbf{p}_{*}$
Tenderization Type	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
၁	-38.53bC±0.86	$-13.69^{aB}\pm0.16$	-0.20 <sup>bA</sup> ±0.02	$1.76^{aA}\pm0.06$	$9.51^{bA}\pm 1.28$	$15.69^{aB}\pm1.36$
DW	$-26.90^{bA}\pm1.75$	$-11.94^{aA}\pm0.53$	$-0.85^{\text{bB}}\pm0.06$	$1.79^{aA}\pm0.23$	$9.05^{bA}\pm1.08$	$15.00^{aB}\pm0.03$
AA	$-33.01^{\text{bB}}\pm0.84$	$-21.78^{aD}\pm0.80$	$-2.06^{aC}\pm0.09$	$-1.89^{aB}\pm0.71$	$1.97^{\text{bB}}\pm0.27$	$9.65^{\mathrm{aC}}\pm0.22$
MTS	$-28.90^{\text{bA}}\pm0.65$	$-15.95^{aC}\pm0.76$	$-0.68^{\text{bB}}\pm0.01$	$2.57^{\mathrm{aA}}\pm0.06$	$8.07^{\mathrm{bA}}\pm1.77$	$20.44^{aA}\pm0.63$

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05) Means within the same row, followed by the different small letters (a-b) are significantly different each process (p<0.05)

#### 3.2.4 Color Measurements

The result of the color experiment of uncooked and cooked samples was given in Table 3.19. It was found that while C had the lowest L\* values in uncooked samples, and DW had the highest after cooking (p<0.05). The similar result was found in the study of Smith & Young (2007) and it was reported that while L\* value of marinated samples decreased, a\* value of sample increased. The sample tenderized with acid had the lowest b\* values in uncooked and cooked samples (p<0.05). The cooking process affected the color of meat by discoloration, probably due to oxidation of heme group of color pigment (García-Segovia et al., 2007).

# 3.2.5 Total Soluble Protein and Free Amino Group Analysis after *in vitro*Digestion

The total soluble protein content values after *in vitro* digestion of the sample were determined by Lowry and OPA methods, and the results are given in Table 3.20. According to these results, the total soluble protein content of the MTS sample was found to be higher than the other samples (p<0.05). The total soluble protein content depends on myofibrillar protein content (Shin et al., 2008). Therefore, it was concluded that meat tenderization with enzymatic solution resulted in more protein degradation after digestion. In another study, a similar result was found: meat tenderized with papain solution had higher total soluble content than the control one (Kim et al., 2015).

Table 3.20 Total soluble protein content (mg/mL) of chicken meat digestion juice

Total soluble protein content
$2.6367^{\mathrm{B}} \pm 0.0670$
$2.4760^{\mathrm{B}} \pm 0.1780$
$2.886^{\mathrm{B}} \pm 0.1429$
$5.2870^{A}\pm0.2990$

Means within the same column, followed by the different capital letters (A-B), are significantly different each sample (p<0.05)

The free amino group released to meat from digestion juice was given in Table 3.21. The results showed that acidic and enzymatic tenderized meat values were significantly different from control (p<0.05). These highest results were again for the MTS sampled followed by the AA samples. Both acidic and enzymatic treatment increased the digestibility of chicken breast protein in terms of the free amino group due to the changes in meat protein structure during the tenderization. (Berge et al., 2001; Fernández-Lucas et al., 2017). Moreover, the protein unfolding provides accessibility of the protease enzyme by making the hidden cleavage side of the meat proteins available, thus improving digestibility (Yin et al., 2020).

Table 3.21 Free amino group (mg/mL) of chicken meat digestion juice

Tenderization Type	Free amino group
$\mathbf{C}$	$1.3668^{\mathrm{C}} \pm 0.0668$
$\mathbf{DW}$	$1.4324^{\text{C}} \pm 0.0599$
$\mathbf{A}\mathbf{A}$	$1.7037^{\mathrm{B}} \pm 0.0760$
MTS	$2.6096^{A} \pm 0.0720$

Means within the same column, followed by the different capital letters (A-C), are significantly different each sample (p<0.05)

#### **CHAPTER 4**

### **CONCLUSION**

Meat is tenderized to make it more palatable. However, the studies on how the changes in palatability effect digestibility still needs investigation. In this thesis a comprehensive analysis was carried out to examine different tenderization methods and their effect on digestion.

Beef and chicken breast samples were tenderized with acid (acetic acid, citric acid, and lactic acid) and enzyme solutions. The effects of these processes were investigated before and after cooking. In addition, the cooked samples were exposed to *in vitro* digestion fluids to examine how the tenderization type affected the product's digestibility. The samples were also investigated through textural properties, color, water holding capacity (WHC), NMR relaxation times (T<sub>2</sub>), morphological properties and total soluble protein content, and a free amino group analysis.

In the first part of the study, it was concluded that the acidic solution decreased the WBS force of the meat samples while cooking process increased the hardness. Because of the denaturation of the meat protein at low pH, water was released then it resulted in longer T<sub>2</sub> values of tenderized samples. The tenderization process affected the pH, WHC, and color of the sample due to marinate uptake.

In the second part of the study, beef was tenderized with commercial meat tenderization solution (MTS) at different times. The results showed that MTS decreased the hardness of the sample after 20 minutes as the proteolytic enzyme in the solution caused degradation of the protein in meat. Besides, the pH and color of the sample changed. While L\* (lightness) values increased the a\* (redness) values decreased in the sample tenderized for 24 hours.

In the third part of the study, the digestion of treated samples took place, and the effects of digestion on the meat sample were investigated. When the hardness of the sample was examined, the hardness of the control and DW samples decreased after digestion. Besides, the results showed that the tenderization increased meat's digestibility. The SEM results also showed that there was a difference between uncooked and cooked samples.

In the last part of the study, the chicken breast was tenderized with acetic acid solution and MTS, and it was digested. The hardness of meat was examined, and the sample tenderized with acid had the lowest hardness in both cooked and uncooked meat. Furthermore, the acidic-treated sample were the longer T<sub>2</sub> values since degradation of meat protein occurred due to low pH, which resulted in the release of water molecules. In addition, the tenderization increased the digestibility of the chicken breast like beef in terms of total soluble protein and a free amino group.

Results showed that acidic marination specifically acetic acid marination had significant effects on the physicochemical properties and on the digestion behavior. MTS solution also improved the physical properties significantly and was shown to have an effect on digestion. As a further study, it would be good to examine how acetic concentration would change the properties and digestion and how different crude enzyme mixes will affect the digestion. Understanding the effect different processes such as ultrasound, high hydrostatic pressure as marination methods and their effect on digestibility can be a further topic to elaborate.

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# **APPENDICES**

# A. Calibration Curves

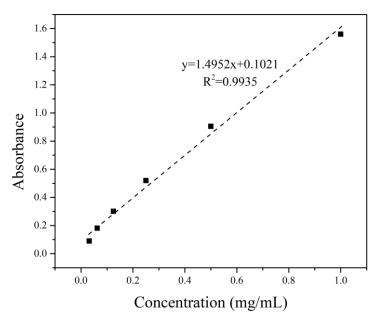


Figure 4.1. Calibration curve of Lowry method prepared by Bovine Serum Albumin (BSA)

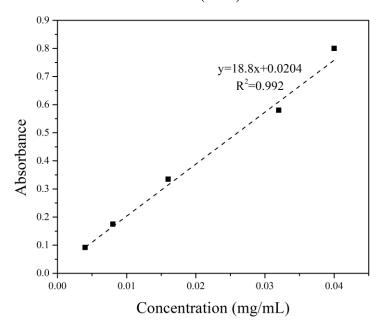


Figure 4.2. Calibration curve of OPA method prepared by glycine

# **B.** Statistical Analyses

Table 4.1 Table 4.2 ANOVA and Tukey's Comparison Test with 95% confidence level for red meat tenderized with acidic solution

#### **UNCOOKED**

## One-way ANOVA: WBS (N) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	6553.48	1638.37	492.88	0.000
Error	20	66.48	3.32		
Total	24	6619.96			

# **Model Summary**

<b>S</b>	R-sq	R-sq(adj)	R-sq(pred)
1.82321	99.00%	98.79%	98.43%
$Pooled\ StDev = 1.82321$			

#### **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
DW	4	50.94 A		
NT	6	21.02	В	
AA	4	6.882	C	
LA	5	5.942	C	
CA	6	5.230	C	

Means that do not share a letter are significantly different.

#### **COOKED**

# One-way ANOVA: WBS (N) versus Tenderization Type

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	9641.8	2410.46	95.29	0.000
Error	24	607.1	25.29		
Total	28	10248.9			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.02940	94.08%	93.09%	91.76%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
NT	6	51.81 A		
DW	8	49.81 A		
CA	3	21.97	В	
AA	5	19.27	В	
LA	7	9.200	C	

# CONTROL

# One-way ANOVA: Hardness (N) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	2844.7	2844.69	94.79	0.000
Error	10	300.1	30.01		
Total	11	3144 8			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.47808	90.46%	89.50%	86.26%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	6	51.81 A	
U	6	21.02	В

Means that do not share a letter are significantly different.

#### DW

## One-way ANOVA: Hardness (N) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.360	3.360	0.10	0.754
Error	10	322.881	32.288		
Total	11	326.241			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.68226	1.03%	0.00%	0.00%

#### **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	4	50.94 A	_
C	8	49.81 A	

Means that do not share a letter are significantly different.

#### AA

# One-way ANOVA: Hardness (N) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	341.00	341.000	90.23	0.000
Error	7	26.45	3.779		
Total	8	367.45			

S	R-sq	R-sq(adj)	R-sq(pred)
1.94400	92.80%	91.77%	88.67%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	5	19.27 A	
U	4	6.882	В

Means that do not share a letter are significantly different.

#### CA

## One-way ANOVA: Hardness (N) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	560.23	560.232	302.71	0.000
Error	7	12.96	1.851		
Total	8	573.19			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.36042	97.74%	97.42%	95.29%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	21.97 A	
U	6	5.230	В

Means that do not share a letter are significantly different.

## LA

## One-way ANOVA: Hardness (N) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	30.96	30.959	27.70	0.000
Error	10	11.17	1.117		
Total	11	42.13			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.05710	73.48%	70.83%	62.82%

# **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	7	9.200 A	
U	5	5.942	В

Means that do not share a letter are significantly different.

#### UNCOOKED

## One-way ANOVA: T2 (ms) versus Tenderization Type

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	34102	8525.5	42.59	0.000
Error	10	2002	200.2		
Total	14	36104			

 S	R-sq	R-sq(adj)	R-sq(pred)
14.1491	94.45%	92.24%	87.52%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
LA	3	161.2 A		
AA	3	140.66 A		
CA	3	125.8 A		
DW	3	55.90	В	
NT	3	41.04	В	

Means that do not share a letter are significantly different.

