

THE EFFECTS OF MULTIPLE FURTHER PROCESSING TECHNIQUES ON OBJECTIVE  
TEXTURAL CHARACTERISTICS OF WOODY BREASTED FILLETS

by

JAMISON WILLIAMS

(Under the Direction of Alexander Stelzleni)

ABSTRACT

To ameliorate textural defects associated with the wooden breast myopathy, multiple forms of further processing were investigated in succession. Breast fillets were sorted to three categories based on wooden breast severity via manual palpation: normal (NORM), moderate (MOD), and severe (SEV). Four further processing treatments were applied to all severities: no treatment (CON), blade tenderization (BT), blade tenderization and vacuum marination (BTM), and blade tenderization, injection enhancement, and vacuum marination (BTIM). Prior to processing treatment application NORM, MOD, and SEV differed for compression scores, 21.46 N, 33.37 N, and 50.05 N, respectively ( $P < 0.05$ ). After processing, SEV fillets subjected to BTIM and BTM did not differ ( $P \geq 0.55$ ) in marinade uptake compared to MOD fillets subjected to BTM, but regardless of processing treatment SEV fillets had impaired uptake compared to NORM fillets ( $P < 0.05$ ). Additionally, peak force, measured via multiple blade shear, for SEV fillets that underwent more aggressive further processing did not differ ( $P \geq 0.07$ ) when compared to MOD fillets regardless of treatment and NORM fillets except those subjected to BTIM. When fillets were subjected to texture profile analysis, cooked fillet hardness was not different between processing treatments ( $P = 0.07$ ). Only a difference within severity was noted with SEV being greater ( $P < 0.05$ ) than NORM which was greater ( $P < 0.05$ ) than MOD.

Overall, the negative attributes associated with WB fillets may be mitigated when breasts undergo multiple forms of processing. Although SEV fillets had deficits when compared to NORM fillets, the industry may be able to salvage borderline or MOD fillets through further processing.

INDEX WORDS: Woody breast, further processing, texture, injection enhancement, marination, blade tenderization, poultry

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JAMISON WILLIAMS

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JAMISON WILLIAMS

Major Professor: Alexander Stelzleni  
Committee: Harshvadha Thippareddi  
John Gonzalez

Electronic Version Approved:

Ron Walcott  
Vice Provost for Graduate Education and Dean of the Graduate School  
The University of Georgia  
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## DEDICATION

I dedicate this work to my mother Shary Williams. Everything I do is with the incredible support of you behind me. Even though at times things may be strenuous, I have watched you overcome any obstacle in your way. This ability to overcome hardships through work and dedication are what drives me to continue forward and hope to achieve great things. Additionally, to Amber Adams, I am sure we will have an amazing future together and cannot wait for you to be my wife. Amber, you have truly kept me grounded and driven at times when I think I am not enough. You have been one of the greatest things in my life, and I am forever grateful. I cannot thank the people around me enough for giving me the support and opportunities needed for me to succeed.

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## CHAPTER 1

### INTRODUCTION

Poultry consumption with the United States continues to increase due to a multitude of factors such as health concerns, cost, and time (Mottet and Tempio, 2017; Cavani et al., 2016). With the increased demand, the poultry industry has changed broiler production to select for broilers strain exhibiting increased feed efficiency, growth rate, and yield, especially in popular cuts such as the breast meat (Petracci et al., 2015; Kuttappan et al., 2016). Though the industry saw increased yield and success, the increased strain on broilers has not gone unnoticed especially with the increased emergence of myopathies (Kuttappan et al., 2016). One of the most notable and detrimental myopathies is woody breast myopathy (**WBM**) which may be accompanied by other common myopathies like white striping (**WS**).

Woody breast myopathy was first documented in 2014 and has become an increasing issue within the poultry industry as there are predicted losses of \$200 million or higher (Sihvo et al., 2014; Kuttappan et al., 2016). The myopathy is characterized as an irregular hardening of the caudal tissue on the pectoralis major of effected broilers (Sihvo et al., 2014). Additionally, the irregular hardening which is accompanied and caused by a diffuse myodegenerative lesion with collagen and lipid infiltration and other compositional changes that lead to issues with recapturing value due to poor technological quality (Soglia et al., 2016a). In addition, poor sensory attributes such as increased hardness and fibrousness tend to lead to an inferior product within consumer perceptions (Mudalal et al., 2015; Puolanne and Ruusunen, 2014). Past research has investigated the remediation of woody fillets through a single method of further processing like marination, tumbling, blade tenderization, or injection enhancement (Bowker et al., 2018;

Maxwell et al., 2018; Tasoniero et al., 2019). Little improvement has been noted with the usage of single method processing procedures which may indicate that no one method can overcome the negative qualities associated with WBM. (Aguirre et al., 2019; Bowker et al. 2018). The stacked effects of multiple further processing techniques are yet to be investigated, so it is currently unknown whether the combined efforts may be able to overcome the deficiencies seen with woody breasted fillets.

The objective of this study was to investigate whether a multi-faceted approach to further processing would be successful in the remediation of fillets affected by the woody breast condition. The increased amount of processing would hopefully ameliorate negative textural issues associated with woody breasted chicken, and the addition of marinades may help with poor water holding capacity seen in effected fillets.

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## CHAPTER 2

### A REVIEW OF THE LITERATURE

#### **2.1 Industry background**

Within recent years, the meat industry has undergone massive changes due to a push for healthier alternatives from red meat species as increasing cardiovascular and other chronic diseases have pushed many to a change in lifestyle (Wolk, 2017). This has pushed the industry to look for healthier processing techniques that add functional ingredients to products such as fiber fortification or probiotics to reduce associated risk of eating a high red meat diet (Toldrá and Reig, 2011). Additionally, as consumers have decreased red meat consumption the industry has and will continue to observe a global increase in poultry consumption due to the perceived leanness and relatively low cost (Mottet and Tempio, 2017; Cavani et al., 2016). With this increased demand, the industry has pushed for high yield lines and extremely fast growth which unfortunately has led to increased incidence of pale, soft, and exudative (**PSE**) meat and increased incidence of myopathies (Barbut et al., 2008; Petracci and Cavani, 2012).

Multiple myopathies have become prevalent in poultry meat: deep pectoral myopathy (**DPM**), white striping (**WS**), woody-breasted (**WB**), and spaghetti meat (**SM**) myopathies. Woody breast poultry products are becoming more common, and according to Petracci and Cavani (2012) have potential to cost the industry millions in profit due to downgrading of product. This massive potential for loss calls for an immediate remedy. Many have investigated the potential genetic components contributing to this myopathy, but no conclusive solution has yet to be determined (Mutryn et al., 2015). A nutritional component may also be associated but again no conclusive solution has yet to be determined (Livingston et al., 2019). The most

immediate form of remediation would be a processing intervention to correct or mitigate the reduced quality of product. Though current research indicates whole muscle remediation may have little effect on the textural issues associated with the myopathy (Bowker et al., 2018; Maxwell et al., 2018; Tasoniero et al., 2019). This review aims to highlight potential causes and solutions for WB chicken and investigate potential antemortem processing techniques to mitigate negative attributes of affected products.

## 2.2 Myopathies

One of the major muscular myopathies plaguing the industry is WS which is categorized as the presence of white striations, typically veins of intramuscular fat, running parallel to the muscle fibers on the ventral side of the *pectoralis major* in broilers (Bauermeister et al., 2009). Kuttappan et al. (2012b) further categorized WS by creating a visual evaluation in which breasts could be labeled as: normal (no distinct white striations), moderate (striations < 1mm thick), and severe (striations > 1 mm thick). As the industry has evolved WS prevalence has increased without a single causative factor. Instead, multiple factors like sex, growth rate, diet, and genetics play pivotal roles in the development of the myopathy (Petracci, 2013; Kuttappan et al. 2012a; 2013; Lorenzi et al., 2014). Additionally, Kuttappan et al. (2012b) reported that broilers from high breast meat yield lines raised under a high energy diet can have WS incidence as high as 50% in some flocks. Understanding the emergence and causes of this myopathy is vital as quality changes associated with WS may be indicative of other emerging myopathies.

Breasts displaying the WS myopathy typically do not have severe quality changes rather they have increased muscle weight and thickness compared to normal fillets (Kuttappan et al., 2013). Brewer et al. (2012) also noticed a significant correlation between increased fillet weights and increased cranial thickness. Though on a visual level these issues may seem miniscule, histological observation indicated that WS involves some degradation of the muscle fibers

allowing for lipidosis, fibrosis, and interstitial inflammation. These alterations in the muscle may contribute to additional differences such as increased pH, elevated lipid content, and increased collagen content within fillets affected by WS (Mudalal et al. 2014; Petracci et al., 2014). Additionally, Sihvo et al. (2014) theorized that degeneration of the myofibers may contribute to increasing pH by altering the glycogen content of fillets decreasing or modifying the onset of acidification postmortem. Bauermeister et al. (2009) reported that the elevated pH seen in WS did not significantly impact WHC of effected breasts, yet Petracci et al. (2013) reported that fillets with WS had decreased WHC and poor texture when compared to normal fillets. Though in prior research, Petracci et al. (2004) reported that increased pH typically was associated with increased WHC. Also, fillets with WS displayed reduced protein resulting in increased cooking losses, low marinade retention, and decreased protein functionality during processing (Petracci et al. 2013, Mudalal et al. 2014, Petracci et al., 2014). With so many macroscopic and microscopic differences, fillets effected by WS are easily downgraded if the striation is severe enough not only due to visual defects but potential functional defects.

Wooden breast myopathy (**WBM**) primarily affects the *pectoralis major* in lines that have selected rapid growth and high-breast-yield broiler chickens, but is not universal to just one area, rather it has been seen on a global scale despite having only recently been discovered (de Brot et al., 2016; Mutryn et al., 2015; Sihvo et al., 2014). As of now the complete pathogenesis of WBM is yet to be understood. Sihvo et al. (2014) reported that the myopathy did not fall within any previous known etiologies marking the diffuse hardening of the breast tissue was extremely irregular and that the myodegenerative lesion associated with WB had an inflammatory response not seen with other myopathies caused by issues like nutritional deficits. The lesion seen within WB samples was notably accompanied by excessive collagen infiltration. There was some vascular wall damage to surrounding veins; however, no damage appeared near

arteries. Mutryn et al. (2015) hypothesized that it is possible that the lesion is not necessarily instigated by tissue degeneration from lack of nutrients but rather from an inability to properly dispose of cellular metabolic waste products. This inability to efficiently dispose of waste products may ultimately be triggering further damage to surrounding tissues. Additionally, a moderate amount of inflammatory cell infiltration was seen near the myodegenerative lesion indicating some inflammatory process may be involved though this is not yet fully understood (Sihvo et al., 2014). This inflammatory response may also contribute to the theorized hypoxic state within the tissue as it may contribute to vascular injury and damage (Chen et al., 2019).

Chen et al. (2019) reported lymphocytic phlebitis may precede lesion development. This conclusion stems from non-modern meat-type broilers exhibiting widespread and sometimes severe lymphocytic phlebitis within breast samples. Though, it is important to note that no sample displayed a severe amount of WB. Chen et al. (2019) concluded that lymphocytic phlebitis may contribute to muscle injury by altering blood perfusion leading to hypoxia, but further investigation is needed to evaluate whether lymphocytic phlebitis contributes to a secondary lesion or whether it plays any role in pathogenesis. These results are strengthened by Sihvo et al. (2018) that targeted early pathogenesis of WBM lesions identifying reduced vessel density and myofiber ultrastructure changes consistent with hypoxia and possibly ischemia. Additionally, Mutryn et al. (2015) identified that broilers exhibiting WB showed altered gene expression that suggested hypoxia, increased amounts of oxidative stress, and even fiber type switching from a fast-to-slow fiber type.