# COOKED

## One-way ANOVA: T2 (ms) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	20359.3	5089.83	52.19	0.000
Error	9	877.7	97.53		
Total	13	21237.1			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	9.87557	95.87%	94.03%	88.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
LA	2	121.8 A		
CA	3	117.52 A		
AA	3	81.58	В	
DW	3	35.982	C	
NT	3	30.79	C	

Means that do not share a letter are significantly different.

## CONTROL

# One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	157.37	157.37	13.00	0.023
Error	4	48.42	12.11		
Total	5	205.79			

S	R-sq	R-sq(adj)	R-sq(pred)
3.47933	76.47%	70.59%	47.06%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	41.04 A	
C	3	30.79	В

Means that do not share a letter are significantly different

## DW

## One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	594.8	594.82	16.12	0.016
Error	4	147.6	36.90		
Total	5	742.4			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
6.07429	80.12%	75.15%	55.27%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping	
U	3	55.90 A		
C	3	35.982	В	

Means that do not share a letter are significantly different.

#### AA

## One-way ANOVA: T2 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	5236.6	5236.6	33.74	0.004
Error	4	620.8	155.2		
Total	5	5857.4			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
12,4575	89.40%	86.75%	76.15%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	140.66 A	
C	3	81.58	В

Means that do not share a letter are significantly different.

#### CA

## One-way ANOVA: T2 (ms) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	101.7	101.7	0.49	0.523
Error	4	833.6	208.4		
Total	5	935.3			

S	R-sq	R-sq(adj)	R-sq(pred)
14.4359	10.88%	0.00%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	125.8 A	
C	3	117.52 A	

Means that do not share a letter are significantly different.

## LA

## One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1866	1865.8	4.55	0.123
Error	3	1229	409.8		
Total	4	3095			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
20.2432	60.28%	47.04%	0.00%

## **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	${f N}$	Mean Grouping
U	3	161.2 A
C	2	121.8 A

 ${\it Means that do not share a letter are significantly different.}$ 

#### UNCOOKED

## One-way ANOVA: T21 (ms) versus Tenderization Type

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	32795.5	8198.87	118.11	0.000
Error	8	555.4	69.42		
Total	12	33350.8			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
8.33180	98.33%	97.50%	95.24%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
LA	2	149.15 A	
CA	2	142.35 A	
AA	3	132.27 A	
DW	3	42.61	В
NT	3	37.07	В

## UNCOOKED

## One-way ANOVA: T22 (ms) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	54570	13642.6	53.91	0.000
Error	8	2025	253.1		
Total	12	56595			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	15.9086	96.42%	94.63%	90.08%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grou	ping	
DW	3	281.27 A			
LA	3	160.4	В		
AA	3	132.27	В	C	
CA	2	123.9	В	C	
NT	2	104.00		C	

Means that do not share a letter are significantly different.

#### **COOKED**

## One-way ANOVA: T21 (ms) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	8392.5	2098.12	110.13	0.000
Error	9	171.5	19.05		
Total	13	8563.9			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
4.36471	98.00%	97.11%	93.57%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
LA	2	79.89 A	
CA	3	67.78 A	
AA	3	37.077	В
NT	3	18.797	C
DW	3	17.227	C

Means that do not share a letter are significantly different.

## COOKED

## One-way ANOVA: T22 (ms) versus Tenderization Type

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	21878	5469.5	39.35	0.000
Error	9	1251	139.0		
Total	13	23129			

 S	R-sq	R-sq(adj)	R-sq(pred)
11.7899	94.59%	92.19%	87.05%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
CA	3	167.5 A	
LA	2	148.80 A	
AA	3	89.49	В
DW	3	76.17	В
NT	3	72.13	В

Means that do not share a letter are significantly different.

## **CONTROL**

## One-way ANOVA: T21 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	501.05	501.055	118.49	0.000
Error	4	16.92	4.229		
Total	5	517.97			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.05640	96.73%	95.92%	92.65%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping	
U	3	37.07 A		
C	3	18.797	В	

Means that do not share a letter are significantly different.

## CONTROL

## One-way ANOVA: T22 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1219.1	1219.09	15.67	0.029
Error	3	233.4	77.80		
Total	4	1452.5			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	8.82027	83.93%	78.58%	62.34%

## **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping
U	2	104.00 A	
C	3	72.13	В

DW

## One-way ANOVA: T21 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	966.47	966.47	95.33	0.001
Error	4	40.55	10.14		
Total	5	1007.02			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.18410	95.97%	94.97%	90.94%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	42.61 A	
C	3	17.227	В

Means that do not share a letter are significantly different.

#### DΜ

## One-way ANOVA: T22 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	63097.0	63097.0	1481.02	0.000
Error	4	170.4	42.6		
Total	5	63267.4			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
6.52714	99.73%	99.66%	99.39%

## **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping	
U	3	281.27 A		
C	3	76.17	В	

Means that do not share a letter are significantly different.

#### AA

## One-way ANOVA: T21 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	13591.7	13591.7	172.25	0.000
Error	4	315.6	78.9		
Total	5	13907.3			

S	R-sq	R-sq $(adj)$	R-sq(pred)
8.88286	97.73%	97.16%	94.89%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean	Grouping
U	3	132.27 A	
C	3	37.077	В

Means that do not share a letter are significantly different.

#### AA

## One-way ANOVA: T22 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	2744.8	2744.8	17.53	0.014
Error	4	626.3	156.6		
Total	5	3371.1			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
12.5130	81.42%	76.78%	58.20%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	132.27 A	
C	3	89.49	В

Means that do not share a letter are significantly different.

## CA

## One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	6672.23	6672.23	288.12	0.000
Error	3	69.47	23.16		
Total	4	6741.70			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
4.81226	98.97%	98.63%	97.60%

# **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	142.35 A	
C	3	67.78	В

Means that do not share a letter are significantly different.

## $\mathsf{C}\mathsf{A}$

## One-way ANOVA: T22 (ms) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	2281	2281.2	5.74	0.096
Error	3	1192	397.5		
Total	4	3474			

 S	R-sq	R-sq(adj)	R-sq(pred)
19.9366	65.67%	54.23%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
C	3	167.5 A
U	2	123.9 A

Means that do not share a letter are significantly different.

## LA

## One-way ANOVA: T21 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	4796.9	4796.9	33.75	0.028
Error	2	284.2	142.1		
Total	3	5081.2			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
11.9215	94.41%	91.61%	77.62%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	149.15 A	
C	2	79.89	В

Means that do not share a letter are significantly different.

## LA

## One-way ANOVA: T22 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	161.5	161.5	0.46	0.546
Error	3	1053.2	351.1		
Total	4	1214.7			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
18.7366	13.29%	0.00%	0.00%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	160.4 A	
C	2	148.80 A	

# UNCOOKED

# One-way ANOVA: Lightness (L\*) versus Tenderization Type

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	446.44	111.611	54.82	0.000
Error	10	20.36	2.036		
Total	14	466 80			

## **Model Summary**

_	S	R-sq	R-sq(adj)	$\mathbf{R}$ -sq(pred)	
	1.42692	95.64%	93.89%	90.19%	

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
DW	3	-42.077 A	
AA	3	-48.207	В
CA	3	-49.44	В
LA	3	-55.930	C
NT	3	-56.980	C

Means that do not share a letter are significantly different.

#### **UNCOOKED**

## One-way ANOVA: Redness (a\*) versus Tenderization Type

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	106.84	26.709	12.52	0.005
Error	6	12.80	2.133		
Total	10	119.63			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.46047	89.30%	82.17%	73.29%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping		
NT	3	8.67 A			
DW	2	7.390 A	В		
CA	2	3.2400	В	C	
LA	2	2.295	В	C	
AA	2	0.8550		C	

Means that do not share a letter are significantly different.

#### UNCOOKED

## One-way ANOVA: Yellowness (b\*) versus Tenderization Type

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	104.762	26.1905	49.78	0.000
Error	8	4.209	0.5261		
Total	12	108.971			

S	R-sq	R-sq(adj)	R-sq(pred)
0.725338	96.14%	94.21%	89.33%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
DW	3	12.130 A		
NT	2	8.395	В	
CA	3	7.180	В	
AA	2	6.050	В	C
LA	3	4.107		C

Means that do not share a letter are significantly different.

Means that do not share a letter are significantly different.

## **COOKED**

# One-way ANOVA: Lightness $(L^*)$ versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	571.75	142.937	15.45	0.000
Error	10	92.50	9.250		
Total	14	664.24			

## **Model Summary**

S	S R-sq		R-sq(pred)
3.04132	86.07%	80.50%	68.67%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping		
DW	3	-36.79 A			
AA	3	-45.31	В		
NT	3	-49.27	В	C	
CA	3	-50.983	В	C	
LA	3	-54.97		C	

Means that do not share a letter are significantly different.

#### **COOKED**

## One-way ANOVA: Redness (a\*) versus Tenderization Type

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	75.175	18.7937	71.36	0.000
Error	7	1.844	0.2634		
Total	11	77.018			

_	S	R-sq	R-sq(adj)	R-sq(pred)
	0.513183	97.61%	96.24%	91.03%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
DW	2	6.005 A		
NT	3	5.810 A		
CA	2	2.215	В	
LA	3	1.570	В	
AA	2	-0.6200	C	

Means that do not share a letter are significantly different.

#### COOKED

## One-way ANOVA: Yellowness (b\*) versus Tenderization Type

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	276.749	69.1872	79.14	0.000
Error	8	6.994	0.8743		
Total	12	283.743			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	0.935013	97.54%	96.30%	94.28%

## **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

Tenderization Type	N	Mean	Grouping
DW	3	16.157 A	
NT	3	9.813	В
CA	2	6.590	C
AA	3	4.997	C
LA	2	3.400	C

Means that do not share a letter are significantly different.

## CONTROL

## One-way ANOVA: Lightness (L\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	89.17	89.166	14.41	0.019
Error	4	24.76	6.189		
Total	5	113.92			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.48786	78.27%	72.84%	51.10%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	-49.27 A	
U	3	-56.980	В

DW

# One-way ANOVA: Lightness (L\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	41.98	41.976	4.91	0.091
Error	4	34.17	8.542		
Total	5	76.14			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
-	2.92268	55.13%	43.91%	0.00%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	-36.79 A	
U	3	-42.077 A	

Means that do not share a letter are significantly different.

## AA

# One-way ANOVA: Lightness (L\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	12.586	12.586	6.40	0.065
Error	4	7.864	1.966		
Total	5	20.450			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.40215	61.55%	51.93%	13.48%

## **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean Grouping	
C	3	-45.31 A	
U	3	-48.207 A	

Means that do not share a letter are significantly different.

#### CA

## One-way ANOVA: Lightness (L\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.588	3.588	1.07	0.360
Error	4	13.443	3.361		
Total	5	17.031			

 S	R-sq	R-sq(adj)	R-sq(pred)
1.83320	21.07%	1.34%	0.00%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	-49.44 A	
C	3	-50.983 A	

Means that do not share a letter are significantly different.

#### LA

## One-way ANOVA: Lightness (L\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.392	1.392	0.17	0.701
Error	4	32.624	8.156		
Total	5	34.016			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.85588	4.09%	0.00%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	-54.97 A	
U	3	-55.930 A	

Means that do not share a letter are significantly different.

## **CONTROL**

## One-way ANOVA: Redness (a\*) versus Cooking

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	12.27	12.269	4.39	0.104
Error	4	11.18	2.796		
Total	5	23.45			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.67220	52.31%	40.39%	0.00%

# **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
U	3	8.67 A	_
C	3	5.810 A	

Means that do not share a letter are significantly different.

## DW

## One-way ANOVA: Redness (a\*) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.918	1.918	1.47	0.349
Error	2	2.607	1.303		
Total	3	4.525			

S	R-sq	R-sq(adj)	R-sq(pred)
1.14163	42.39%	13.59%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
U	2	7.390 A
C	2	6.005 A

Means that do not share a letter are significantly different.

## AA

## One-way ANOVA: Redness (a\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	2.17562	2.17562	1023.82	0.001
Error	2	0.00425	0.00213		
Total	3	2.17987			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0460977	99.81%	99.71%	99.22%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	0.8550 A	
C	2	-0.6200	В

Means that do not share a letter are significantly different.

## CA

## One-way ANOVA: Redness (a\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.0506	1.0506	3.76	0.192
Error	2	0.5585	0.2792		
Total	3	1.6091			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.528417	65.29%	47.94%	0.00%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
U	2	3.2400 A	
C	2	2.215 A	

## LA One-way ANOVA: Redness (a\*) versus Cooking Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.6308	0.63075	6.59	0.083
Error	3	0.2870	0.09568		
Total	4	0.9178			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.309327	68.72%	58.30%	0.00%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
U	2	2.295 A	
C	3	1.570 A	

Means that do not share a letter are significantly different.

## CONTROL

# One-way ANOVA: Yellowness (b\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	2.414	2.4140	3.83	0.145
Error	3	1.890	0.6300		
Total	4	4.304			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.793750	56.09%	41.45%	0.00%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	9.813 A	
U	2	8.395 A	

Means that do not share a letter are significantly different.

# DW

## One-way ANOVA: Yellowness (b\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	24.321	24.321	14.14	0.020
Error	4	6.879	1.720		
Total	5	31.200			

S	R-sq	R-sq(adj)	R-sq(pred)
1.31136	77.95%	72.44%	50.39%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	16.157 A	
U	3	12.130	В

Means that do not share a letter are significantly different.

#### AA

## One-way ANOVA: Yellowness (b\*) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.331	1.3314	2.77	0.195
Error	3	1.443	0.4812		
Total	4	2.775			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	0.693654	47.98%	30.64%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
U	2	6.050 A
C	3	4.997 A

Means that do not share a letter are significantly different.

## CA

## One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.4177	0.41772	4.71	0.118
Error	3	0.2662	0.08873		
Total	4	0.6839			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.297881	61.08%	48.10%	0.00%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	7.180 A	
C	2	6.590 A	

Means that do not share a letter are significantly different.

#### LA

## One-way ANOVA: Yellowness (b\*) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.5993	0.5993	2.48	0.213
Error	3	0.7245	0.2415		
Total	4	1.3237			

S	R-sq	R-sq(adj)	R-sq(pred)
0.491415	45.27%	27.03%	0.00%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	4.107 A	
C	2	3.400 A	

Means that do not share a letter are significantly different.

## UNCOOKED

# One-way ANOVA: WHC (%) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	2	158.37	79.185	14.10	0.009
Error	5	28.07	5.615		
Total	7	186.44			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.36956	84.94%	78.92%	60.91%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
AA	2	28.33 A	
LA	3	20.67	В
CA	3	16.889	В

Means that do not share a letter are significantly different.

# COOKED

## One-way ANOVA: WHC (%) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	2	37.70	18.85	1.15	0.402
Error	4	65.41	16.35		
Total	6	103.11			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	4.04374	36.57%	4.85%	0.00%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean Grouping	
AA	3	14.22 A	
LA	2	14.00 A	
CA	2	9.000 A	

## AA

# One-way ANOVA: WHC (%) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	238.95	238.95	10.67	0.047
Error	3	67.19	22.40		
Total	4	306.13			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
4.73234	78.05%	70.74%	47.44%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	28.33 A	
C	3	14.22	В

Means that do not share a letter are significantly different.

## CA

# One-way ANOVA: WHC (%) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	74.681	74.681	54.99	0.005
Error	3	4.074	1.358		
Total	4	78.756			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.16534	94.83%	93.10%	87.87%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	16.889 A	
C	2	9.000	В

Means that do not share a letter are significantly different.

#### LA

# One-way ANOVA: WHC (%) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	53.33	53.333	7.20	0.075
Error	3	22.22	7.407		
Total	4	75.56			

S	R-sq	R-sq(adj)	R-sq(pred)
2.72166	70.59%	60.78%	25.59%

**Grouping Information Using the Tukey Method and 95% Confidence** 

Cooking	N	Mean Grouping
U	3	20.67 A
C	2	14.00 A

Means that do not share a letter are significantly different.

#### **UNCOOKED**

# One-way ANOVA: pH of Meat versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	22.3652	5.59129	674.73	0.000
Error	10	0.0829	0.00829		
Total	14	22.4480			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	0.0910311	99.63%	99.48%	99.17%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
NT	3	6.0867 A	
DW	3	5.8433 A	
AA	3	3.8167	В
LA	3	3.4033	C
CA	3	3.2900	C

Means that do not share a letter are significantly different.

## **COOKED**

# One-way ANOVA: pH of Meat versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	4	35.6335	8.90837	1433.75	0.000
Error	10	0.0621	0.00621		
Total	14	35.6956			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.0788247	99.83%	99.76%	99.61%

## **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

Tenderization Type	N	Mean	Grouping	
NT	3	6.5600 A		
DW	3	6.4733 A		
AA	3	3.81000	В	
LA	3	3.2867	C	
CA	3	3.1200	C	

## CONTROL

# One-way ANOVA: pH of Meat versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.336067	0.336067	195.77	0.000
Error	4	0.006867	0.001717		
Total	5	0.342933			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.0414327	98.00%	97.50%	95.49%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	6.5600 A	
U	3	6.0867	В

Means that do not share a letter are significantly different.

#### DW

## One-way ANOVA: pH of Meat versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.59535	0.595350	66.64	0.001
Error	4	0.03573	0.008933		
Total	5	0.63108			

## **Model Summary**

	S R-sq	R-sq(adj)	R-sq(pred)
0.0945163	94.34%	92.92%	87.26%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	6.4733 A	
U	3	5.8433	В

Means that do not share a letter are significantly different.

#### AA

## One-way ANOVA: pH of Meat versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.000067	0.000067	0.25	0.643
Error	4	0.001067	0.000267		
Total	5	0.001133			

S	R-sq	R-sq(adj)	R-sq(pred)
0.0163299	5.88%	0.00%	0.00%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	3.8167 A	
C	3	3.81000 A	

Means that do not share a letter are significantly different.

#### CA

## One-way ANOVA: pH of Meat versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.04335	0.04335	1.87	0.243
Error	4	0.09280	0.02320		
Total	5	0.13615			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
 0.152315	31.84%	14.80%	0.00%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
U	3	3.2900 A
C	3	3.1200 A

Means that do not share a letter are significantly different.

#### LA

## One-way ANOVA: pH of Meat versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.020417	0.020417	9.57	0.036
Error	4	0.008533	0.002133		
Total	5	0.028950			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.0461880	70.52%	63.15%	33.68%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	3.4033 A	
C	3	3.2867	В

Means that do not share a letter are significantly different.

## **COOKING LOSS**

## One-way ANOVA: Cooking loss versus Tenderization Type

Source	DF	Adj SS	Adj MS	F-Value	<b>P-Value</b>
Tenderization Type	4	1386.93	346.73	31.27	0.000
Error	9	99.78	11.09		
Total	13	1486.71			

S	R-sq	R-sq(adj)	R-sq(pred)
3.32971	93.29%	90.31%	83.37%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

## **Tenderization**

Type	N	Mean	Groupin	g	
DW	3	47.69	A		_
C	3	43.684	A	В	
CA	3	37.176		В	
AA	2	25.14			C
LA	3	22.34			C

Means that do not share a letter are significantly different.

## MARINATE UPTAKE

## One-way ANOVA: marinate uptake versus Tenderization type

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization type	3	10952.1	3650.68	90.62	0.000
Error	7	282.0	40.29		
Total	10	11234.1			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)	
6.34723	97.49%	96.41%	94.35%	

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Tenderization type	N	Mean	Grouping	
LA	3	76.66	A	
AA	3	72.70	A	
CA	3	70.90	A	
DW	2	-8.195	В	

Means that do not share a letter are significantly different.

# Table 4.3 ANOVA and Tukey's Comparison Test with 95% confidence level for enzymatic tenderization applications

## UNCOOKED

## One-way ANOVA: WBS versus tenderization time

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
tenderization time	7	4016.1	573.73	26.46	0.000
Error	22	477.0	21.68		
Total	29	4493.0			

S	R-sq	R-sq(adj)	R-sq(pred)
4.65618	89.38%	86.01%	79.69%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization time	N	Mean	Grouping
0	5	56.16 A	
10	3	50.64 A	
50	4	30.163	В
60	4	29.62	В
20	4	29.12	В
30	3	28.83	В
1440	3	28.82	В
40	4	24.163	В

Means that do not share a letter are significantly different.

## COOKED

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
tenderization time	7	1650.7	235.81	11.35	0.000
Error	28	581.8	20.78		
Total	35	2232.5			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	4.55820	73.94%	67.43%	58.22%

# **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

Tenderization time	N	Mean	Grouping	
0	5	49.39 A		
50	4	36.31	В	
40	5	35.60	В	
20	5	34.77	В	
10	4	33.14	В	
1440	3	29.377	В	
30	6	28.10	В	
60	4	27.59	В	

Means that do not share a letter are significantly different.