Although the microscope etiology remains obscure, there are some distinguishing traits seen within WB fillets. Many note that a distinguishable characteristic of affected breasts is increased weight often being significantly different from breasts considered normal or even displaying what is considered moderate severity (Dalle Zotte et al., 2017; Zhuang and Bowker,

2018; Clark and Velleman, 2016). Wooden breast fillets display increased pH when compared to normal fillets which may be attributed to the poor glycogen storing capacity and altered glucose metabolism seen in these birds leading to a reduced pH decline (Soglia et al. 2016; Abasht et al., 2016). Beyond decreased pH, there are notable differences in chemical composition and muscle morphometry between WB fillets and normal ones.

Breasts exhibiting WBM are notably different in not only physical appearance, but also chemical composition changes with severity. Multiple studies have noted differences in moisture content as WB fillets exhibit a decreased moisture percentage when compared to normal fillets; however, this may be attributed to other disparities seen within the breast's chemical compositions (Baldi et al., 2019). A possible explanation may be due to increased lipid content seen within WB fillets which is theorized to be caused due to excessive lipidoses due to the myodegenerative lesion. Additionally, WB fillets exhibit reduced protein content throughout the breast again due to the lesion. Along with this it seems that much of the protein that is still within the breast may have impaired functionality. Wooden breast fillets also exhibit increased collagen when compared to their normal counterparts which may explain some of the excessive hardening. Additionally, collagen formation was irregular and not organized as well as tightly packed which may explain the fibrotic nature of the breast as this leads to highly cross-linked and non-pliable collagen (Velleman et al., 2017). Baldi et al. (2019) also noted increased intramuscular collagen content in WB fillets compared to normal fillets as well as increased mature collagen content which may indicate increased toughness and poor sensory attributes in affected breasts.

Along with notable differences in breast composition, WB fillets display some histological and morphological differences as well. Dalle Zote et al. (2017) noted that affected breasts typically displayed a larger cross-sectional area when compared to their normal

counterparts with many of those fibers being considered giant-type fibers with many of these fibers also displaying increased cross-sectional areas. Due to these differences' fiber counts were reduced within the WB fillets compared to normal fillets. Dalle Zote et al. (2017) additionally noted that these cells were in a hyper-contracted state compromising cell organelles and cell stability. Additionally, NMR analysis indicated that WB fillets had decreased amounts of tightly bound water especially within the superficial muscle which may be due to the poor structure associated with the myodegenerative lesion (Baldi et al., 2019). Along with this decreased tightly bound water an excess of extra-myofibrillar water was seen in WB fillets when compared to normal fillets due to increased exudate. The most abnormal find in NMR analysis was an increased amount of bound water; however, Baldi et al. (2019) theorized the water may not be representative of protein hydration but rather water binding with connective tissue components like collagen. Additionally, relaxation times support the aforementioned results as higher relaxation times for bound water indicate that the water bound to proteins was more loosely bound than water that may be connected to connective tissues.

### **2.3 Genetics**

As mentioned, the continued push for large, fast-growing broilers has increasingly led to meat quality issues especially regarding modern myopathies (Dransfield and Sosnicki, 1999; Kuttappan et al., 2017). As myopathy prevalence has increased a surge in viewing the potential genetic components and solutions has grown. Mutryn et al. (2015) investigated and identified the biological mechanisms that contribute to the development of WB. Through RNA-sequencing multiple genes were found that may indicate an increased hypoxic and oxidative state within WB broilers indicating that the lesion may be exacerbated by cellular death via hypoxia. Additionally, genes regulating calcium sequestration and regulation are irregular compared to normal breasts. Mutryn et al. (2015) attribute these defects to causing hypercontraction of the

surrounding muscle tissue ultimately leading to necrosis and myodegeneration. Genes relating to increased collagen accretion and fibrosis are also upregulated in WB birds agreeing with excessive collagen infiltration seen by Sihvo et al. (2014). Overall, though Mutryn et al. (2015) could not conclusively identify a cause of the myopathy the results highlight additional factors into what may be causing this issue and where to start looking for a solution.

## 2.4 Nutritional Aspects

Beyond looking at simple genetic explanations, the poultry industry has heavily investigated nutritional deficiencies as a source of emerging myopathies. One of the largest reasons for this investigation is evidence of myodegeneration within muscles of birds suffering from either vitamin E ( $\alpha$ -tocopherol), sulfur amino acid, and selenium deficiencies (Klasing and Korver, 2020). This disease known as nutritional myopathy has similar presentation and histological attributes as WBM (Kuttappan et al., 2013). A problematic issue with this theory would be that modern diets are well equipped to meet all requirements and prevent nutritional myopathy as vitamin E and sulfur containing amino acids are all present at sufficient levels (National Research Council, 1994). Guetchom et al. (2012) reported that an addition of vitamin E to the diet would reduce muscle fiber damage, but Kuttappan et al. (2012a) reported contrasting results in which even excessive feeding of vitamin E could not prevent the occurrence of WS or muscle damage. Although these results differ, it becomes important to note that vitamin E deficiency does not only present as muscle degeneration (Klasing and Korver, 2020). Other symptoms such as encephalomalacia and exudative diathesis are typically not seen in flocks plagued by WS and WBM. Though selenium deficiency presents in a similar manner the role of selenium in regard to the myodegeneration of the *pectoralis major* remains largely uninvestigated (Sivho et al., 2014).

Nutritional deficiencies are not the only aspect of the diet researchers have investigated as high performance and high growth diets may exacerbate myopathies. Kuttappan et al. (2013) reported that birds fed high energy and protein rich diets had increased incidence rates of breast myopathies. This revelation reinforces the idea that fast growing and large breasted birds may be the largest factor in WBM. Given that no conclusive evidence shows where WBM originates, producers may be cautioned against pushing for increased efficiency and growth rate at this time while further investigations are conducted.

### **2.5 Meat Quality**

As the industry has progressed and demand has increased, the need for a larger, faster-growing broiler has become a necessity, although this necessity has come with many drawbacks. Dransfield and Sosnicki (1999) reported that increased yield would negatively impact muscle tissue. One consequence of increased muscle size was that the muscles would effectively outgrow the vascular system leading to necrosis or myodegeneration (Wilson, 1990). An irregular fiber type pattern was also noted in poultry as the *pectoralis major* is almost entirely composed of Type IIB glycolytic fibers that may contribute to decreased quality due to irregular postmortem events (Dransfield and Sosnicki, 1999;). Typically, rigor mortis onset is an extended process taking a long time, but due to the high number of glycolytic fibers poultry meat may complete the process in just a few hours or sooner (Dransfield and Sosnicki, 1999).

### **2.6 Meat Color**

With WB being a relatively new myopathy, color differences between normal and wooden breast fillets are not yet fully understood. Mudalal et al. (2015) reported that breast affected by the WS and WB conditions were increasingly lighter than their normal counterparts but recorded that there were ultimately no relevant color changes. In contrast, Zhuang et al. (2018) reported that WB fillets had significant differences in color when compared to normal

breasts. Wooden-breast fillets showed significant differences in  $L^*$ ,  $a^*$ , and  $b^*$  values when compared to normal fillets. Effected breasts displayed a decreased  $L^*$  value while having increased  $a^*$  and  $b^*$  indicating these breasts tended to be paler in comparison to normal fillets. Additionally, they theorized the increased redness seen by the increased  $a^*$  values may be attributed to the increased hemorrhaging seen in WB fillets. These results agree with previous results by Sihvo et al. (2014) who reported that breasts had a lighter pigmentation with multiple hemorrhages seen among the surface of the breast. Additionally, Zhuang et al. (2018) reported that though no observational data was collected visually all breasts affected by WB had a noticeable visual defect when compared to normal breasts. They also acknowledged that changes with coloration were only present on the ventral, or skin side, of the breast as the dorsal, or bone side, displayed  $L^*$ ,  $a^*$ , and  $b^*$  values similar to that of normal breasts.

In addition to some noted differences in raw breast color, newer data suggests that cooked breasts may also display some noticeable color differences. Zhuang and Bowker (2018) found that within cooked breasts those categorized as WB fillets had a 6 unit decrease in  $L^*$  values on the ventral side when compared to normal fillets. As well as decreased  $L^*$ , WB fillets were noted to have increased  $a^*$  and  $b^*$  as high 0.5 and 2 units respectively, indicating a significant difference in surface color after cooking. These differences indicate a darkened breast with a more yellow and red or brown appearance. These color differences were not entirely unexpected due to such drastic differences within raw product color, and the presence of petechial hemorrhages along the ventral side of the breast may provide some explanation for the increased  $a^*$ . Zhuang and Bowker (2018) theorized that not only would the areas of hemorrhages cause differences in surface discoloration, but altered surface properties such as white striations, increased connective tissues, and a gelatinous fluid underneath the epimysium may account for some darkening as well (Sihvo et al., 2014, Dalle Zote et al., 2017, Kuttappan et al., 2017). One

of the biggest concerns would be consumer acceptance of whole muscled products exhibiting this surface discoloration as consumers negatively associated abnormal coloration like excessive greying or tanning with inferior quality (Fletcher, 1999). As of now, no consumer panels have been performed on WB fillets; however, Kuttappan et al. (2012b) reported that increased severity of WS negatively impacted consumers intent to purchase affected breasts as consumers associated them with a fattier inferior product. These issues may cause future problems as Fletcher (2002) noted that meat color continues to be the largest factor influencing consumer purchasing habits.

## **2.7 Meat Texture**

Meat texture is ranked as a major quality concern with broiler breast fillets as it is major sensory factor affecting overall consumer satisfaction and future purchasing habits (Sams, 1999). Additionally, consumers have pushed for further processed products like nuggets, luncheon meats, and many other ready-to-cook or ready-to-eat products due to increased desire for convenience and ease of cooking. Beyond a shift in consumer preferences, texture is not a simple attribute as it can be affected by multiple other quality concerns like PSE, pH, additional myopathies, or even improper processing. To improve texture the industry has implemented multiple strategies to reduce water loss, increase muscle fiber disruption, or alter pH and subsequently WHC (Alvarado and McKee, 2007). The desire for chicken meat has drastically changed the industry as mentioned since faster growing and higher yielding birds may ultimately lead to an increase in muscle myopathies especially those like WB which drastically alter texture.

The increased emergence of WB has negatively impacted the poultry industry as the meat affected by this myopathy must be downgraded or even tossed aside due to its inherently negative qualities (Pettracci et al., 2015; Kuttappan et al., 2016). The lesions seen within the

tissue and the excessive infiltration of collagen lead to an increased hardness that could render the breast almost inedible as a whole muscle product if the severity is great enough as consumers may find the texture unfavorable. Brambila et al. (2018) reported that multiple sensory characteristics specifically springiness, cohesiveness, hardness, and fibrousness may be negatively impacted by WB condition. Aguirre et al. (2018) had similar results to that of Brambila et al. (2018) noting a negative impact on the textural characteristics. All textural and sensory characteristics were derived from Meilgaard et al. (2007), Rutledge (2004), and Brambila (2018). Springiness was defined as the force with which a sample would return to original size/shape after initial compression and was ranked from mushy to very springy. Cohesiveness was defined as the distance a panelist could bite into a sample prior to the sample breaking, cracking, or crumbling and was ranked from not cohesive to very cohesive. Hardness was defined as the amount of force required to compress a sample with one's molar in the first two bites and was ranked from not hard to very hard. Fibrousness was defined as the amount of grinding needed to break through filaments or strands of muscle fiber and was ranked from low fibrous to high fibrous. Additionally, WB fillets typically had increased textural profile analysis values indicating a hardened or firm texture when compared to normal fillets (Aguirre et al., 2018).

Additionally, objective analyses yield similar results as WB fillets exhibit exacerbated traits of excessive hardening when compared to normal breasts. Chatterjee et al. (2016) noted that for raw breasts multiple forms of textural analysis, Meullenet-Owens Razor Shear (MORS) and textural profile analysis (TPA), yield similar results indicating that breasts exhibiting WBM were tougher than normal breasts. Using the MORS technique, they were able to note that regardless of degree of woodiness, fillets would have increased shear force values. Additionally, they noted an increase in shear energy needed to sever muscle fibers in woody fillets compared

to normal fillets. A limitation noted within the study though was the inability of the MORS method to differentiate between moderate and severe woodiness. These results conform with similar findings as Mudalal et al. (2015) reported increased shear force values in WB fillets compared to normal fillets as well when using Warner-Bratzler shear force analysis.

Additionally, Bowker et al. has reported increased compression force values in raw WB fillets when compared to normal fillets.

When examining additional textural traits, TPA has become the preferred analysis as it yields more information than force measurements. Texture profile analysis allows for the acquisition of data on a products hardness, cohesion, adhesiveness, chewiness, gumminess, springiness, and resilience; although, some traits are independent of one another and cannot be measured together (Texture Profile Technologies, unpublished). Chatterjee et al. (2018) noted increased hardness, the amount of force required to compress a material, in cooked WB fillets when compared to normal fillets, but noted no difference in hardness between raw fillets. They also noted a difference in cohesion, the strength of the internal bonds within the product, as cooked WB fillets had increased cohesion when compared to normal fillets. Additionally, they noted increased springiness, resilience, and chewiness in cooked breasts versus raw breasts but noted no differences between WB severity. Similar to MORS, TPA was unable to differentiate between WB severity. This may present some issues with objective measurement of WB fillets as the myopathy may not be fully elucidated through only objective analysis.

Further issues with TPA may be noted as the method, though approved for usage in meat products, was never fully intended for the product. Texture profile analysis involves the usage a rheometer which is intentionally made for non-Newtonian fluids but has frequently been adapted for many other products as it can test: tensile strength, compression, elasticity, etc. if the appropriate setting and head is attached (Fisher and Windhab, 2011). Many note that frequently

this analysis has many variables that are not consistently replicated therefore providing large source of error as it may not fully capture the selected trait and results may not be replicated due to lack of standardization throughout the industry (Joyner, 2018). Texture Profile Technologies (unpublished) notes that along with this, many frequently misuse the device as sample preparation, selection, and machine settings are vital to proper analysis. For example, gumminess and chewiness may not be measured together. Additionally, many stress the sample too much as they have found that between 25 and 50% strain is enough to obtain accurate results but many apply so much force that sample is deformed or torn interfering with the analysis (Texture Profile Technologies, unpublished). All of these limitations must be considered when using this analysis as large variability in results and analysis may result in findings that are difficult to replicate or compare.

## **2.8 Marination**

Marination is the addition of liquid, brine, or other formulations to meat prior to cooking and typically aims to improve the sensory properties of meat (Owens, 2010, Alvarado and Mckee, 2007, Barbanti and Pasquini, 2005). Marination has become increasingly popular within the poultry industry to ensure consistent and acceptable final product quality. In fact, Smith and Acton (2001) reported that up to 50% of total raw poultry meat within the United States of America undergoes some form of marination. Under normal circumstances marination will add value to products by increasing positive attributes such as tenderness, water holding capacity, and flavor (Owens, 2010). One of the largest reasons marination is typically successful is due to the functional ingredients especially sodium chloride (salt) and phosphates, as they can have the largest impact on tenderness, flavor, and juiciness (Barbanti and Pasquini, 2005).

### *Functional Ingredients*

Salt is most likely one of the most used ingredients in the food industry today due to its essential role in flavor, but salt has also been used for millennia in many other areas of the industry as a preservative. Salt is essential in many meat products as it imparts desirable flavors and highlights ones found naturally in meat. Additionally, salt is commonly used to improve texture especially within sausages as it increases myosin solubility, opening the core of proteins to allow increased access to water binding sites (Alvarado and Mckee, 2007). Salt not only increases WHC by exposing additional binding sites, but it also increases the space between the thin and thick filaments found in the muscle allowing more water to occupy the space. By increasing myosin solubility salt can increase the amount of water and fats bound within the product especially when examining a comminuted product (Owens, 2010). Though not as important in many modern products, salt can act as a preservative decreasing water activity within products by driving out excess moisture which will inhibit the growth of some pathogenic bacteria (Lücke, 1994). Typically, marinated products may contain approximately 2% salt in the finished products, though this value can vary wildly depending on the product, producer, and targeted audience (Alvarado and Mckee, 2007). One of the largest reasons for this range is since excessive amounts of salt could ruin the palatability of a product, therefore doing more harm than good.

A typical marinade would generally not only contain salt but phosphates as well. Phosphates have a wide variety of uses within meat processing; however, the most common usage is to improve WHC of meat products especially when used in conjunction with salt (Hamm, 1961). In short, the mechanism of this increased WHC involves the extraction of myofibrillar proteins which allows a lattice like network of unwound proteins to form allowing for the binding of phosphates and additional water within the protein network. As water is bound the meat continues to swell sometimes upward of 50% on a microscopic level if phosphates

levels are high enough thereby increasing WHC (Offer and Trinick, 1983; Trout and Schmidt, 1983). Along with improved WHC, phosphates are known to increase the overall pH of marinated meats which is vital to some theories as increased pH may influence increased WHC and retention (Trout and Schmidt, 1983). Additionally, some protection from lipid oxidation and microbial contamination can be seen with the usage of phosphates, but these are secondary roles for the use of this ingredient (Gomez et al., 2020).

Phosphates impact more than just sensory attributes as some color stabilization and changes are noted with the usage of phosphates. In lean products, phosphates add additional juiciness which improves the perceived texture and palatability of marinated products versus unmarinated products (Alvarado and Mckee, 2007). With that some reported usage of phosphates can lead to a rubbery texture that consumers would find extremely unappealing (Owens, 2010). Along with possible textural issues some note that some sensory attributes may be negatively impacted by the presence of phosphates. One of the largest benefits of phosphates as previously mentioned was the increased WHC of treated products. This improvement in WHC leads to decreased cook loss and therefore a perceived juicier product. Improved juiciness or water retention as mentioned prior correlates to an improved overall eating experience where all positive attributes are heightened. However, with these beneficial attributes, one of the largest drawbacks to phosphate usage is a perceived soapiness in both off-flavor and odor. Though, the soapiness does not necessarily impact other sensory attributes it can be off putting to a sensitive consumer base. Additionally, Allen et al. (1998) noted that breasts exhibiting a higher pH may be lightened in color which may impact consumer purchasing habits. These lighter breasts also have poor color retention over display periods although secondary effects of phosphates may impact the color change allowing them to retain the lightened color longer (Kim and Marshall, 1999).

With these benefits and drawbacks in mind, producers face the challenge of whether to include phosphates within marinades to improve product consistency and reception.

Although phosphates are a common product in marinades, many countries have begun to move away from them, and those who still allow them to have stringent guidelines on their usage. The United States Department of Agriculture (USDA) limits phosphate usage depending on product with whole muscle products not being allowed greater than 0.5% or 5000 ppm (USDA, 1995). Though many producers have marinades that typically consist of 0.15 to 0.40% phosphates in final the final product. These guidelines are important because of possible health concerns attached to the consumption of excessive amounts of these products.

## **2.9 Processing Methods**

### *Vacuum Marination*

Wooden breast fillet marination may be an alternative method to recuperate profit and ultimately reduce loss. One of the main issues noted with WB fillets is reduced water holding capacity due to increased final pH, decreased muscle protein content, and increased connective tissue (Kuttappan, 2012a; Solgia et al. 2016). This reduction greatly affects marination uptake and retention which can negatively impact the final product yield and ultimately affect cook loss (Bowker et al., 2018). Multiple studies have reported a drastic difference in cook loss between normal and WB fillets noting that the increase in ultimate pH may be the main cause of this due to decreased WHC (Mudalal et al., 2015; Bowker et al., 2018). Additionally, Bowker et al. (2018) noted that this reduced marinade uptake and retention did show some intramuscular locational difference as the ventral side of breasts had greater loss than the dorsal sides. This intramuscular locational difference is in line with previous research indicating that the lesion and subsequent characteristics are mainly seen on the ventral side of the breasts (Clark and

Velleman, 2016). Previous research also indicates that marinades may struggle to penetrate the epimysium of the ventral side of the breasts, which in WB fillets may have thickened epimysial connective tissue, while the dorsal side offers more exposed tissue allowing for easier uptake (Bowker et al., 2010; Yoon et al., 2016).

Along with impaired marinade retention, WB fillets exhibited impaired textural attributes even after vacuum marination. Maxwell et al. (2018) noted that prior to treatment, breasts had increased hardness, springiness, cohesiveness, fibrousness, and chewiness values when examined by a sensory panel of trained panelists. Additionally, post marination WB fillets had decreased hardness, springiness, cohesiveness, fibrousness, and chewiness, but the decrease was not enough to overcome the difference between a WB fillet and normal fillet. Along with decreased attributes, WB fillets saw increased juiciness when compared to normal fillets. Objective analysis displayed abnormal results as WB fillets had a lower initial shear force value when compared to normal fillets which was not seen in previous literature (Dalle Zotte et al., 2017; Sanchez-Brambila et al., 2017). This was attributed to Warner-Bratzler shear force analysis being unable to easily detect WBM. Maxwell et al. (2018) did note a difference post marination with WB fillets exhibiting increased shear values at all locations when compared to normal fillets. Jarvis et al. (2020) reported similar results finding that regardless of marinade type severe woody breast fillets could not overcome their inherent textural deficits showing increased hardness, chewiness, and crunchiness.

As previously stated, WB fillets additionally have notable color differences when compared to normal fillets due to increased hemorrhaging (Mudalal et al. 2015; Zhuang and Bowker, 2018). Since color is a primary factor in consumer purchasing, Zhuang and Bowker (2018) investigated using marination as an intervention to mitigate the darkened color of the breasts. Though distinct color differences were seen between normal and WB fillets, marination

did not improve color characteristics and finished products may remain unacceptable in the public eye. This may warrant further analysis as consumer preferences were not analyzed.

### *Injection Marination*

Continuing upon remediation strategies a producer can use multi-needle injection to distribute marinade directly and evenly among the breast either alone or in some combination with a vacuum tumble. Alvarado and Sams (2004) reported that normal breasts subjected to tumbling, injection, or injection and tumbling all were significantly more tender when compared to an unprocessed breast. Additionally, they noted that the injection alone had the lowest shear value indicating it would be perceived as the most tender when compared to all other treatments. The reason they believe injection yielded the best results was due to fiber disruption from fluid pressure and more extensive and profuse brine distribution. However, they note that the combination of tumbling and injection and tumbling alone may have had increased values as the stimulation of muscle can increase toughness if done prior to rigor completion. As of yet, little research has been done to show the effect of multi-needle injection on WB fillets, but similar results should be expected as muscle fiber disruption may reduce shear value and reduce tough texture seen in WB fillets.

Injection marination can also be used to not only remediate issues with palatability but may affect other quality factors such as pH and even color remediation. Broiler breasts exhibiting the PSE condition tend to be lighter in color and have poor water holding capacity when compared to normal breasts. Alvarado and Sams (2003) reported that breasts exhibiting the PSE condition that underwent injection marination with a sodium bicarbonate marinade typically had reduced effects on color and increased final pH. However, functional property loss especially that of WHC was not affected by injection marination as PSE breasts still had increased cook loss and expressible moisture when compared to normal fillets. This phenomenon

has been seen in other research regarding WB fillets as even after marination the breasts will still have excessive cook loss and marinade retention when compared to normal fillets (Mudalal et al. 2015; Bowker et al. 2018). Further research is needed in this area to fully understand why functional properties remain unchanged and decreased, but it could be hypothesized that the myodegeneration and decreased functional protein content cannot be easily remediated through simple marination as no functional protein is added back to the system.