0

# One-way ANOVA: WBS versus Cook

# **Analysis of Variance**

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
Cook	1	114.6	114.58	2.91	0.127
Error	8	315.5	39.44		
Total	9	430.1			

S	R-sq	R-sq(adj)	R-sq(pred)
6.28012	26.64%	17.47%	0.00%

## Grouping Information Using the Tukey Method and 95% Confidence

Cook	N	Mean Grouping
U	5	56.16 A
C	5	49.39 A

Means that do not share a letter are significantly different.

10

## One-way ANOVA: WBS versus Cook

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	524.7	524.70	14.43	0.013
Error	5	181.8	36.35		
Total	6	706.5			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
6.02918	74.27%	69.13%	46.48%

# **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cook	N	Mean	Grouping
U	3	50.64 A	
C	4	33.14	В

Means that do not share a letter are significantly different.

20

## One-way ANOVA: WBS versus Cook

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	70.98	70.98	3.43	0.106
Error	7	144.85	20.69		
Total	8	215.82			

## **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
4.54887	32.89%	23,30%	0.00%

# **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cook	N	Mean Grouping
C	5	34.77 A
U	4	29.12 A

 ${\it Means that do not share a letter are significantly different.}$ 

30

## One-way ANOVA: WBS versus Cook

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	674.37	674.37	27.98	0.006
Error	4	96.42	24.11		
Total	5	770.80			

	S	R-sq	R-sq(adj)	R-sq(pred)
4.90	0977	87.49%	84.36%	71.85%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cook	N	Mean	Grouping	
С	3	50.03 A		
U	3	28.83	В	
Means that do not share a letter are significantly different.				

40

## One-way ANOVA: WBS versus Cook

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	290.50	290.500	29.25	0.001
Error	7	69.53	9.933		
Total	8	360.03			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.15170	80.69%	77.93%	69.44%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cook	N	Mean	Grouping
C	5	35.60 A	
U	4	24.163	В

Means that do not share a letter are significantly different.

50

## One-way ANOVA: WBS versus Cook

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	75.71	75.707	8.69	0.026
Error	6	52.26	8.710		
Total	7	127.97			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.95133	59.16%	52.35%	27.40%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cook	N	Mean	Grouping	
С	4	36.31 A		
U	4	30.163	В	

60

# One-way ANOVA: WBS versus Cook

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	8.242	8.242	0.57	0.480
Error	6	87.184	14.531		
Total	7	95.426			

## **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	3.81191	8.64%	0.00%	0.00%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cook	${f N}$	Mean Grouping
U	4	29.62 A
C	4	27.59 A

Means that do not share a letter are significantly different.

1440

# One-way ANOVA: WBS versus Cook

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cook	1	0.4648	0.4648	0.06	0.815
Error	4	29.6491	7.4123		
Total	5	30.1139			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.72255	1.54%	0.00%	0.00%

## **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cook	N	Mean Grouping
C	3	29.377 A
U	3	28.82 A

Means that do not share a letter are significantly different.

#### **UNCOOKED**

## One-way ANOVA: T2 (ms) versus MTS Treatment Duration (min)

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	107.72	15.388	2.69	0.048
Error	16	91.39	5.712		
Total	23	199.11			

S	R-sq	R-sq(adj)	R-sq(pred)
2.39001	54.10%	34.02%	0.00%

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grouping
40	3	48.23 A	
10	3	45.39 A	В
50	3	45.230 A	В
0	3	44.27 A	В
1440	3	43.089 A	В
60	3	43.06 A	В
30	3	42.97 A	В
20	3	40.56	В

Means that do not share a letter are significantly different.

## **COOKED**

# One-way ANOVA: T2 (ms) versus MTS Treatment Duration (min)

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	138.06	19.723	4.74	0.005
Error	16	66.64	4.165		
Total	23	204.70			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.04078	67.45%	53.20%	26.76%

## **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

MTS Treatment Duration (min)	N	Mean	Grouping	
0	3	31.24 A		
1440	3	27.23 A	В	
40	3	26.71 A	В	
60	3	26.11 A	В	
10	3	25.70 A	В	
50	3	24.449	В	
30	3	24.39	В	
20	3	22.61	В	

Means that do not share a letter are significantly different

## 0 MIN

## One-way ANOVA: T2 (ms) versus Cooking

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	254.59	254.593	39.10	0.003
Error	4	26.05	6.512		
Total	5	280.64			

S	R-sq	R-sq(adj)	R-sq(pred)
2,55180	90.72%	88.40%	79.12%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	44.27 A	
C	3	31.24	В

Means that do not share a letter are significantly different.

#### **10 MIN**

# One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	581.72	581.72	49.75	0.002
Error	4	46.77	11.69		
Total	5	628.49			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.41945	92.56%	90.70%	83.26%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	45.39 A	
C	3	25.70	В

Means that do not share a letter are significantly different.

## 20 MIN

## One-way ANOVA: T2 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	483.29	483.286	134.75	0.000
Error	4	14.35	3.587		
Total	5	497.63			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.89381	97.12%	96.40%	93.51%

## **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	40.56 A	
C	3	22.61	В

Means that do not share a letter are significantly different.

#### **30 MIN**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	517.64	517.639	100.50	0.001
Error	4	20.60	5.151		
Total	5	538.24			

_	S	R-sq	R-sq(adj)	R-sq(pred)
	2.26948	96.17%	95.22%	91.39%

## **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	42.97 A	
C	3	24.39	В

Means that do not share a letter are significantly different.

## 40 MIN

## One-way ANOVA: T2 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	694.39	694.386	153.96	0.000
Error	4	18.04	4.510		
Total	5	712.43			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.12369	97.47%	96.83%	94.30%

## **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping
U	3	48.23 A	
C	3	26.71	В

Means that do not share a letter are significantly different.

## 50 MIN

## One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	647.775	647.775	823.22	0.000
Error	4	3.148	0.787		
Total	5	650.922			

#### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.887061	99.52%	99.40%	98.91%

#### **Tukey Pairwise Comparisons**

## Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	45.230 A	
C	3	24.449	В

## 60 MIN

## One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	430.63	430.632	90.87	0.001
Error	4	18.96	4.739		
Total	5	449.59			

## **Model Summary**

	S R-sq	R-sq(adj)	R-sq(pred)
2.1769	1 95.78%	94.73%	90.51%

## **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	43.06 A	
C	3	26.11	В

Means that do not share a letter are significantly different.

#### 24 H

# One-way ANOVA: T2 (ms) versus Cooking

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	377.29	377.294	149.10	0.000
Error	4	10.12	2.530		
Total	5	387.42			

## **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.59075	97.39%	96.73%	94.12%

#### **Tukey Pairwise Comparisons**

## **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping
U	3	43.089 A	
C	3	27.23	В

Means that do not share a letter are significantly different.

#### **UNCOOKED**

## One-way ANOVA: Lightness (L\*) versus MTS Treatment Duration (min)

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	354.08	50.582	17.20	0.000
Error	16	47.06	2.941		
Total	23	401.14			

S	R-sq	R-sq(adj)	R-sq(pred)
1.71505	88.27%	83.13%	73.60%

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grouping
1440	3	-39.357 A	
0	3	-49.820	В
20	3	-50.463	В
30	3	-50.86	В
60	3	-51.03	В
50	3	-51.25	В
40	3	-51.297	В
10	3	-51.413	В

Means that do not share a letter are significantly different.

## UNCOOKED

## One-way ANOVA: Redness (a\*) versus MTS Treatment Duration (min)

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	97.72	13.9598	17.10	0.000
Error	16	13.06	0.8165		
Total	23	110.78			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.903581	88.21%	83.05%	73.47%

## **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

MTS Treatment Duration (min)	N	Mean	Grouping
20	3	11.6167 A	
0	3	11.167 A	
10	3	11.027 A	
30	3	10.017 A	
40	3	9.713 A	
60	3	9.597 A	
50	3	9.103 A	
1440	3	4.743	В

Means that do not share a letter are significantly different.

## UNCOOKED

# One-way ANOVA: Yellowness (b\*) versus MTS Treatment Duration (min)

## **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	12.07	1.7247	2.45	0.066
Error	16	11.28	0.7050		
Total	23	23.35			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.839633	51.70%	30.57%	0.00%

# Grouping Information Using the Tukey Method and 95% Confidence

#### **MTS Treatment**

<b>Duration (min)</b>	N	Mean Grouping	
20	3	11.617 A	
10	3	11.337 A	
0	3	10.443 A	
50	3	10.147 A	
60	3	10.143 A	
1440	3	9.917 A	
40	3	9.903 A	
30	3	9.3667 A	

Means that do not share a letter are significantly different.

#### COOKED

# One-way ANOVA: Lightness (L\*) versus MTS Treatment Duration (min)

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	52.58	7.512	1.04	0.443
Error	16	115.57	7.223		
Total	23	168.15			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
2.68757	31.27%	1.20%	0.00%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean Grouping	
0	3	-48.347 A	
40	3	-48.43 A	
10	3	-48.49 A	
20	3	-49.64 A	
1440	3	-50.89 A	
50	3	-51.513 A	
30	3	-51.78 A	
60	3	-51.953 A	

Means that do not share a letter are significantly different.

### COOKED

# One-way ANOVA: Redness (a\*) versus MTS Treatment Duration (min)

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	24.197	3.4568	10.30	0.000
Error	16	5.372	0.3358		
Total	23	29.569			

S	R-sq	R-sq(adj)	R-sq(pred)
0.579443	81.83%	73.88%	59.12%

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grou	ping	
0	3	7.070 A			
60	3	7.053 A			
1440	3	6.630 A	В		
50	3	5.680 A	В	C	
40	3	5.660 A	В	C	
20	3	5.057	В	C	
10	3	4.717		C	
30	3	4.2367		C	

Means that do not share a letter are significantly different.

#### **COOKED**

# One-way ANOVA: Yellowness (b\*) versus MTS Treatment Duration (min)

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	18.39	2.627	2.34	0.076
Error	16	17.97	1.123		
Total	23	36.36			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.05984	50.57%	28.95%	0.00%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grouping
40	3	13.553 A	
20	3	12.080 A	В
10	3	11.997 A	В
0	3	11.977 A	В
50	3	11.627 A	В
1440	3	11.45 A	В
60	3	10.780 A	В
30	3	10.480	В

Means that do not share a letter are significantly different.

#### 0 MIN

### One-way ANOVA: Lightness (L\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.256	3.2561	6.10	0.069
Error	4	2.136	0.5340		
Total	5	5.392			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.730764	60.39%	50.48%	10.87%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
C	3	-48.347 A
U	3	-49.820 A

Means that do not share a letter are significantly different.

#### 0 MIN

#### One-way ANOVA: Redness (a\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	25.174	25.174	20.87	0.010
Error	4	4.825	1.206		
Total	5	29.999			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.09830	83.92%	79.89%	63.81%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping	
U	3	11.167 A		
C	3	7.070	В	

Means that do not share a letter are significantly different.

#### 0 MIN

### One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.527	3.527	2.38	0.197
Error	4	5.917	1.479		
Total	5	9.444			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.21626	37.34%	21.68%	0.00%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
C	3	11.977 A	
U	3	10.443 A	

Means that do not share a letter are significantly different.

#### **10 MIN**

### One-way ANOVA: Lightness (L\*) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	12.85	12.85	1.25	0.327
Error	4	41.26	10.32		
Total	5	54.11			

S	R-sq	R-sq(adj)	R-sq(pred)
3.21176	23.74%	4.68%	0.00%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	-48.49 A	
U	3	-51.413 A	

Means that do not share a letter are significantly different.

#### 10 MIN

### One-way ANOVA: Redness (a\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	59.724	59.7241	96.44	0.001
Error	4	2.477	0.6193		
Total	5	62.201			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.786946	96.02%	95.02%	91.04%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	11.027 A	
C	3	4.717	В

Means that do not share a letter are significantly different.

#### 10 MIN

### One-way ANOVA: Yellowness (b\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.6534	0.6534	0.45	0.541
Error	4	5.8661	1.4665		
Total	5	6.5195			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.21101	10.02%	0.00%	0.00%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	11.997 A	
U	3	11.337 A	

#### 20 MIN

### One-way ANOVA: Lightness (L\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.017	1.017	0.62	0.477
Error	4	6.608	1.652		
Total	5	7.625			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	1.28533	13.34%	0.00%	0.00%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	-49.64 A	
U	3	-50.463 A	

Means that do not share a letter are significantly different.

#### **20 MIN**

### One-way ANOVA: Redness (a\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	64.550	64.5504	170.46	0.000
Error	4	1.515	0.3787		
Total	5	66.065			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
 0.615373	97.71%	97.13%	94.84%

#### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	${f N}$	Mean	Grouping
U	3	11.6167 A	
C	3	5.057	В

Means that do not share a letter are significantly different.

#### **20 MIN**

### One-way ANOVA: Yellowness (b\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.3220	0.3220	1.09	0.356
Error	4	1.1857	0.2964		
Total	5	1.5077			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.544442	21.36%	1.70%	0.00%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	12.080 A	
U	3	11.617 A	

Means that do not share a letter are significantly different.

#### **30 MIN**

# One-way ANOVA: Lightness (L\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.260	1.260	0.16	0.708
Error	4	31.221	7.805		
Total	5	32.481			

### **Model Summary**

_	$\mathbf{S}$	R-sq	R-sq(adj)	R-sq(pred)
	2,79379	3.88%	0.00%	0.00%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	-50.86 A	
C	3	-51.78 A	

Means that do not share a letter are significantly different.

#### **30 MIN**

### One-way ANOVA: Redness (a\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	50.113	50.1126	73.99	0.001
Error	4	2.709	0.6773		
Total	5	52.822			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.823003	94.87%	93.59%	88.46%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	10.017 A	
C	3	4.2367	В

Means that do not share a letter are significantly different.

#### **30 MIN**

### One-way ANOVA: Yellowness (b\*) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.859	1.8593	3.42	0.138
Error	4	2.171	0.5429		
Total	5	4.031			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.736795	46.13%	32.66%	0.00%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
С	3	10.480 A	
U	3	9.3667 A	

Means that do not share a letter are significantly different.

#### 40 MIN

#### One-way ANOVA: Lightness (L\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	12.30	12.298	2.64	0.179
Error	4	18.61	4.652		
Total	5	30.91			

### **Model Summary**

_	$\mathbf{S}$	R-sq	R-sq(adj)	R-sq(pred)
	2.15686	39.79%	24.74%	0.00%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
C	3	-48.43 A	
U	3	-51.297 A	

Means that do not share a letter are significantly different.

# 40 MIN

### One-way ANOVA: Redness (a\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	24.644	24.6443	65.44	0.001
Error	4	1.506	0.3766		
Total	5	26.151			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.613650	94.24%	92.80%	87.04%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	9.713 A	
C	3	5.660	В

#### 40 MIN

### One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	19.984	19.9838	65.27	0.001
Error	4	1.225	0.3062		
Total	5	21.208			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.553338	94.23%	92.78%	87.01%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
C	3	13.553 A	
U	3	9.903	В

Means that do not share a letter are significantly different.

#### 50 MIN

### One-way ANOVA: Lightness (L\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.1067	0.1067	0.04	0.851
Error	4	10.6955	2.6739		
Total	5	10.8022			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.63520	0.99%	0.00%	0.00%

### **Tukey Pairwise Comparisons**

#### **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean Grouping	
U	3	-51.25 A	
C	3	-51.513 A	

Means that do not share a letter are significantly different.

#### 50 MIN

# One-way ANOVA: Redness (a\*) versus Cooking

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	17.5788	17.5788	499.87	0.000
Error	4	0.1407	0.0352		
Total	5	17.7195			

S	R-sq	R-sq(adj)	R-sq(pred)
0.187528	99.21%	99.01%	98.21%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	9.103 A	
C	3	5.680	В

Means that do not share a letter are significantly different.

#### 50 MIN

#### One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.286	3.2856	5.06	0.088
Error	4	2.595	0.6488		
Total	5	5.881			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.805502	55.87%	44.84%	0.70%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
C	3	11.627 A	
U	3	10.147 A	

Means that do not share a letter are significantly different.

#### 60 MIN

### One-way ANOVA: Lightness (L\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1.270	1.270	0.26	0.634
Error	4	19.207	4.802		
Total	5	20.477			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	2.19131	6.20%	0.00%	0.00%

# **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping	
U	3	-51.03 A	
C	3	-51.953 A	

Means that do not share a letter are significantly different.

#### 60 MIN

### One-way ANOVA: Redness (a\*) versus Cooking

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	9.703	9.7028	26.26	0.007
Error	4	1.478	0.3695		
Total	5	11.181			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.607893	86.78%	83.47%	70.25%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	9.597 A	
C	3	7.053	В

Means that do not share a letter are significantly different.

#### 60 MIN

### One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	0.6080	0.6080	1.13	0.348
Error	4	2.1559	0.5390		
Total	5	2.7639			

#### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.734143	22.00%	2.50%	0.00%

### **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	${f N}$	Mean Grouping	
C	3	10.780 A	
U	3	10.143 A	

Means that do not share a letter are significantly different.

#### 24 H

### One-way ANOVA: Lightness (L\*) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	199.53	199.527	24.26	0.008
Error	4	32.89	8.223		
Total	5	232.42			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.86762	85.85%	82.31%	68.16%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	-39.357 A	
C	3	-50.89	В

# 24 H One-way ANOVA: Redness (a\*) versus Cooking

Analysis	of	Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	5.339	5.3393	5.64	0.076
Error	4	3.784	0.9460		
Total	5	9.123			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.972634	58.52%	48.15%	6.68%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping	
C	3	6.630 A	
U	3	4.743 A	

Means that do not share a letter are significantly different.

#### 24 F

### One-way ANOVA: Yellowness (b\*) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	3.542	3.542	1.74	0.257
Error	4	8.136	2.034		
Total	5	11.678			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.42616	30.33%	12.91%	0.00%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping
C	3	11.45 A
U	3	9.917 A

Means that do not share a letter are significantly different.

#### **UNCOOKED**

# One-way ANOVA: T21 (ms) versus MTS Treatment Duration (min)

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	620.64	88.662	26.83	0.000
Error	12	39.66	3.305		
Total	19	660.30			

S	R-sq	R-sq(adj)	R-sq(pred)
1.81802	93.99%	90.49%	77.97%

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	$\mathbf{G}$	rouping	
30	2	43.34 A			
40	3	41.630 A	В		
20	2	39.670 A	В	C	
50	3	39.237 A	В	C	
60	3	38.653 A	В	C	
1440	3	36.377	В	C	
0	2	33.67		C	
10	2	22.560			D

Means that do not share a letter are significantly different.

#### **COOKED**

# One-way ANOVA: T21 (ms) versus MTS Treatment Duration (min)

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	249.98	35.711	26.74	0.000
Error	13	17.36	1.336		
Total	20	267.34			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.15565	93.51%	90.01%	83.23%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grouping
50	3	18.133 A	
60	3	17.597 A	
1440	3	17.327 A	
40	3	17.170 A	
30	3	11.081	В
20	2	11.020	В
0	2	10.285	В
10	2	9.939	В

Means that do not share a letter are significantly different.

#### 0 MIN

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	546.86	546.858	57.68	0.017
Error	2	18.96	9.480		
Total	3	565.82			

S	R-sq	R-sq(adj)	R-sq(pred)
3.07901	96.65%	94.97%	86.60%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	33.67 A	
C	2	10.285	В

Means that do not share a letter are significantly different.

#### **10 MIN**

#### One-way ANOVA: T21 (ms) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	159.277	159.277	145.02	0.007
Error	2	2.197	1.098		
Total	3	161.474			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.04799	98.64%	97.96%	94.56%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	22.560 A	
C	2	9.939	В

Means that do not share a letter are significantly different.

#### 20 MIN

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	820.822	820.822	5168.91	0.000
Error	2	0.318	0.159		
Total	3	821.140			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.398497	99.96%	99.94%	99.85%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	39.670 A	
C	2	11.020	В

Means that do not share a letter are significantly different.

#### **30 MIN**

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	1248.80	1248.80	206.32	0.001
Error	3	18.16	6.05		
Total	4	1266.96			

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_	S	R-sq	R-sq(adj)	R-sq(pred)
_	2.46021	98.57%	98.09%	94.85%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	2	43.34 A	
C	3	11.081	В

Means that do not share a letter are significantly different.

#### 40 MIN

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	897.437	897.437	780.69	0.000
Error	4	4.598	1.150		
Total	5	902.036			

#### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	1.07217	99.49%	99.36%	98.85%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	41.630 A	
C	3	17.170	В

Means that do not share a letter are significantly different.

#### 50 MIN

### One-way ANOVA: T21 (ms) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	668.026	668.026	1711.42	0.000
Error	4	1.561	0.390		
Total	5	669.587			

#### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.624767	99.77%	99.71%	99.48%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean Grouping
U	3	39.237 A
C	3	18.133 B

# 60 MIN

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	665.075	665.075	495.78	0.000
Error	4	5.366	1.341		
Total	5	670.441			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.15822	99.20%	99.00%	98.20%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	38.653 A	
C	3	17.597	В

Means that do not share a letter are significantly different.

#### 24 11

### One-way ANOVA: T21 (ms) versus Cooking

#### **Analysis of Variance**

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	544.354	544.354	371.18	0.000
Error	4	5.866	1.467		
Total	5	550.220			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.21101	98.93%	98.67%	97.60%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean	Grouping
U	3	36.377 A	
C	3	17.327	В

Means that do not share a letter are significantly different.