### *Blade Tenderization*

A final processing aid that may be useful in the mitigation of the increased hardness seen with WB fillets is blade tenderization. Though blade tenderization is not extremely common within the poultry industry, the technique is commonly applied to cuts from other species especially bovine animals. The process consists of piercing meat products with a series of blades or needles which weakens the structure of the product by severing myofibrillar bonds and connective tissues (Jeremiah et al., 1999; Bekhet et al., 2014). Within cattle, Jeremiah et al. (1999) found that blade tenderization was successful at improving tenderness within certain muscle groups especially those from the hindquarter, but the method was not as effective in other muscle groups that were more tender initially. Additionally, blade tenderization has been an extremely successful process for simply reducing variability in product tenderness within muscle grouping (Pietrasik et al., 2010).

Although improvements in tenderness are seen with blade tenderization, it becomes important to note some negatives of the technique. Blade tenderized products typically have increased drip loss that could vary from 1-2% depending on muscle location (Jeremiah et al., 1999). Along with increased drip loss, products that have undergone blade tenderization have been perceived as less juicy when subjected to sensory evaluations although in beef animals no reduction in cook loss percentage has been noted (Savell, et al., 1977; Jeremiah et al., 1999;

Behkit et al., 2014). Blade tenderization has also been noted to act synergistically with other processing methods like vacuum marination or injection enhancement, further improving textural attributes as well as offsetting negative moisture attributes that may be seen in some products (Motycka and Betchel, 1983; Bowker et al., 2007; Pietrasik et al., 2010). Although blade tenderization has been deemed effective in red meat species little information is available for its effect on poultry products especially those exhibiting WBM.

As previously discussed, no form of processing has been able to fully remediate the negative quality attributes noted in WB fillets. Tasoniero, et al. (2019) found that blade tenderization warranted further investigation as a processing method and showed potential as a corrective process through objective measurements. Raw breasts that underwent blade tenderization were notably more tender than untreated fillets but were still not comparable to normal or breasts exhibiting a moderate severity of WB when undergoing shear force analysis. However, cooked breasts displayed similar values (Severe WB = 280 N vs. Norm breasts = 270 N) after shearing analysis indicating that blade tenderization may provide some form of remediation to negative textural attributes. Additionally, some discrepancies were noted between texture analyses as compression analysis was only able to differentiate between normal and WB fillets whereas the multiblade shear force analysis was able to isolate normal breasts, moderate WB fillets, and severe WB fillets. This discrepancy was assumed to be due to differences in deformation ease and behavior during the analyses.

Additionally, locational differences along the breasts were noted as the cranial and medial portion of the breast were tougher than the caudal end in raw fillets; whereas cooked breasts showed decreasing toughness over multiple areas with the cranial/medial portion being the most tough followed by the cranial end which was followed by the caudal end. The abnormal toughness in varying location indicated that wooden breast lesions are not uniform throughout

the breast. This idea was reinforced by Dalle Zote et al., (2017) who noted that the myodegenerative lesions were typically present in the caudal end of effected breasts.

Additionally, Clark and Velleman (2016) noted increased amounts of pathological fibrosis within the anterior ends of breasts with abnormal collagen distribution throughout impacted fillets.

Though textural analyses revealed locational issues within WB fillets, overall blade tenderization improved overall objective texture of impacted breasts indicating that though it may not have fully resolved some morphological issues it is a promising form of reprocessing.

### **2.10 Conclusion**

With ever increasing incidence of the WBM, intervention has become vital. The extreme economic losses noted with the myopathy are rapidly increasing so some form of remediation to at least recoup some profit would be ideal. There are seemingly insurmountable textural issues associated with affected breasts that the industry must address and correct to achieve a product that may be satisfactory to a consumer base. The purpose of this research was to examine if a combination of common reprocessing techniques may mitigate some of the textural issues associated with WBM. After processing, it becomes important to note if textural improvements were seen on both an objective level and consumer satisfaction level especially in a myopathy that can be difficult to capture objectively. Ideally, a remediation of any severity would be beneficial to the industry as it provides opportunity for some regained profit until the source of the myopathy has been identified.

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## CHAPTER 3

### FURTHER PROCESSING OF WOODY BREASTED FILLETS AS A FORM OF REMEDICATION FOR NEGATIVE TEXTURAL ATTRIBUTES

#### 3.1 Introduction

Poultry meat disappearance in the United States has increased since the 1970's due to a multitude of factors such as health concerns, cost, and time (USDA-ERS, 2024; Mottet and Tempio, 2019; Cavani et al., 2016) and projected to continue to increase globally (OECD-FAO, 2023). Continued increased demand has led the poultry industry to change broiler production to select for broilers with increasing feed efficiency, growth rate, and yield especially in the breast (Petracci et al., 2015; Kuttappan et al., 2016). Though the industry saw increased yield and success, the increased strain on broilers has not gone unnoticed especially with the increased emergence of myopathies (Kuttappan et al., 2016). One of the most notable and detrimental myopathies is woody breast myopathy (**WBM**) which may be accompanied by other myopathies like white striping (**WS**).

Woody breast myopathy was first documented in 2014 and has become an ever-increasing issue within the poultry industry with predicted losses of \$200 million USD and greater (Sihvo et al., 2014; Kuttappan et al., 2016). The WBM is characterized as an irregular hardening of the caudal tissue on the pectoralis major of effected broilers (Sihvo et al., 2014). Additionally, the irregular hardening which is caused by a diffuse myodegenerative lesion with collagen and lipid infiltration causes poor water holding capacity making remediation of the woody breasted (**WB**) fillet difficult. These factors cause WB fillets to be extremely difficult to repurpose as a whole muscle product as they greatly affect consumer satisfaction (Mudalal et al.,

2015; Puolanne and Ruusunen, 2014). Past research has investigated the remediation of WB fillets via a single method of further processing like marination, tumbling, blade tenderization, or injection enhancement (Bowker et al., 2018; Maxwell et al., 2018; Tasoniero et al., 2019). Previous work has resulted in only slight textural improvements, most notably with the use of blade tenderization, but the poor protein functionality and subsequent poor water holding capacity has been difficult to overcome even with tumbling or injection enhancement (Aguirre et al., 2019; Bowker et al. 2018). The combined effects of multiple further processing techniques are yet to be investigated, so it is unknown whether stacked further processing may be able to overcome the deficiencies seen with WB fillets.

Utilizing multiple stacked levels of further processing in a sensical order may ameliorate negative textural properties associated with WBM. Incorporating enhancement solutions with stacked processing may improve moisture retention, increasing processing yields and further improving textural properties. Blade tenderization has been shown to improve WB objective tenderness but also led to increased cook losses (Tasoniero et al., 2019). Following blade tenderization with vacuum tumbling with enhancement solution and/or multi-needle enhancement may take advantage of the avenues created by the blades to allow greater enhancement solution retention by piercing through the lesions of connective and adipose tissue. Therefore, the objective of this study was to determine if a multi-level (stacked) approach to further processing could be used to improve moisture retention and processing yields while subsequently ameliorate the negative tenderness and textural attributes associated with broiler fillets affected by the woody breast condition.

## **3.2 Materials and Methods**

### *Sample Collection*

Across three replicate days (minimum 2 weeks between replicates), in cooperation with a national broiler processor, chicken breasts ( $N > 600$ ) were selected approximately 4 hours postmortem by knowledgeable plant personnel for 3 woody breast severity categories: normal (**NOR**), moderate woodiness (**MOD**), and severe woodiness (**SEV**). University of Georgia personnel picked up the breast samples within 30 minutes of selection and transported the samples in iced coolers to the University of Georgia Meat Science and Technology Center (47 km). Immediately upon arrival at the University of Georgia fillets were further examined by trained personnel via manual palpation and visual characterization and severity was confirmed, recategorized, or questionable samples (borderline classification, ripped, torn, or missing section) were discarded. After classification confirmation on each replicate day 50 fillets in each severity category were randomly assigned to either texture analysis ( $n = 20$ ), sensory analysis ( $n = 20$ ), raw proximate analysis ( $n = 5$ ), or raw nuclear magnetic resonance imaging (**NMR**) water analysis ( $n = 5$ ). The fillets assigned to texture and sensory analysis were then randomly sorted into 4 processing treatments: control (**CON**), blade tenderization (**BT**), blade tenderization and vacuum marination (**BTM**), and blade tenderization, multi-needle injection enhancement, and vacuum marination (**BTIM**). Fillets were trimmed of all external fat and bone that may be left after the deboning process. Breast pH was recorded on proximate samples by direct probe (Edge® Multiparameter DO Meter, Hanna Instruments, Woonsocket, RI) and then proximate and NMR samples were vacuum sealed (B-620 series; 30-50 cm<sup>3</sup> O<sub>2</sub>/m<sup>2</sup>/24 h/101,325 Pa/ 23°C; Cryovac Sealed Air Corporation, Duncan, SC, USA) and frozen ( $-20 \pm 2$  °C) until further analysis.

Texture and sensory samples were weighed and had hemorrhagic lesion (**HL**) and white striping (**WS**) scores recorded (Kuttappan et al., 2012, 2017). Objective lean color ( $L^*$ ,  $a^*$ ,  $b^*$ ) was recorded on texture samples using a portable colorimeter (HunterLab; Miniscan EZ 4500L,

Reston, VA, 10° viewing area, 2.54 cm aperture size; A illuminant) calibrated using saturated white and black tiles. Color was taken on the ventral side of the fillet and was measured in triplicate then averaged for each individual fillet. After color evaluation, texture samples were compressed at the cranial end of the fillet using a 12 mm probe head on a TA-XT Texture Analyzer (Texture Technologies Corp.; Hamilton, MA) to 30% compression of its original height with a 245 N load cell with 5 mm/sec crosshead speed and 10 g trigger force. Afterwards, samples were placed on plastic trays and covered in polyvinylchloride (**PVC**) overwrap and placed in a storage cooler ( $0 \pm 1^\circ\text{C}$ ) overnight until further processing.

### *Sample Processing*

The day after initial sample collection texture and sensory samples were moved into a processing room ( $6 \pm 2^\circ\text{C}$ ) for further processing. Each processing day 151.4 L of fresh enhancement solution was made for a final product target of 0.75% NaCl and 0.30% sodium tripolyphosphates (**STPP**) at 10% total solution retention. The enhancement solution was allowed to mix thoroughly while samples were prepared. All samples were tagged with a unique identifier number on the anterior bone side of the breast using a Tyvek tag and tagging gun. All samples were weighed, and texture fillets had objective lean color recorded, and then CON samples were recovered in PVC to prevent sample drying and placed in the storage cooler. All other samples (BT, BTM, and BTIM) were then passed through a Ross Tenderizer TC700M (Ross Industries; Midland, VA) receiving one pass on both the ventral and dorsal side of the fillet. Post blade tenderization, BT texture fillets had objective lean color and weight recorded, were recovered with PVC and placed in the storage cooler. Post blade tenderization, BTM fillets were sorted into batches based on severity, were weighed and placed in a vacuum tumbler (Koch LT-5 vacuum tumbler, Koch Industries; Wichita, KS) for 30 minutes at 131 kPa in enhancement solution targeting 10% retention. Fillets within the BTIM treatment were sorted into batches

based on severity and injected to target 10% enhancement solution pick-up (Inject Star BI-72 pickle injector, Inject Star; Brookfield, CT). After injection, fillets were allowed to drip for 5 minutes and were re-weighed. Average injection pick-up was calculated by weighing each individual breast and calculating pick-up which was then averaged for that batch. Average pick-up from multi-needle injection was used to calculate how much additional solution would be added to the vacuum tumbler to achieve the targeted 10% pick up. The measured enhancement solution was added to BTM fillets, and they were vacuum tumbled for 30 minutes at 131 kPa. After processing of BTM and BTIM samples, fillets were allowed to drip for 5 minutes prior to being reweighed and objective color was recorded on the texture samples followed by being recovered with PVC and being returned to the storage cooler overnight. Sensory fillets were vacuum sealed and frozen ( $-20 \pm 2$  °C) for further analysis. Due to COVID-19 restrictions the sensory samples were not able to be analyzed in a timeframe consistent and in accordance to acceptable sensory guidelines.

#### *Texture Analysis*

Approximately 10 hours post-processing texture samples were reweighed to calculate overnight marinade retention and drip loss. Drip loss was calculated as  $((\text{post-treatment weight} - \text{pre-cook weight}) / \text{post-treatment weight})$ . Thermocouples (copper-constantan; Omega Engineering; Samford, CT) were inserted into the geometric center of the thickest portion of the breast and temperature was recorded using a Digi-Sense 12-channel scanning thermometer (model 9200-00; Cole Palmer Vernon Hills, IL). Fillets were placed in unsealed, heat stable vacuum pouches and were cooked in a circulating immersion water bath set to 82 °C to a final internal temperature of 76 °C. Samples, 10 at a time were randomly selected for each cooking cycle to ensure water temperature did not drop and the bags containing the samples were slowly lowered into the water bath to ensure removal of excess air from each bag and each bag was

fixed to a crossbar ensuring the sample stayed submerged, was not in contact with the bottom, sides, or other samples, and the thermocouple was held in place. Between cooking cycles, water was added to the water bath to bring it back to volume and the water bath was allowed to come to temperature before new samples were added.

After cooking, samples were immediately pulled from vacuum bags, blotted dry of excess moisture, and were reweighed for cooked weight prior to being set on an absorbent pad and covered in PVC overwrap to cool for approximately 45 min. Once cooled, cooked objective color was taken following previous methods. After color analysis, samples were again covered and placed in a cooler ( $2 \pm 2$  °C) overnight for texture analysis.

The next day samples were removed from the cooler and allowed to acclimate to room temperature (30 minutes) while the texture analyzer was enabled. Samples underwent Texture Profile Analysis (**TPA**) using the same analyzer and probe as raw compression. Samples were subjected to a two-stroke compression test with a 2 second pause to allow the sample to relax between strokes with probe targeting the cranial portion of the fillet approximately 2 cm lateral to the raw compression sample location. Samples were compressed to 20% sample height with a pre- and post-test speed of 5 mm/sec and testing speed of 1 mm/sec using 245 N load cell with a trigger force of 0.049 N. Texture Profile Analysis values were obtained from the force-time curve output calculated by Exponent Connect (Texture Technologies Corp.; Hamilton, MA) for hardness, cohesiveness, springiness, resilience, and chewiness.

After TPA analysis, samples were subjected to multiblade analysis (Tasoniero et al., 2019) as a secondary textural analysis. Samples were sheared by attaching a multiblade probe head to the TA-TX Texture Analyzer which sheared the samples to 30% of sample height at a crosshead speed of 5 mm/sec with a trigger force of 0.049 N. Peak force and energy were measured via the Exponent Connect software again using the force-time curve obtained during

analysis. Afterwards, samples were vacuum sealed and frozen at  $-20 \pm 2$  °C for cooked NMR water analysis.

### *Proximate Analysis*

Samples were thawed in a cooler overnight ( $2 \pm 2$  °C) and trimmed of all external fat and bone prior to being minced, flash frozen in liquid nitrogen, and homogenized (Waring commercial blenders, Model 34BL97, Dynamics Corporation of America, New Hartford, CT). For moisture loss and ash,  $3 \pm 0.1$  g of sample was placed in ceramic crucibles that were in a desiccator for 24 hours and dried (90 °C) in a forced air oven (Fisher Scientific; Hampton, NH) for 24 hours and placed in a desiccator for 10 min to equalize then weighed as outlined in AOAC (1990). To ensure proper drying, samples were dried an additional 24 hours to ensure all excess moisture was driven off prior to ash analysis. Moisture loss was calculated as:  $((\text{wet sample weight} - \text{dry sample weight}) / (\text{wet sample weight})) \times 100$ . Dried samples were then placed in an ash oven (Fisher Scientific; Isotemp muffle furnace, Hampton, NH) at 550 °C for 12 hours then allowed to cool overnight in a desiccator prior to weighing. Ash content was calculated as:  $((\text{dry sample weight} - \text{ash weight}) / (\text{dry sample weight})) \times 100$ .

To determine total lipid content an ANKOM XT15 Extraction System (Macedon, NY) was used following guidelines of AOCS (2009). Lipid content was determined by placing  $1 \pm 0.1$  g of homogenized sample within an ether permeable filter bag (ANKOM XT4; ANKOM Technology; Macedon, NY). Samples were dried of all moisture at 90 °C for 48 hours prior to ether extraction. After extraction, samples were again placed in an oven (90 °C) for 12 hours to ensure all moisture was removed prior to placing in a desiccator for 10 min then weighing to get final sample weight. Total lipid content was calculated as:  $((\text{wet sample weight} - \text{dry sample weight}) / (\text{wet sample weight})) \times 100$ . To complete compositional analysis protein was calculated by:  $(100\% - \text{moisture loss} - \text{lipid content} - \text{ash})$ .

Collagen analysis was performed following Hill (1996) and AOCA (2005). Samples were freeze dried for 18 hours using a Labconco FreeZone 2.5L freeze drier (Labconco Corp., Kansas City, MO) prior to analysis. Approximately  $1 \pm 0.01$  g of freeze-dried sample was weighed into a tube in duplicate prior to adding  $\frac{1}{4}$  strength Ringer's solution. Samples were then incubated in a water bath (77 °C) for 80 minutes, hand swirling every 10 minutes. After incubation, tubes were centrifuged (Eppendorf Centrifuge 5810R; Eppendorf Corp.; Enfield, CT) at 3000 rpm for 12 minutes at 20 °C. After centrifugation, sample supernatant was aliquoted into a separate tube and the pellet was again suspended in  $\frac{1}{4}$  Ringer's solution and centrifuged again. To the tube containing supernatant, 18M H<sub>2</sub>SO<sub>4</sub> was added, and the solution was carefully shaken. The insoluble pellet was resuspended in 3.5 M H<sub>2</sub>SO<sub>4</sub> and shaken until the pellet was broken up. Both the soluble supernatant and insoluble pellet were placed in a drying oven (105 °C) for 16 hours for hydrolysis. Afterwards, samples were allowed cool prior to adjusting sample pH to 6.0 using varying concentrations of NaOH and HCl. Soluble samples were then diluted to 250 mL and insoluble to 500 mL with deionized water, and the sample was filtered through hardened, ashless, fast filter paper and samples were organized into soluble and insoluble fractions. Prior to analysis, a 6 µg/mL hydroxyproline stock was made and a standard curve was titrated with each set of samples to compare values of that reading against the curve. Sufficient sample was aliquoted to a tube and chloramine-T oxidant reagent was added to all samples and standards and allowed to incubate at room temperature for 20 minutes. Afterwards, 7,12-Dimethylbenz(a)anthracene (**DMBA**) was added to the tubes which were then vortexed to mix and incubated while covered for 15 minutes in a water bath (60 °C). After incubation, tubes remained covered and were cooled in cold water. Samples were then read at 558 nm on a spectrophotometer (Jasco V-630 Spectrophotometer; Jasco Inc.; Easton, MA) against a blank containing no sample. Afterwards insoluble, soluble, and percent soluble collagen were

calculated against the standard curve, ranging from 0.0  $\mu\text{g/mL}$  to 2.4  $\mu\text{g/mL}$  final hydroxyproline concentration.

### *NMR Analysis*

Myowater properties of intact fillets were assessed using transverse relaxation time (T2) measurements collected with a LF 90II minispec NMR analyzer (Bruker BioSpin, Rheinstetten, Germany). Transverse relaxation times (T2) were acquired using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence (Carr and Purcell, 1954; Meiboom et al., 1958) ( $\tau = 1$  ms; total number of acquired echoes = 200). To obtain the relaxation time and population percentage for each of the three water populations (T2B, T21, and T22), the T2 data were then analyzed using distributed exponential fitting analysis carried out with Contin software (version V3.00, Bruker BioSpin, Rheinstetten, Germany). Plots of relaxation amplitudes for individual relaxation processes vs relaxation times revealed the presence of the three relaxation populations (no. points of relaxation spectrum = 500; relaxation spectrum = 0.1 – 1000 ms). For each of the three water populations, relaxation times were calculated from the peak position and proportions of protons exhibiting those relaxation times were calculated from the corresponding area under each peak.

### *Statistical Analysis*

Data were analyzed using the PROC MIXED function of SAS (V 9.4) as a randomized complete blocked design where woody breast severity and processing treatment were fixed effects and replicate (block) was the random variable. For all analyses fillet was considered the observational and experimental unit. Initial weights, descriptive woody breast characterization, raw color was analyzed for the main effects of woody breast severity and further processing treatment while raw NMR, and proximate analysis was only analyzed for woody breast severity. For textural analyses, initial compression was included in the model as a covariate for hardness, cohesion, springiness, resilience, chewiness, multiblade shear, and total energy. Least square

means were separated using the LSmeans pdiff option with a Tukey HSD adjustment for the main effects of woody breast severity and treatment and their interaction. Means were considered different at  $\alpha \leq 0.05$ .

### 3.3 Results and Discussion

Descriptive characteristics of fillets are shown in Table 1. Assessments showed that there were differences within WB severity ( $P < 0.01$ ), while there was no difference between processing treatments ( $P = 0.24$ ) or the interaction of WB severity and processing treatments ( $P = 0.36$ ). Upon visual inspection SEV fillets exhibited increased WS and HL scores when compared to both NOR and MOD, while MOD fillets had greater values when compared to NOR fillets ( $P < 0.01$ ) in WS score only. Additionally, breasts were notably larger with SEV fillets having increased initial weights when compared to both MOD and NOR groups, and MOD fillets having greater initial weights compared to NOR fillets ( $P < 0.01$ ). These observations agreed with previous research indicating breast affected by the woody breast myopathy were typically larger than their NOR counter parts (Brambila et al., 2018; Mudalal et al., 2015; Kuttapan et al. 2017). Additionally, as severity increased so did WS and HL and score reinforcing previous hypotheses that these myopathies are commonly seen together though they are still independent defects (Bowker et al., 2018; Caldas-Cueva et al., 2020; Kuttappan et al., 2017). These descriptive characteristics also verify the adequacy in the selection and categorization of effected fillets.

Though not commonly used as a selection characteristic, breasts were subjected to raw compression to confirm presence of increased hardness (Table 1). Compression showed differences ( $P < 0.01$ ) only within the WB severity main effect while processing treatment and the interaction had no differences ( $P = 0.45$ ). Severe fillets exhibited increased compression scores when compared to both MOD and NOR fillets ( $P < 0.05$ ). Additionally, MOD fillets

exhibited increased compression values when compared to NOR fillets ( $P < 0.05$ ). Mudalal et al. (2014) reported similar results noting increased hardness in the caudal portion of breasts exhibiting WBM. Within context of this study initial compression values served as a great indicator of severity and may be a more objective method of separation versus manual palpation. Pang et al. (2020) concluded similarly that of all objective methods that compression was the best predictor of woody breast severity. Such methods are critical to further research to provide a more standardized method that is not reliant upon skill or experience of grader as mentioned by Bowker et al. (2019).

Objective color analysis presented distinct but not unexpected differences ( $P < 0.01$ ) between WB severity main effect as well the treatment main effect ( $P < 0.01$ ) in  $a^*$  values only (Table 1). When examining  $L^*$ , SEV breasts were lighter or whiter than both MOD and NOR fillets ( $P < 0.01$ ). Moderate fillets were also lighter or whiter than NOR fillets ( $P < 0.05$ ). Severe fillets also displayed increased redness or  $a^*$  values compared to both MOD and NOR fillets ( $P < 0.01$ ). Moderate fillets were also redder and had increased  $a^*$  values when compared to NOR fillets ( $P < 0.05$ ). Notably,  $a^*$  values also displayed differences between processing treatments with BT being redder ( $P < 0.05$ ) than BTIM fillets while control and BTM fillets did not differ ( $P \geq 0.20$ ) from each other or BTIM. Additionally, both MOD and SEV fillets had increased  $b^*$  values or were more yellow than their NOR counterpart ( $P < 0.01$ ). All findings agreed with previous research as WB fillets typically have increased petechial hemorrhaging accounting for a diffuse pattern of blood (Shivo et al. 2014). Additionally, Soglia et al. (2016b) noted that due to differences within fiber membrane integrity that WB fillets exhibit greater moisture loss which can result in higher reflectance values which may impact  $L^*$  by increasing the light coloration of the product.