#### UNCOOKED

### One-way ANOVA: T22 (ms) versus MTS Treatment Duration (min)

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	29600.4	4228.63	142.38	0.000
Error	15	445.5	29.70		
Total	22	30045.9			

S	R-sq	R-sq(adj)	R-sq(pred)
5.44982	98.52%	97.83%	96.47%

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grouping
1440	3	124.43 A	
60	3	117.20 A	
40	3	116.37 A	
50	3	113.43 A	
0	2	54.00	В
10	3	49.29	В
30	3	43.45	В
20	3	41.96	В

Means that do not share a letter are significantly different.

#### **COOKED**

# One-way ANOVA: T22 (ms) versus MTS Treatment Duration (min)

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MTS Treatment Duration (min)	7	5338.6	762.65	25.02	0.000
Error	15	457.2	30.48		
Total	22	5795.7			

# **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	5.52059	92.11%	88.43%	81.54%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

MTS Treatment Duration (min)	N	Mean	Grou	ping
1440	3	67.73 A		
60	3	60.98 A		
50	3	57.29 A		
40	3	56.57 A	В	
0	2	39.11	В	C
10	3	31.15		C
30	3	30.91		C
20	3	26.84		C

Means that do not share a letter are significantly different.

#### 0 MIN

### One-way ANOVA: T22 (ms) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	221.86	221.86	7.82	0.108
Error	2	56.74	28.37		
Total	3	278.60			

 S	R-sq	R-sq(adj)	R-sq(pred)
5.32640	79.63%	69.45%	18.53%

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean Grouping
U	2	54.00 A
C	2	39.11 A

Means that do not share a letter are significantly different.

#### **10 MIN**

#### One-way ANOVA: T22 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	493.8	493.77	17.66	0.014
Error	4	111.8	27.95		
Total	5	605.6			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	5.28713	81.54%	76.92%	58.46%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping	
U	3	49.29 A		
C	3	31.15	В	

Means that do not share a letter are significantly different.

#### 20 MIN

### One-way ANOVA: T22 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	342.77	342.77	22.48	0.009
Error	4	61.00	15.25		
Total	5	403.77			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	3.90509	84.89%	81.12%	66.01%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	${f N}$	Mean	Grouping
U	3	41.96 A	
C	3	26.84	В

Means that do not share a letter are significantly different.

#### **30 MIN**

### One-way ANOVA: T22 (ms) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	235.88	235.88	16.11	0.016
Error	4	58.57	14.64		
Total	5	294.45			

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_	S	R-sq	R-sq(adj)	R-sq(pred)
_	3.82664	80.11%	75.13%	55.24%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	43.45 A	
C	3	30.91	В

Means that do not share a letter are significantly different.

#### 40 MIN

### One-way ANOVA: T22 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	5363.5	5363.46	111.18	0.000
Error	4	193.0	48.24		
Total	5	5556.4			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
6.94572	96.53%	95.66%	92.19%

### **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping
U	3	116.37 A	
C	3	56.57	В

Means that do not share a letter are significantly different.

#### 50 MIN

### One-way ANOVA: T22 (ms) versus Cooking

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	4728.67	4728.67	338.19	0.000
Error	4	55.93	13.98		
Total	5	4784.60			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.73928	98.83%	98.54%	97.37%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	113.43 A	
C	3	57.29	В

#### 60 MIN

### One-way ANOVA: T22 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	4740.47	4740.47	313.45	0.000
Error	4	60.49	15.12		
Total	5	4800.96			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.88890	98.74%	98.42%	97.16%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Cooking	N	Mean	Grouping
U	3	117.20 A	
C	3	60.98	В

Means that do not share a letter are significantly different.

#### 24 H

### One-way ANOVA: T22 (ms) versus Cooking

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cooking	1	4823.5	4823.47	63.23	0.001
Error	4	305.1	76.28		
Total	5	5128.6			

### **Model Summary**

	S R-sq	R-sq(adj)	R-sq(pred)
8.7341	2 94.05%	92.56%	86.61%

#### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Cooking	N	Mean	Grouping
U	3	124.43 A	
C	3	67.73	В

Means that do not share a letter are significantly different.

# Table 4.4 ANOVA and Tukey's Comparison Test with 95% confidence level for digestion of red meat

### DIGESTED

#### One-way ANOVA: WBS versus Tenderization type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization type	5	1331.1	266.227	31.84	0.000
Error	27	225.7	8.361		
Total	32	1556.9			

_	S	R-sq	R-sq(adj)	R-sq(pred)
	2.89151	85.50%	82.82%	78.64%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization type	N	Mean		Group	oing	
CA	3	35.01 A				
LA	5	32.05 A	В			
DW	7	27.83	В	C		
MTS	6	25.65		C	D	
NT	5	20.32			D	E
AA	7	15.633				E

Means that do not share a letter are significantly different.

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
D	6	25.65 A	
U	7	19.334	В

Means that do not share a letter are significantly different.

#### **COOKED**

### One-way ANOVA: WBS versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	9664.7	1932.94	82.35	0.000
Error	26	610.3	23.47		
Total	31	10275.0			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
4.84481	94.06%	92.92%	91.71%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
C	6	51.81 A		
DW	8	49.81 A		
MTS	3	29.377	В	
CA	3	21.97	В	
AA	5	19.27	В	
LA	7	9.200	C	

Means that do not share a letter are significantly different.

# NT

### One-way ANOVA: WBS versus digestion

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
digestion	1	2730.4	2730.39	78.93	0.000
Error	10	345.9	34.59		
Total	11	3076.3			

S	R-sq	R-sq(adj)	R-sq(pred)
5.88158	88.76%	87.63%	83.81%

Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
U	6	51.81 A	
D	6	21.64	В

Means that do not share a letter are significantly different.

### DW

### One-way ANOVA: WBS versus digestion

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.30592	83.13%	81.83%	77.80%

#### Means

digestion	N	Mean	StDev	95% CI
D	7	27.83	3.29	(23.50, 32.17)
U	8	49.81	6.56	(45.76, 53.87)

 $Pooled\ StDev = 5.30592$ 

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
U	8	49.81 A	
D	7	27.83	В

Means that do not share a letter are significantly different.

#### AA

# One-way ANOVA: WBS versus digestion

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
digestion	1	38.58	38.584	6.80	0.026
Error	10	56.77	5.677		
Total	11	95.36			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	2.38275	40.46%	34.51%	13.66%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
U	5	19.27 A	
D	7	15.633	В

Means that do not share a letter are significantly different.

#### CA

# One-way ANOVA: WBS versus digestion

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
digestion	1	255.06	255.062	58.27	0.002
Error	4	17.51	4.377		
Total	5	272.57			

S	R-sq	R-sq(adj)	R-sq(pred)
 2.09217	93.58%	91.97%	85.55%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
D	3	35.01 A	
U	3	21.97	В

Means that do not share a letter are significantly different.

### LA

### One-way ANOVA: WBS versus digestion

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
digestion	1	1522.59	1522.59	289.03	0.000
Error	10	52.68	5.27		
Total	11	1575.27			

### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	2.29520	96.66%	96.32%	94.89%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean	Grouping
D	5	32.05 A	
U	7	9.200	В

Means that do not share a letter are significantly different.

#### MTS

### One-way ANOVA: WBS versus digestion

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
digestion	1	27.83	27.826	3.94	0.087
Error	7	49.39	7.055		
Total	8	77.21			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
 2.65617	36.04%	26.90%	4.54%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

digestion	N	Mean Grouping	
U	3	29.377 A	
D	6	25.65 A	

### UNDIGESTED

### One-way ANOVA: T21 (ms) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	9848.4	1969.68	123.11	0.000
Error	11	176.0	16.00		
Total	16	10024.4			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3,99990	98.24%	97.45%	94.41%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

TenderizationbType	N	Mean	Grouping
LA	2	79.89 A	
CA	3	67.78 A	
AA	3	37.077	В
NT	3	18.797	C
MTS	3	17.327	C
DW	3	17.227	C

Means that do not share a letter are significantly different.

### UNDIGESTED

### One-way ANOVA: T22 (ms) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	23028	4605.5	11.32	0.000
Error	12	4884	407.0		
Total	17	27912			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
20.1747	82.50%	75.21%	60.63%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence $\,$

Tenderization Type	N	Mean	Grouping		
CA	3	167.5 A			
LA	3	125.0 A	В		
AA	3	89.49	В	C	
DW	3	76.17	В	C	
NT	3	72.13	В	C	
MTS	3	67.73		C	

Means that do not share a letter are significantly different.

### DIGESTED

### One-way ANOVA: T21 (ms) versus Tenderization Type

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	154.50	30.900	4.64	0.023
Error	9	59.97	6.663		
Total	14	214.47			

S	R-sq	R-sq(adj)	R-sq(pred)
2.58133	72.04%	56.50%	0.00%

# **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
AA	2	23.20 A		
NT	3	15.200 A	В	
CA	2	14.23 A	В	
MTS	3	14.107	В	
LA	2	13.34	В	
DW	3	13.027	В	

Means that do not share a letter are significantly different.

#### **DIGESTED**

# One-way ANOVA: T22 (ms) versus Tenderization Type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	725.6	145.11	4.21	0.025
Error	10	344.8	34.48		
Total	15	1070.3			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.87170	67.79%	51.68%	19.72%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
AA	2	66.45 A		
MTS	3	55.30 A	В	
CA	3	50.57 A	В	
LA	2	49.77 A	В	
DW	3	48.62 A	В	
NT	3	43.29	В	

Means that do not share a letter are significantly different.

#### NT

# One-way ANOVA: T21 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	19.404	19.4040	21.78	0.010
Error	4	3.563	0.8909		
Total	5	22.967			

S	R-sq	R-sq(adj)	R-sq(pred)
0.943857	84.48%	80.61%	65.09%

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	18.797 A	
D	3	15.200	В

Means that do not share a letter are significantly different.

#### DW

#### One-way ANOVA: T21 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	26.460	26.460	24.34	0.008
Error	4	4.348	1.087		
Total	5	30.808			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.04256	85.89%	82.36%	68.25%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	17.227 A	
D	3	13.027	В

Means that do not share a letter are significantly different.

#### AA

### One-way ANOVA: T21 (ms) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	231.07	231.07	17.05	0.026
Error	3	40.66	13.55		
Total	4	271.73			

### **Model Summary**

	S	R-sq	R-sq(adj)	R-sq(pred)
3.	68147	85.04%	80.05%	43.29%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	37.077 A	
D	2	23.20	В

Means that do not share a letter are significantly different.