Proximate analyses were performed to further confirm categorization of breasts and to investigate compositional differences between severities (Table 1). Since no treatment was performed on any samples selected for proximate analysis, differences only in WB severity main effect were noted ( $P < 0.01$ ). With fillet pH, SEV and MOD fillets did not differ ( $P \geq 0.36$ ), but both had increased ( $P < 0.01$ ) pH values when compared to NOR fillets. When looking at chemical composition, SEV fillets had an increased ( $P < 0.05$ ) percent moisture when compared to both MOD and NOR fillets. Additionally, MOD fillets had an increased moisture content when compared to NOR fillets ( $P < 0.05$ ). For lipid content or percent fat, SEV fillets did not differ ( $P \geq 0.83$ ) from MOD fillets but when compared to NOR fillets had increased ( $P < 0.05$ ) lipid content. Moderate fillets did not differ ( $P \geq 0.09$ ) in lipid content when compared to NOR fillets. For crude protein, NOR fillets had an increased ( $P < 0.01$ ) percentage of protein when compared to MOD and SEV fillets, which differed from one another with MOD fillets having greater ( $P < 0.01$ ) amounts of protein compared to SEV fillets. For ash content, NOR fillets had increased ( $P < 0.01$ ) amounts of ash when compared to SEV fillets but did not differ ( $P = 0.06$ ) from MOD fillets which did not differ ( $P = 0.19$ ) in ash content compared to SEV fillets.

Collagen was also analyzed with proximates to investigate differences as noted by Soglia et al. (2016a; Table 1). Within WB severity no differences were seen in either soluble collagen content or the percentage of soluble collagen ( $P = 0.10$ ). Total collagen amount and insoluble collagen content did have differences ( $P < 0.01$ ) within the WB severity main effect. Total collagen revealed that SEV fillets had increased ( $P < 0.05$ ) amounts of collagen when compared to both MOD and NOR fillets which did not differ ( $P = 0.28$ ) from one another. Insoluble collagen content showed similar results with SEV fillets having greater ( $P < 0.01$ ) amounts of insoluble collagen when compared to MOD and NOR fillets which again did not differ ( $P = 0.28$ ) from one another. Proximate results show similar trends as seen by Soglia et al. (2016a;

2016b) and Petracci et al. (2014). These notable differences may contribute to the poor technological quality of WB chicken.

After processing, fillets were again monitored to discern differences between severities and to investigate whether processing treatments impacted the poor WHC and subsequently marinade retention seen in WB fillets (Table 2). Post-treatment weights displayed differences amongst WB severity ( $P < 0.01$ ) but saw no difference ( $P = 0.73$ ) between processing treatments or the interaction between WB severity and treatment ( $P = 0.47$ ). Between severities, SEV breasts again had increased initial and post processing weights compared to both MOD and NOR fillets. Moderate fillets also had increased initial and post-treatment weights compared to NOR fillets. These results agree with Brambila et al. (2018) as an increase in WB severity typically displays increased fillet weights. The lack of difference between processing treatments was also not unexpected as the equal distribution of WB severity into treatment groups would account for a normalized division in processing treatments.

Total marinade uptake had differences in WB severity and treatment main effects as well as their interaction ( $P < 0.01$ , Fig. 1). The most notable change amongst the interaction was the effect of increased processing on marinade uptake. No difference was seen between all severities subjected to either no treatment or BT ( $P = 1.00$ ). Severe fillets in both the BTM and BTIM groups did not differ ( $P \geq 0.55$ ) compared to MOD fillets that underwent BTM, though total marinade pick up was still lower ( $P < 0.01$ ) than NOR fillets. Moderate fillets in the BTM treatment displayed did not differ ( $P \geq 0.31$ ) from both MOD subjected to BTIM fillets and NOR subjected to BTM fillets, but these fillets still had decreased ( $P < 0.01$ ) marinade uptake when compared to NOR fillets in all processing treatments groups. Moderate fillets in the BTIM treatment showed no difference ( $P \geq 0.12$ ) in total marinade pick up to NOR fillets in both BTM

and BTIM treatment groups indicating remediation with multiple methods of processing may improve WHC of fillets that do not exhibit SEV signs of woody breast.

Studies by Bowker et al. (2018) show similar findings that further processing may improve the retention and possibly WHC of WB fillets though they note that locational differences can greatly impact the marinade retention. This could be due to addition of items like salt and phosphate that swell the proteins to increase bound water (Smith and Acton, 2010; Petracci et al., 2013). Though it should be noted it has been theorized that the diminished WHC capacity may be due to inherent compositional differences in protein, fat, and collagen content (Soglia et al., 2016a). The increased retention seen in the BTIM group regardless of severity indicated that additional processing may provide a better method of marinade uptake especially in fillets with poor WHC. Bowker et al. (2018) noted that breasts undergoing vacuum tumble alone typically on display marinade uptake in superficial areas of the breast versus injection marination which allows for deeper marinade penetration. The combination of BT, vacuum marination, and injection enhancement may allow for the marinade to fully penetrate breasts allowing for increased marinade uptake by creating more deeper marinade penetration and increased extraction and subsequent protein swelling via the addition of salt and phosphates (Petracci et al., 2013).

Post-processing breasts displayed differences ( $P < 0.01$ ) in overnight purge loss within woody breast severity but not between treatment or main effect interactions ( $P = 0.48$ , Table 2). Similar with other findings, breasts classified as SEV or MOD had increased ( $P < 0.01$ ) amounts of purge loss compared to NOR breasts. Woody breasted fillets had an approximately 43% increase in purge loss when compared to the normal counterpart. Possible explanations are due to the decreased WHC seen with this myopathy due to myodegeneration near lesion sites. Additionally, others have observed poor marinade retention and increased purge loss due to

varying factors such as proximate differences, decreased protein functionality, lesion site location, and variance within WB severity (Bowker et al., 2018; Soglia et al., 2016a).

To confirm differences in marinade retention, breasts were examined during injection pick-up as well to see if any differences existed (Table 2). During post-injection, differences ( $P < 0.01$ ) within the WB severity main effect were noted. Post injection, NOR fillets had increased ( $P < 0.01$ ) marinade pick-up compared to both MOD and SEV fillets. Moderate fillets displayed greater ( $P < 0.05$ ) amounts of injection pick-up when compared to SEV fillets which had the lowest pick-up.

Objective color post-processing exhibited differences in both WB severity and treatment main effects ( $P < 0.01$ ) and for  $L^*$  differences ( $P < 0.05$ ) within their subsequent interaction were noted (Table 2, Fig. 2). Examining  $L^*$ , SEV and MOD fillets subjected to BT did not differ ( $P = 1.00$ ) in brightness from SEV and MOD fillets in the CON treatment and BTM for SEV fillets. Severe and MOD fillets subjected to BT were also lighter ( $P < 0.01$ ) in color when compared to NOR fillets across all processing treatments and MOD fillets subjected to BTM or BTIM. Severe fillets subjected to BTM treatment did not differ ( $P \geq 0.65$ ) in  $L^*$  values compared to both MOD and NOR fillets subjected to CON and BT treatments. Again, SEV fillets subjected to BTM were lighter or whiter ( $P < 0.05$ ) when compared to MOD and NOR fillets subjected to either BTM or BTIM treatments. Severe fillets that underwent BTIM treatments did not differ ( $P \geq 0.70$ ) compared to NOR fillets subjected to CON or BT treatments and MOD fillets subjected to BTM or BTIM treatments. Additionally, These SEV fillets from the BTIM treatment were darker ( $P < 0.01$ ) in color compared to SEV and MOD fillets subjected to BT. Lastly NOR fillets subjected to BTM and BTIM were darker ( $P < 0.01$ ) compared to all other fillets of varying severity and treatment. The color change may be due to addition of the marinade as phosphates could potentially affect the coloration of breasts by minorly altering  $L^*$  and decreasing  $a^*$  (Kim

and Marshall, 1999). An additional observation is that it seems as both MOD and SEV fillets undergo further processing the inherent lightness seen in WBM decreased and the fillets become similar to untreated NOR fillets.

Examining  $a^*$ , SEV fillets were redder and had greater ( $P < 0.01$ )  $a^*$  values compared to both MOD and NOR fillets while MOD fillets were redder ( $P < 0.05$ ) than NOR fillets. Control, BT, and BTM treatments had no difference ( $P \geq 0.32$ ) in redness or  $a^*$  values, yet all were redder and had increased ( $P < 0.01$ )  $a^*$  values compared to the BTIM treatment. Additionally, SEV and MOD fillets had increased ( $P < 0.01$ )  $b^*$  compared to NOR fillets but did not differ from one another ( $P \geq 0.99$ ). Post-processing  $b^*$  values between CON, BT, and BTM groups were not different ( $P \geq 0.72$ ), but CON and BT treatments had increased ( $P < 0.05$ )  $b^*$  values compared to the BTIM treatment. Additionally, BTM and BTIM groups  $b^*$  values were not different from one another ( $P \geq 0.10$ ). Differences amongst processing treatments can be accounted for the inclusion of phosphates within the applied marinade. Kim and Marshall (1999) reported phosphates having multiple effects on color by reducing redness but noted that multiple factors could cause conflicting results. Additionally, the BTIM treatment had the greatest marinade uptake which may allow the marinade to exhibit an exacerbated effect on the fillets. Consumers may also prefer the darker coloration of the BTM and BTIM fillets as some perceive it as a higher quality product although it is important to highlight that very few studies have examined consumer perception of WB fillets (Kuttappan et al., 2017).

Post processing breasts were cooked prior to textural analysis (Table 3). Pre-cook weights showed similar results to initial weights and processing weights. Within the pre-cook weights only a difference in the WB severity main effect was seen ( $P < 0.01$ ). Again, SEV fillets had increased ( $P < 0.01$ ) weights compared to MOD and NOR fillets, while MOD fillets had increased ( $P < 0.01$ ) weights compared to NOR fillets. Additionally, cook times showed

differences ( $P < 0.01$ ) only within the main effect of WB severity. Both SEV and MOD breasts had increased cook times ( $P < 0.01$ ) from NOR fillets with an approximately 8 min difference, while they did not differ ( $P = 0.99$ ) from one another. This difference in cook time may be accounted for by the difference in weigh between severities.

After cooking, cooked weights had differences ( $P < 0.01$ ) in both WB severity and treatment main effects as well as differences ( $P < 0.05$ ) within their interaction (Table 3, Fig.3). Severe fillets subjected to BTIM did not differ ( $P \geq 0.10$ ) from SEV fillets subjected to BTM and CON as well as MOD fillets subjected to BTIM and BTM; however, SEV fillets from the BTIM treatment and MOD fillets from BTIM or BTM were greater ( $P < 0.05$ ) in weight compared to all NOR fillets, CON and BT MOD fillets and SEV fillets subjected to BT. Additionally, SEV fillets subjected to BTM did not differ ( $P \geq 0.06$ ) from SEV fillets of all processing treatments and MOD fillets of all processing treatments but weighed more ( $P < 0.01$ ) than from NOR fillets of all processing treatments. Severe fillets subjected to BT did not differ ( $P \geq 0.41$ ) in post-cook weight compared to SEV fillets subjected to BTM and CON treatments, MOD fillets subjected to BT and CON treatments, and NOR fillets subjected to all processing treatments except CON. Severe fillets subjected to BT were heavier than NOR fillets subjected to no processing technique ( $P < 0.05$ ). These differences are not unexpected as woody breasted fillets are heavier than normal fillets (Brambila et al., 2018). Additionally, some of these differences may be caused by excessive cook loss seen in WB fillets as seen within this study (Fig. 3) and previous research.

As noted by Mudalal et al. (2015), WBM severely impairs WHC and therefore negatively impacts cook loss causing excessive moisture loss during the cooking process. For cook loss, differences ( $P < 0.01$ ) in the WB severity and processing treatment main effects were noted as well as their subsequent interaction ( $P < 0.05$ , Fig. 4). As expected, both SEV and MOD fillets

subjected to BT or CON treatments exhibited the greatest percentage of cook loss ( $P \geq 0.68$ ). Severe fillets subjected to BTM did not differ ( $P \geq 0.48$ ) in cook loss compared to MOD fillets in CON and BT treatments as well as NOR fillets subjected to BT and CON treatments; though, both SEV fillets subjected to BTM and MOD fillets in the CON treatment still exhibited greater ( $P < 0.05$ ) cooking loss compared to NOR and MOD fillets subjected to BTM or BTIM and SEV fillets subjected to BTIM. Additionally, SEV fillets in the BTIM treatment group did not differ ( $P \geq 0.17$ ) from MOD fillets subjected to BTM and BTIM as well as NOR fillets in all processing treatment groups except BTIM which exhibited the reduced ( $P < 0.05$ ) cook loss. Normal fillets subjected to BTIM did not differ in cook loss compared to NOR and MOD fillets in BTM and BTIM treatment groups ( $P \geq 0.99$ ). These differences are aligned with previous research as the increase in further processing and inclusion of phosphates and salt increases the amount of bound water within the system resulting in reduced cook loss (Petracci et al., 2013).

Post-cook color was taken as the effects that WBM may have on cooked color are not yet understood. Post-cook  $L^*$  and  $a^*$  showed differences ( $P < 0.05$ ) between WB severity main effects but no differences ( $P = 0.06$ ) were seen based upon treatment main effect; post-cook  $b^*$  showed differences ( $P < 0.05$ ) in both WB severity and treatment main effects. Within severity, SEV fillets had greater  $L^*$  values and subsequently were lighter than both MOD and NOR fillets ( $P < 0.01$ ). MOD fillets had greater  $L^*$  values compared to NOR fillets ( $P < 0.01$ ). Additionally, SEV fillets had increased ( $P < 0.05$ )  $a^*$  and  $b^*$  values and were redder and more yellow than both MOD and NOR fillets which were not different ( $P \geq 0.12$ ) from one another. Within processing treatment, BT and CON treatments  $b^*$  values were not different ( $P \geq 0.95$ ) from one another, but both exhibited greater ( $P < 0.01$ )  $b^*$  values than BTM and BTIM treatments. Both BTM and BTIM treatments did not differ from one another ( $P \geq 0.98$ ). Zhuang and Bowker (2018) reported similar results noting that woody breast fillets exhibit different cooked color

characteristics when compared to NOR breasts. In this study, MOD fillets were lighter than NOR fillets which was not unexpected as MOD fillets exhibited greater amounts of WS which may account for noted difference. As Zhuang and Bowker (2018) theorized the petechial hemorrhaging seen with WBM may account for the increased redness or brownness seen within cooked fillets. The reduced  $b^*$  caused by phosphates within the marinade was seen within the cooked product leading to a reduction in the yellow coloration of the fillets subjected to both the BTM and BTIM treatments.

In addition to normal cooking data, breasts underwent NMR analysis to investigate the myowater properties within the fillets (Table 3). Examining the fractional myowater distribution, differences ( $P < 0.01$ ) in only the WB severity main effect were noted. The proportion of tightly bound water, P2B, did not differ ( $P \geq 0.24$ ) in both NOR and MOD fillets which exhibited a greater ( $P < 0.01$ ) percentage of tightly bound water when compared to SEV fillets. Normal fillets exhibited an increased ( $P < 0.01$ ) proportion of intra-myofibrillar water, P21, when compared to both MOD and SEV fillets which did not differ ( $P \geq 0.61$ ) from one another. Due to the decrease in immobilized water SEV and MOD fillets had an increased ( $P < 0.01$ ) proportion of extra-myofibrillar water, P22, compared to NOR fillets. Similar findings are seen with Soglia et al. (2016b) reporting that fillets effected by woody breast had increased amounts of extra-myofibrillar water fractions compared to normal breasts.

In addition to measuring the distribution of myowater, relaxation times were measured to examine the mobility of the water fractions (Table 3). Examining bound water relaxation times, T2B was observed having no main effect difference ( $P = 0.11$ ) in either WB severity or TRT, but the subsequent interaction had notable differences ( $P < 0.05$ ). The relaxation time for intra-myofibrillar water, T21, had main effect differences ( $P < 0.01$ ) in both WB severity and treatment, but no difference ( $P = 0.07$ ) was seen in the subsequent interaction. Relaxation times

for extra-myofibrillar water, T22, also had main effect differences for WB severity and treatment as well as the subsequent interaction ( $P < 0.05$ ). For T2B, SEV fillets subjected to BTM had a longer ( $P < 0.01$ ) relaxation time compared to NOR fillets subjected to BTIM, but neither differed ( $P \geq 0.08$ ) from any other severity or treatment. When examining the mobility of intra-myofibrillar water, T21, SEV fillets had increased ( $P < 0.01$ ) relaxation times compared to both NOR and MOD fillets, while MOD fillets only had increased ( $P < 0.01$ ) mobility compared to NOR fillets. The intra-myofibrillar relaxation time revealed that BTIM had increased ( $P < 0.01$ ) mobility compared to BTM, BT, and CON treatments with BTM being greater ( $P < 0.02$ ) than BT and CON which did not differ ( $P = 0.99$ ) from one another. Examining extra-myofibrillar relaxation times, SEV fillets subjected to BTM or BTIM did not differ ( $P \geq 0.20$ ) from both NOR and MOD fillets subjected to BTIM but had increased ( $P < 0.01$ ) relaxation times compared to all NOR and MOD fillets subjected to BTM, BTM, or CON treatments. Normal fillets subjected to BTIM did not differ ( $P \geq 0.14$ ) from BTIM and BTM treatments across all severities but had increased ( $P < 0.05$ ) mobility compared to BT and CON treatments in NOR and MOD fillets. Severe fillets subjected to BT did not differ ( $P \geq 0.14$ ) from CON, BT, and BTM treatments across NOR and MOD fillets, but had decreased ( $P < 0.01$ ) relaxation times compared to SEV fillets subjected to BTM and BTIM. These distributions are not surprising as increased processing may increase the mobility of bound water and free water proportions as additional moisture is added to the system. In conjunction with additional moisture, the increased fiber disruption through the multiple forms of processing or fiber degeneration near the lesion site may increase the mobility due to poor binding (Soglia et al., 2016a). The inclusion of phosphates and salts may have increased water mobility and functionality by altering protein status and causing additional swelling which may lead to additional water within these fractions (Petracci et al., 2013).

Filletts were subjected to multiple objective textural analyses to evaluate effectivity of further processing methods as a form of remediation for the increased hardness and impaired sensory characteristics of WB fillets (Table 4). One of the primary forms of textural analysis used within research application and the industry to perform objective textural trials is TPA (Chatterjee et al., 2016). Within this study hardness, resilience, cohesion, springiness, and chewiness traits were all investigated through TPA analysis. As an additional method, multi-blade shear was used to elucidate any further differences between processed fillets.

For adhesion and chewiness, differences ( $P < 0.01$ ) were only noted within the main effect of WB severity with no differences ( $P = 0.25$ ) seen in treatment main effects. Severe fillets exhibited reduced ( $P < 0.01$ ) adhesion when compared to MOD and NOR fillets which did not differ ( $P = 0.31$ ) from one another. Similar to adhesion, SEV fillets exhibited increased ( $P < 0.01$ ) chewiness when compared to MOD and NOR fillets which again did not differ ( $P = 0.22$ ) from one another.

For hardness, resilience, cohesion, and springiness, differences were seen in both WB severity and treatment main effects ( $P < 0.05$ ). Within the WB severity main effect, SEV fillets had increased ( $P < 0.01$ ) hardness values compared to both NOR and MOD fillets which did not differ ( $P = 0.20$ ) from one another. Examining resilience, SEV fillets exhibited increased ( $P < 0.01$ ) resilience to deformation when compared to both MOD and NOR fillets which did not differ ( $P = 0.25$ ) from one another. For cohesion similar results are noted with SEV fillets having greater ( $P < 0.01$ ) cohesion compared to MOD and NOR fillets which did not differ ( $P = 0.97$ ) from one another. Within springiness, the same trend was observed with SEV fillets exhibiting greater ( $P < 0.01$ ) springiness, or rebound, compared to NOR and MOD fillets which again did not differ ( $P = 0.93$ ).

Within treatment main effects, for hardness fillets subjected to BT did not differ ( $P = 0.98$ ) from CON treatments, but were more tough ( $P < 0.05$ ), or harder, when compared to BTM and BTIM fillets. For resiliency, fillets subjected to BT were less resilient ( $P < 0.01$ ) or more apt to deformation compared to all other processing treatments which did not differ ( $P = 0.11$ ) from one another. For cohesion, BTIM and CON treatments did not differ ( $P = 0.64$ ), but fillets subjected to BTIM were more cohesive ( $P < 0.01$ ) from BTM and BT treatments which did not differ ( $P = 0.07$ ) from fillets in the CON treatment. Fillets within BTIM and BTM treatments exhibited increased ( $P < 0.05$ ) springiness values compared to fillets that underwent BT, but they did not differ ( $P = 0.99$ ) from one another. Additionally, no differences in springiness were noted between CON fillets and all other processing treatment groups ( $P = 0.31$ ).

Using multiblade shear allowed for additional textural measurements that may help elucidate the effect that stacked processing had on the fillets. Both WB severity and treatment differences ( $P < 0.05$ ) were observed for multiblade shear force, but only WB severity differences ( $P < 0.01$ ) were noted in total energy measurements. For multiblade shear force, SEV fillets had the greatest shear force value compared to both MOD and NOR fillets ( $P < 0.01$ ). Moderate fillets also exhibited increased shear force values when compared to NOR fillets ( $P < 0.01$ ). For the treatment main effect, CON, BT, and BTM fillets all exhibited increased ( $P < 0.05$ ) shear force values when compared to BTIM fillets, but they did not differ ( $P = 0.25$ ) from one another. Additionally, in total energy required to shear the breast SEV fillets had required greater amounts of energy compared to MOD and NOR fillets ( $P < 0.01$ ). Moderate fillets also exhibited needing greater ( $P < 0.01$ ) amounts of total energy for shearing when compared to NOR fillets.

### 3.4 CONCLUSION

While further sensory evaluation is needed this research indicates that by stacking multiple forms of common processing techniques it may be possible to overcome some of the technological challenges associated with moderate to severe grades of wooden breast. The increased destruction and manipulation of proteins may have allowed for deeper marinade penetration offsetting many of the compositional differences noted between normal and woody fillets. By using additional objective measurements, like raw compression, in the sorting process a better method for sample selection may be noted from this study. Further sensory studies should be performed to investigate if these improvements are noted in consumer perceptions as well. Attention should also be given to the formation of a more standardized method to select and characterize the woody breast myopathy between breasts to reduce the reliance upon skilled sorters.