#### CA

### One-way ANOVA: T21 (ms) versus Process

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	3441.55	3441.55	120.66	0.002
Error	3	85.57	28.52		
Total	4	3527.12			

S	R-sq	R-sq(adj)	R-sq(pred)
5.34068	97.57%	96.77%	93.59%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	67.78 A	
D	2	14.23	В

Means that do not share a letter are significantly different.

### LA

### One-way ANOVA: T21 (ms) versus Process

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	4428.24	4428.24	91.76	0.011
Error	2	96.52	48.26		
Total	3	4524.75			

#### **Model Summary**

_	S	R-sq	R-sq(adj)	R-sq(pred)
	6.94684	97.87%	96.80%	91.47%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	2	79.89 A	
D	2	13.34	В

Means that do not share a letter are significantly different.

#### MTS

### One-way ANOVA: T21 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	15.553	15.553	11.73	0.027
Error	4	5.304	1.326		
Total	5	20.857			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.15156	74.57%	68.21%	42.78%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	$\mathbf{N}$	Mean	Grouping
C	3	17.327 A	
D	3	14.107	В

NT

### One-way ANOVA: T22 (ms) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	1247.0	1247.04	17.62	0.014
Error	4	283.2	70.79		
Total	5	1530.2			

### **Model Summary**

	S R	R-sq R-sq	q(adj) R-	sq(pred)
8.413	389 81.4	19% 76	5.87%	58.36%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	72.13 A	
D	3	43.29	В

Means that do not share a letter are significantly different.

#### DW

### One-way ANOVA: T22 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	1138.8	1138.78	38.56	0.003
Error	4	118.1	29.53		
Total	5	1256.9			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
5.43410	90.60%	88.25%	78.86%

### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Process	N	Mean	Grouping
C	3	76.17 A	
D	3	48.62	В

Means that do not share a letter are significantly different.

#### AA

### One-way ANOVA: T22 (ms) versus Process

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	637.0	637.0	5.29	0.105
Error	3	361.1	120.4		
Total	4	998.1			

S	R-sq	R-sq(adj)	R-sq(pred)
10.9706	63.82%	51.77%	10.63%

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean Grouping	
C	3	89.49 A	
D	2	66.45 A	

Means that do not share a letter are significantly different.

#### CA

#### One-way ANOVA: T22 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	20507.8	20507.8	117.68	0.000
Error	4	697.0	174.3		
Total	5	21204.8			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
13.2008	96.71%	95.89%	92.60%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	167.5 A	
D	3	50.57	В

Means that do not share a letter are significantly different.

#### LA

### One-way ANOVA: T22 (ms) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	6785	6785	5.79	0.095
Error	3	3513	1171		
Total	4	10298			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
34.2205	65.89%	54.52%	23.21%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean Grouping	
С	3	125.0 A	
D	2	49.77 A	

Means that do not share a letter are significantly different.

#### MTS

# One-way ANOVA: T22 (ms) versus Process

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	231.8	231.76	3.61	0.130
Error	4	256.4	64.11		
Total	5	488.2			

S	R-sq	R-sq(adj)	R-sq(pred)
8.00699	47.47%	34.34%	0.00%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean Grouping	
C	3	67.73 A	
D	3	55.30 A	

Means that do not share a letter are significantly different.

# One-way ANOVA: Lowry (mg/mL) versus Tenderization Type

Analysis of Varian	ıce	
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Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	11.0412	2.20825	261.36	0.000
Error	12	0.1014	0.00845		
Total	17	11.1426			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0919187	99.09%	98.71%	97.95%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grou	ping	
MTS	3	3.1768 A			
DW	3	1.2328	В		
CA	3	1.1191	В	C	
NT	3	1.1102	В	C	
LA	3	1.0344	В	C	
AA	3	0.9452		C	

Means that do not share a letter are significantly different.

# One-way ANOVA: OPA (g/100mL) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	5	0.010998	0.002200	54.11	0.000
Error	12	0.000488	0.000041		
Total	17	0.011486			

S	R-sq	R-sq(adj)	R-sq(pred)
0.0063759	95.75%	93.98%	90.44%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
MTS	3	0.08848 A		
CA	3	0.05736	В	
AA	3	0.03404	C	
NT	3	0.03129	C	D
DW	3	0.02172	C	D
LA	3	0.01587		D

Means that do not share a letter are significantly different.

Table 4.5 ANOVA and Tukey's Comparison Test with 95% confidence level for chicken

#### UNCOOKED

### One-way ANOVA: WBS (N) versus Tenderization Type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	620.75	206.918	45.49	0.000
Error	16	72.78	4.549		
Total	19	693.54			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.13281	89.51%	87.54%	85.04%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
MTS	8	19.148 A		
DW	5	17.088 A	В	
NT	4	13.605	В	
AA	3	2.703	C	

Means that do not share a letter are significantly different.

### COOKED

# One-way ANOVA: WBS (N) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	3623.2	1207.74	156.23	0.000
Error	18	139.2	7.73		
Total	21	3762.4			

S	R-sq	R-sq(adj)	R-sq(pred)
2.78040	96.30%	95.69%	94.64%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
DW	8	37.92 A	
MTS	4	27.21	В
NT	5	18.580	C
AA	5	4.590	D

Means that do not share a letter are significantly different.

#### **DIGESTED**

# One-way ANOVA: WBS (N) versus Tenderization Type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	3077.7	1025.90	181.90	0.000
Error	22	124.1	5.64		
Total	25	3201.8			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
2.37486	96.12%	95.60%	94.67%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
DW	8	33.75 A	
MTS	6	25.82	В
NT	6	9.862	C
AA	6	8.777	C

Means that do not share a letter are significantly different.

### CONTROL

### One-way ANOVA: WBS (N) versus Treatment

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	207.44	103.720	45.77	0.000
Error	12	27.19	2.266		
Total	14	234.63			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
1.50528	88.41%	86.48%	81.09%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
C	5	18.580 A	
U	4	13.605	В
D	6	9.862	C

#### DW

# One-way ANOVA: WBS (N) versus Treatment

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	1409.0	704.492	76.92	0.000
Error	18	164.9	9.159		
Total	20	1573.8			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
3.02633	89.53%	88.36%	86.11%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
C	8	37.92 A	
D	8	33.75	В
U	5	17.088	C

Means that do not share a letter are significantly different.

# AA

#### One-way ANOVA: WBS (N) versus Treatment

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	88.798	44.3991	66.21	0.000
Error	11	7.376	0.6705		
Total	13	96.174			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.818868	92.33%	90.94%	88.62%

### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Treatment	N	Mean	Grouping	
D	6	8.777 A		
C	5	4.590	В	
U	3	2.703	C	

Means that do not share a letter are significantly different.

#### MTS

### One-way ANOVA: WBS (N) versus Treatment

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	237.0	118.513	13.01	0.001
Error	15	136.6	9.106		
Total	17	373.6			

S	R-sq	R-sq(adj)	R-sq(pred)
3.01762	63.44%	58.57%	45.97%

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
C	4	27.21 A	
D	6	25.82 A	
U	8	19.148	В

Means that do not share a letter are significantly different.

#### UNCOOKED

# One-way ANOVA: T2 (ms) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	2899.1	966.37	59.97	0.000
Error	7	112.8	16.11		
Total	10	3011.9			

#### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
4.01423	96.25%	94.65%	86.88%

#### **Tukey Pairwise Comparisons**

**Grouping Information Using the Tukey Method and 95% Confidence** 

Tenderization Type	N	Mean	Grouping
AA	2	85.25 A	
MTS	3	46.01	В
DW	3	42.89	В
NT	3	41.3155	В

Means that do not share a letter are significantly different.

#### COOKED

#### One-way ANOVA: T2 (ms) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	4386.8	1462.28	72.80	0.000
Error	8	160.7	20.09		
Total	11	4547.5			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
4.48168	96.47%	95.14%	92.05%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
AA	3	67.15 A	
DW	3	23.709	В
MTS	3	22.908	В
NT	3	22.406	В

#### **DIGESTED**

### One-way ANOVA: T2 (ms) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	869.89	289.963	177.18	0.000
Error	8	13.09	1.637		
Total	11	882.98			

### **Model Summary**

	S R-so	$\mathbf{R}$ -sq(adj)	R-sq(pred)
1.2792	26 98.52%	6 97.96%	96.66%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Group	ping
AA	3	41.861 A		
NT	3	24.778	В	
DW	3	22.93	В	C
MTS	3	20.053		C

Means that do not share a letter are significantly different.

#### **CONTROL**

#### One-way ANOVA: T2 (ms) versus Process

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	2	636.689	318.345	729.74	0.000
Error	6	2.617	0.436		
Total	8	639.307			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.660486	99.59%	99.45%	99.08%

### **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Process	N	Mean	Group	ing	
U	3	41.3155 A			
D	3	24.778	В		
C	3	22.406		C	

Means that do not share a letter are significantly different.

### DW

### One-way ANOVA: T2 (ms) versus Process

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	2	767.00	383.501	60.90	0.000
Error	6	37.79	6.298		
Total	8	804.79			

S	R-sq	R-sq(adj)	R-sq(pred)
2.50950	95.30%	93.74%	89.44%

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping	
U	3	42.89 A		
C	3	23.709	В	
D	3	22.93	В	

Means that do not share a letter are significantly different.

AA

### One-way ANOVA: T2 (ms) versus Process

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	2	2377.0	1188.48	25.06	0.002
Error	5	237.1	47.42		
Total	7	2614.1			

**Model Summary** 

 S	R-sq	R-sq(adj)	R-sq(pred)
6.88624	90.93%	87.30%	74.18%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
U	2	85.25 A	
C	3	67.15 A	
D	3	41.861	В

Means that do not share a letter are significantly different.

MTS

# One-way ANOVA: T2 (ms) versus Process

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	2	1215.90	607.950	402.18	0.000
Error	6	9.07	1.512		
Total	8	1224.97			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.22949	99.26%	99.01%	98.33%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping	
U	3	46.01 A		
C	3	22.908	В	
D	3	20.053	В	

# UNCOOKED

# One-way ANOVA: pH of Meat versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	9.32949	3.10983	612.77	0.000
Error	8	0.04060	0.00507		
Total	11	9.37009			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0712390	99.57%	99.40%	99.03%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type N Mean Grouping
MTS 3 6.2600 A
NT 3 6.2567 A
DW 3 6.16667 A
AA 3 4.1933 B

Means that do not share a letter are significantly different.

#### **COOKED**

### One-way ANOVA: pH of Meat versus Tenderization Type

# **Analysis of Variance**

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	14.5149	4.83829	883.71	0.000
Error	8	0.0438	0.00548		
Total	11	14.5587			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0739932	99.70%	99.59%	99.32%

# **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Tenderization Type	N	Mean	Grouping
DW	3	6.6567 A	
NT	3	6.5867 A	
MTS	3	6.53000 A	
AA	3	4.0533	В

Means that do not share a letter are significantly different.

### CONTROL

# One-way ANOVA: pH of Meat versus Process

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0.16335	0.163350	56.65	0.002
Error	4	0.01153	0.002883		
Total	5	0.17488			

S	R-sq	R-sq(adj)	R-sq(pred)
0.0536967	93.41%	91.76%	85.16%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	6.5867 A	
U	3	6.2567	В

Means that do not share a letter are significantly different.

#### DW

#### One-way ANOVA: pH of Meat versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0.36015	0.360150	56.42	0.002
Error	4	0.02553	0.006383		
Total	5	0.38568			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0798958	93.38%	91.72%	85.10%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	6.6567 A	
U	3	6.16667	В

Means that do not share a letter are significantly different.

#### ΔΔ

### One-way ANOVA: pH of Meat versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0.02940	0.029400	6.63	0.062
Error	4	0.01773	0.004433		
Total	5	0.04713			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.0665833	62.38%	52.97%	15.35%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	${f N}$	Mean Grouping	
U	3	4.1933 A	
C	3	4.0533 A	

#### MTS

#### One-way ANOVA: pH of Meat versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0.10935	0.109350	14.78	0.018
Error	4	0.02960	0.007400		
Total	5	0.13895			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0860233	78.70%	73.37%	52.07%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	6.53000 A	
U	3	6.2600	В

Means that do not share a letter are significantly different.

#### UNCOOKED

# One-way ANOVA: Lightness (L\*) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	237.335	79.112	64.22	0.000
Error	8	9.855	1.232		
Total	11	247.190			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.10989	96.01%	94.52%	91.03%

#### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Tenderization Type	N	Mean	Grouping
DW	3	-26.90 A	
MTS	3	-28.903 A	
AA	3	-33.007	В
NT	3	-38.530	C

Means that do not share a letter are significantly different.

### UNCOOKED

#### One-way ANOVA: Redness (a\*) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	4.89683	1.63228	425.81	0.000
Error	5	0.01917	0.00383		
Total	8	4.91600			

S	R-sq	R-sq(adj)	R-sq(pred)
0.0619139	99.61%	99.38%	98.96%

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
NT	2	-0.1950 A	
MTS	2	-0.6800	В
DW	2	-0.8450	В
AA	3	-2.0633	C

Means that do not share a letter are significantly different.

#### UNCOOKED

# One-way ANOVA: Yellowness (b\*) versus Tenderization Type

**Analysis of Variance** 

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	81.25	27.082	15.86	0.002
Error	7	11.95	1.708		
Total	10	93.20			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.30673	87.17%	81.68%	71.01%

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
NT	3	9.507 A	
DW	3	9.047 A	
MTS	3	8.07 A	
AA	2	1.970	В

Means that do not share a letter are significantly different.

### COOKED

### One-way ANOVA: Lightness (L\*) versus Tenderization Type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	165.538	55.1795	144.70	0.000
Error	8	3.051	0.3813		
Total	11	168.589			

### **Model Summary**

 S	R-sq	R-sq(adj)	R-sq(pred)
0.617515	98.19%	97.51%	95.93%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping	
DW	3	-11.937 A		
NT	3	-13.6933	В	
MTS	3	-15.950	C	
AA	3	-21.783	D	

#### COOKED

# One-way ANOVA: Redness (a\*) versus Tenderization Type

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	32.672	10.8908	58.35	0.000
Error	6	1.120	0.1867		
Total	9	33.792			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.432043	96.69%	95.03%	92.50%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
MTS	2	2.5650 A	
DW	3	1.787 A	
NT	2	1.7550 A	
AA	3	-1.890	В

Means that do not share a letter are significantly different.

#### **COOKED**

### One-way ANOVA: Yellowness (b\*) versus Tenderization Type

# **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	142.280	47.4267	72.93	0.000
Error	7	4.552	0.6503		
Total	10	146.832			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.806390	96.90%	95.57%	92.97%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
MTS	3	20.443 A	
NT	3	15.687	В
DW	3	15.0033	В
AA	2	9.645	C

Means that do not share a letter are significantly different.

### CONTROL

# One-way ANOVA: Lightness (L\*) versus Process

# **Analysis of Variance**

inalysis of variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	925.290	925.290	2445.49	0.000
Error	4	1.513	0.378		
Total	5	926.803			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.615115	99.84%	99.80%	99.63%

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean Grouping
C	3	-13.6933 A
U	3	-38.530 B

Means that do not share a letter are significantly different.

#### CONTROL

#### One-way ANOVA: Redness (a\*) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	3.80250	3.80250	1690.00	0.001
Error	2	0.00450	0.00225		
Total	3	3.80700			

### **Model Summary**

	S R-sq	R-sq(adj)	R-sq(pred)
0.047434	2 99.88%	99.82%	99.53%

#### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	2	1.7550 A	
U	2	-0.1950	В

Means that do not share a letter are significantly different.

#### CONTROL

### One-way ANOVA: Yellowness (b\*) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	57.289	57.289	32.81	0.005
Error	4	6.985	1.746		
Total	5	64.274			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
1.32149	89.13%	86.41%	75,55%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	15.687 A	
U	3	9.507	В

Means that do not share a letter are significantly different.

#### DW

### One-way ANOVA: Lightness (L\*) versus Process

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	336.002	336.002	200.10	0.000
Error	4	6.717	1.679		
Total	5	342.718			

	S	R-sq	R-sq(adj)	R-sq(pred)
_	1.29581	98.04%	97.55%	95.59%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	-11.937 A	
U	3	-26.90	В

Means that do not share a letter are significantly different.

#### DW

### One-way ANOVA: Redness (a\*) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	8.3108	8.31080	223.98	0.001
Error	3	0.1113	0.03711		
Total	4	8.4221			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.192628	98.68%	98.24%	96.94%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	1.787 A	
U	2	-0.8450	В

Means that do not share a letter are significantly different.

#### DW

### One-way ANOVA: Yellowness (b\*) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	53.223	53.2228	91.43	0.001
Error	4	2.329	0.5821		
Total	5	55.551			

#### **Model Summary**

	S	R-sq	R-sq(adj)	R-sq(pred)
0.76	52977	95.81%	94.76%	90.57%

# **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	15.0033 A	
U	3	9.047	В

#### AA

# One-way ANOVA: Lightness (L\*) versus Process

#### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	188.945	188.945	281.49	0.000
Error	4	2.685	0.671		
Total	5	191.630			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.819288	98.60%	98.25%	96.85%

#### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
С	3	-21.783 A	
U	3	-33.007	В

Means that do not share a letter are significantly different.

#### AA

### One-way ANOVA: Redness (a\*) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	0.04507	0.04507	0.18	0.696
Error	4	1.01907	0.25477		
Total	5	1.06413			

### **Model Summary**

	S	R-sq	R-sq(adj)	R-sq(pred)
0.	504744	4.24%	0.00%	0.00%

#### **Tukey Pairwise Comparisons**

# **Grouping Information Using the Tukey Method and 95% Confidence**

Process	N	Mean Grouping	
C	3	-1.890 A	
U	3	-2.0633 A	

Means that do not share a letter are significantly different.

#### AA

### One-way ANOVA: Yellowness (b\*) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	58.9056	58.9056	979.72	0.001
Error	2	0.1202	0.0601		
Total	3	59.0259			

 S	R-sq	R-sq(adj)	R-sq(pred)
0.245204	99.80%	99.69%	99.19%

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	2	9.645 A	
U	2	1.970	В

Means that do not share a letter are significantly different.

#### MTS

#### One-way ANOVA: Lightness (L\*) versus Process

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	251.683	251.683	505.78	0.000
Error	4	1.990	0.498		
Total	5	253.674			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.705419	99.22%	99.02%	98.23%

### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
С	3	-15.950 A	
U	3	-28.903	В

Means that do not share a letter are significantly different.

#### MTS

### One-way ANOVA: Redness (a\*) versus Process

#### **Analysis of Variance**

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
Process	1	10.5300	10.5300	4955.31	0.000
Error	2	0.0043	0.0021		
Total	3	10.5343			

#### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0460977	99.96%	99.94%	99.84%

#### **Tukey Pairwise Comparisons**

### **Grouping Information Using the Tukey Method and 95% Confidence**

Process	N	Mean	Grouping
C	2	2.5650 A	
U	2	-0.6800	В

Means that do not share a letter are significantly different.

# MTS

# One-way ANOVA: Yellowness (b\*) versus Process

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Process	1	229.525	229.525	129.85	0.000
Error	4	7.071	1.768		
Total	5	236.596			

S	R-sq	R-sq(adj)	R-sq(pred)
1.32952	97.01%	96.26%	93.28%

### **Tukey Pairwise Comparisons**

### Grouping Information Using the Tukey Method and 95% Confidence

Process	N	Mean	Grouping
C	3	20.443 A	
U	3	8.07	В

Means that do not share a letter are significantly different.

# One-way ANOVA: Lowry (mg/mL) versus Tenderization Type

### **Analysis of Variance**

Source	$\mathbf{DF}$	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	15.7128	5.23761	143.88	0.000
Error	8	0.2912	0.03640		
Total	11	16.0041			

# **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.190796	98.18%	97.50%	95.91%

# **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence

Tenderization Type	N	Mean	Grouping
MTS	3	5.287 A	
AA	3	2.8863	В
NT	3	2.6367	В
DW	3	2.476	В

Means that do not share a letter are significantly different.

# One-way ANOVA: OPA (mg/mL) versus Tenderization Type

### **Analysis of Variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tenderization Type	3	2.95647	0.985489	207.46	0.000
Error	8	0.03800	0.004750		
Total	11	2.99447			

### **Model Summary**

S	R-sq	R-sq(adj)	R-sq(pred)
0.0689227	98.73%	98.25%	97.14%

### **Tukey Pairwise Comparisons**

# Grouping Information Using the Tukey Method and 95% Confidence $\,$

Tenderization Type	N	Mean	Grouping
MTS	3	2.6096 A	
AA	3	1.7037	В
DW	3	1.4324	C
NT	3	1.3668	C

# C. Extra Figures

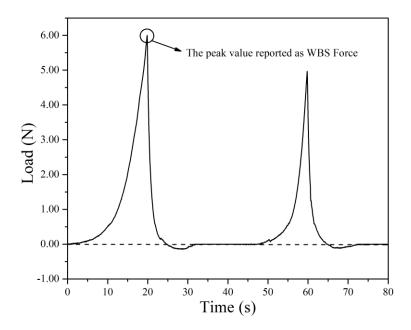


Figure 4.3. Example texture profile analysis curve that is output of WBS measurement