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**Table 1.** Least Squares Means for the main effect descriptive statistics for normal (NOR), moderate (MOD), and severe (SEV) wooden breast samples prior to processing including not processed (CON), blade tenderization (BT), blade tenderization and vacuum tumbling (BTM), and blade tenderization plus multi-needle injection and vacuum tumbling (BTIM)

Item	Wooden Breast Severity				SEM	P-value
	NOR	MOD	SEV			
<i>Descriptive</i> <sup>1</sup>						
Initial Wt, g	386.79 <sup>c</sup>	492.50 <sup>b</sup>	537.02 <sup>a</sup>	9.99	<0.0001	
White Striping Score <sup>2</sup>	1.03 <sup>c</sup>	1.28 <sup>b</sup>	1.73 <sup>a</sup>	0.06	<0.0001	
Hemorrhagic Score <sup>3</sup>	1.00 <sup>b</sup>	1.17 <sup>b</sup>	1.63 <sup>a</sup>	0.09	<0.0001	
Compression <sup>4</sup> , N	21.49 <sup>c</sup>	33.37 <sup>b</sup>	50.05 <sup>a</sup>	2.18	<0.0001	
pH	5.74 <sup>b</sup>	5.92 <sup>a</sup>	6.00 <sup>a</sup>	0.06	<0.0001	
<i>Color</i> <sup>1</sup>						
L*	65.63 <sup>c</sup>	68.64 <sup>b</sup>	69.70 <sup>a</sup>	0.29	<0.0001	
a*	9.89 <sup>c</sup>	10.61 <sup>b</sup>	11.16 <sup>a</sup>	0.14	<0.0001	
b*	15.22 <sup>b</sup>	17.12 <sup>a</sup>	17.82 <sup>a</sup>	0.26	<0.0001	
<i>Proximate Analysis</i> <sup>5</sup>						
Moisture, %	75.48 <sup>c</sup>	76.42 <sup>b</sup>	77.37 <sup>a</sup>	0.23	<0.0001	
Protein, %	22.03 <sup>a</sup>	20.83 <sup>b</sup>	19.87 <sup>c</sup>	0.21	<0.0001	
Fat, %	1.26 <sup>b</sup>	1.58 <sup>ab</sup>	1.67 <sup>a</sup>	0.14	0.0229	
Ash, %	1.23 <sup>a</sup>	1.17 <sup>ab</sup>	1.09 <sup>b</sup>	0.02	0.0008	
<i>Collagen</i> <sup>5</sup>						
Insoluble, mg/g	5.55 <sup>b</sup>	6.30 <sup>b</sup>	7.87 <sup>a</sup>	0.34	<0.0001	
Soluble, mg/g	2.57	2.79	3.04	0.18	0.2108	
<i>NMR Water</i> <sup>5,6</sup>						
P2B, %	2.40	2.26	2.39	0.18	0.4383	
P21, %	90.76 <sup>a</sup>	88.16 <sup>b</sup>	84.37 <sup>c</sup>	1.16	<0.0001	
P22, %	7.14 <sup>b</sup>	9.58 <sup>b</sup>	13.25 <sup>a</sup>	1.34	<0.0001	
T2B, ms	3.14	3.13	2.84	0.20	0.3174	
T21, ms	48.73 <sup>b</sup>	50.33 <sup>b</sup>	53.78 <sup>a</sup>	0.98	0.0011	
T22, ms	164.34 <sup>b</sup>	174.54 <sup>ab</sup>	191.78 <sup>a</sup>	8.73	0.0082	
Item	Processing Treatment				SEM	P-value
	CON	BT	BTM	BTIM		
<i>Descriptive</i> <sup>1</sup>						
Initial Wt, g	470.06	480.82	470.44	467.08	10.95	0.7198
Wooden Breast Score <sup>2</sup>	1.24	1.42	1.42	1.31	0.07	0.2407
Hemorrhagic Score <sup>3</sup>	1.24	1.29	1.27	1.27	0.10	0.9810
Compression <sup>3</sup> , N	34.50	37.15	35.33	32.90	2.38	0.4562
<i>Color</i> <sup>1</sup>						
L*	68.42	68.03	67.75	67.76	0.34	0.4369
a*	10.40 <sup>ab</sup>	10.85 <sup>a</sup>	10.69 <sup>ab</sup>	10.27 <sup>b</sup>	0.17	0.0455
b*	16.86	17.21	16.70	16.11	0.30	0.0731

<sup>abc</sup>Means within a row with differing superscripts are different,  $P < 0.05$ .

<sup>1</sup>Based on total sample evaluation (N = 360).

<sup>2</sup>White striping score was manually scored based on 3-point scale where 1 = normal, 2 = moderate, and 3 = severe condition (Kuttappan et al., 2012).

<sup>3</sup>Hemorrhagic lesion visually scored based on 3-point scale where 1 = none, 2 = moderate, 3 = severe (Kuttappan et al., 2017).

<sup>4</sup>Compression was conducted on the superficial cranial region of each breast (N = 360) approximately 1 cm from the point of the breast on the sagittal plane.

<sup>5</sup>Based on a subsample of breast (N = 45) representing each wooden breast classification.

<sup>6</sup>Nuclear magnetic resonance was conducted on cooked breasts (N = 45) to examine the mobility and physiochemical properties of the water fraction within the fillets.

**Table 2.** Least Squares Means for the main effect processing characteristics for normal (NOR), moderate (MOD), and severe (SEV) wooden breast samples after processing including not processed (CON), blade tenderization (BT), blade tenderization and vacuum tumbling (BTM), and blade tenderization plus multi-needle injection and vacuum tumbling (BTIM)

Item	Wooden Breast Severity				SEM	P-value
	NOR	MOD	SEV			
Initial Processing Wt, g	385.52 <sup>c</sup>	488.73 <sup>b</sup>	533.20 <sup>a</sup>		10.31	<0.0001
Post Processing Wt, g	400.62 <sup>c</sup>	505.13 <sup>b</sup>	546.32 <sup>a</sup>		11.15	<0.0001
Injection Pickup <sup>1</sup> , %	6.76 <sup>a</sup>	5.80 <sup>b</sup>	4.96 <sup>c</sup>		0.23	<0.0001
Total Pickup <sup>2</sup> , %	4.14 <sup>a</sup>	3.31 <sup>b</sup>	2.47 <sup>c</sup>		0.44	<0.0001
Purge Loss <sup>3</sup> , %	0.32 <sup>b</sup>	0.76 <sup>a</sup>	0.71 <sup>a</sup>		0.13	0.0022
<i>Color</i>						
L*	64.06 <sup>c</sup>	67.14 <sup>b</sup>	68.29 <sup>a</sup>		0.33	<0.0001
a*	10.64 <sup>c</sup>	11.19 <sup>b</sup>	11.79 <sup>a</sup>		0.14	<0.0001
b*	16.88 <sup>b</sup>	18.46 <sup>a</sup>	18.43 <sup>a</sup>		0.26	<0.0001
	Processing Treatment				SEM	P-value
	CON	BT	BTM	BTIM		
Initial Processing Wt, g	467.77	477.62	467.36	463.85	11.23	0.7256
Post Processing Wt, g	467.78	477.82	494.02	496.47	12.07	0.0921
Total Pickup, %	0.00 <sup>c</sup>	0.00 <sup>c</sup>	5.97 <sup>b</sup>	7.25 <sup>a</sup>	0.46	<0.0001
Purge Loss <sup>3</sup> , %	0.45	0.65	0.63	0.66	0.14	0.4834
<i>Color</i>						
L*	68.13 <sup>a</sup>	68.21 <sup>a</sup>	65.07 <sup>b</sup>	64.57 <sup>b</sup>	0.36	<0.0001
a*	11.68 <sup>a</sup>	11.61 <sup>a</sup>	11.29 <sup>a</sup>	10.26 <sup>b</sup>	0.16	<0.0001
b*	19.14 <sup>a</sup>	18.70 <sup>a</sup>	17.41 <sup>b</sup>	16.43 <sup>b</sup>	0.30	<0.0001

<sup>abc</sup>Means within a row with differing superscripts are different,  $P < 0.05$ .

<sup>1</sup>Injection pickup is the amount of marinade pickup from multi-needle injection only in BTIM prior to vacuum tumbling.

<sup>2</sup>Total pick up for the main effect of severity includes processing treatments CON and BT that were not subjected to marinade and had 0% pickup.

<sup>3</sup>Purge loss percentage is the amount of moisture loss occurring during an 8-hr rest between processing treatment application and cooking.

**Table 3.** Least Squares Means for the main effect cooking characteristics for normal (NOR), moderate (MOD), and severe (SEV) wooden breast samples after processing including not processed (CON), blade tenderization (BT), blade tenderization and vacuum tumbling (BTM), and blade tenderization plus multi-needle injection and vacuum tumbling (BTIM)

Item	Wooden Breast Severity				SEM	P-value
	NOR	MOD	SEV			
Pre-cook Wt, g	399.41 <sup>c</sup>	503.13 <sup>b</sup>	541.55 <sup>a</sup>		11.34	<0.0001
Post-cook Wt, g	332.91 <sup>b</sup>	408.33 <sup>a</sup>	416.94 <sup>a</sup>		9.47	<0.0001
Cook Time, min	42.82 <sup>b</sup>	54.21 <sup>a</sup>	54.00 <sup>a</sup>		1.80	<0.0001
Cook Loss, %	16.56 <sup>c</sup>	19.06 <sup>b</sup>	23.05 <sup>a</sup>		0.49	<0.0001
<i>NMR Water</i> <sup>1</sup>						
P2B, %	0.49 <sup>a</sup>	0.45 <sup>a</sup>	0.37 <sup>b</sup>		0.03	<0.0001
P21, %	63.72 <sup>a</sup>	60.94 <sup>b</sup>	59.79 <sup>b</sup>		1.84	0.0029
P22, %	35.78 <sup>b</sup>	38.58 <sup>a</sup>	39.84 <sup>a</sup>		1.81	0.0022
T2B, ms	0.32	0.32	0.33		0.01	0.1143
T21, ms	28.49 <sup>c</sup>	29.58 <sup>b</sup>	30.65 <sup>a</sup>		0.57	<0.0001
T22, ms	190.79 <sup>b</sup>	193.39 <sup>b</sup>	216.90 <sup>a</sup>		20.38	0.0101
<i>Color</i>						
L*	81.05 <sup>a</sup>	78.60 <sup>b</sup>	76.39 <sup>c</sup>		0.57	<0.0001
a*	7.00 <sup>b</sup>	7.14 <sup>b</sup>	7.94 <sup>a</sup>		0.64	<0.0001
b*	18.15 <sup>b</sup>	18.83 <sup>b</sup>	20.23 <sup>a</sup>		1.24	<0.0001
Item	Processing Treatment				SEM	P-value
	CON	BT	BTM	BTIM		
Pre-cook Wt, g	465.68	472.63	492.58	494.57	12.23	0.0639
Post-cook Wt, g	358.57 <sup>b</sup>	359.11 <sup>b</sup>	403.56 <sup>a</sup>	423.00 <sup>a</sup>	10.26	<0.0001
Cook Time, min	51.98	50.46	50.53	48.41	1.89	0.1772
Cook Loss, %	22.54 <sup>a</sup>	23.56 <sup>a</sup>	17.84 <sup>b</sup>	14.28 <sup>c</sup>	0.57	<0.0001
<i>NMR Water</i> <sup>1</sup>						
P2B, %	0.41	0.44	0.45	0.46	0.03	0.2332
P21, %	60.55	61.72	62.45	61.22	1.86	0.5413
P22, %	39.01	37.84	37.09	38.32	1.84	0.5383
T2B, ms	0.32	0.32	0.33	0.32	0.01	0.1968
T21, ms	26.18 <sup>c</sup>	26.14 <sup>c</sup>	31.72 <sup>b</sup>	34.27 <sup>a</sup>	0.57	<0.0001
T22, ms	156.01 <sup>c</sup>	162.34 <sup>c</sup>	221.59 <sup>b</sup>	261.49 <sup>a</sup>	20.53	<0.0001
<i>Color</i>						
L*	79.06	78.47	78.19	79.00	0.60	0.3870
a*	7.30	7.56	7.47	7.12	0.65	0.0606
b*	19.58 <sup>a</sup>	19.79 <sup>a</sup>	18.52 <sup>b</sup>	18.39 <sup>b</sup>	1.25	0.0003

<sup>abc</sup>Means within a row with differing superscripts are different,  $P < 0.05$ .

<sup>1</sup> Nuclear magnetic resonance was conducted on cooked breasts (N = 45) to examine the mobility and physiochemical properties of the water fraction within the fillets.

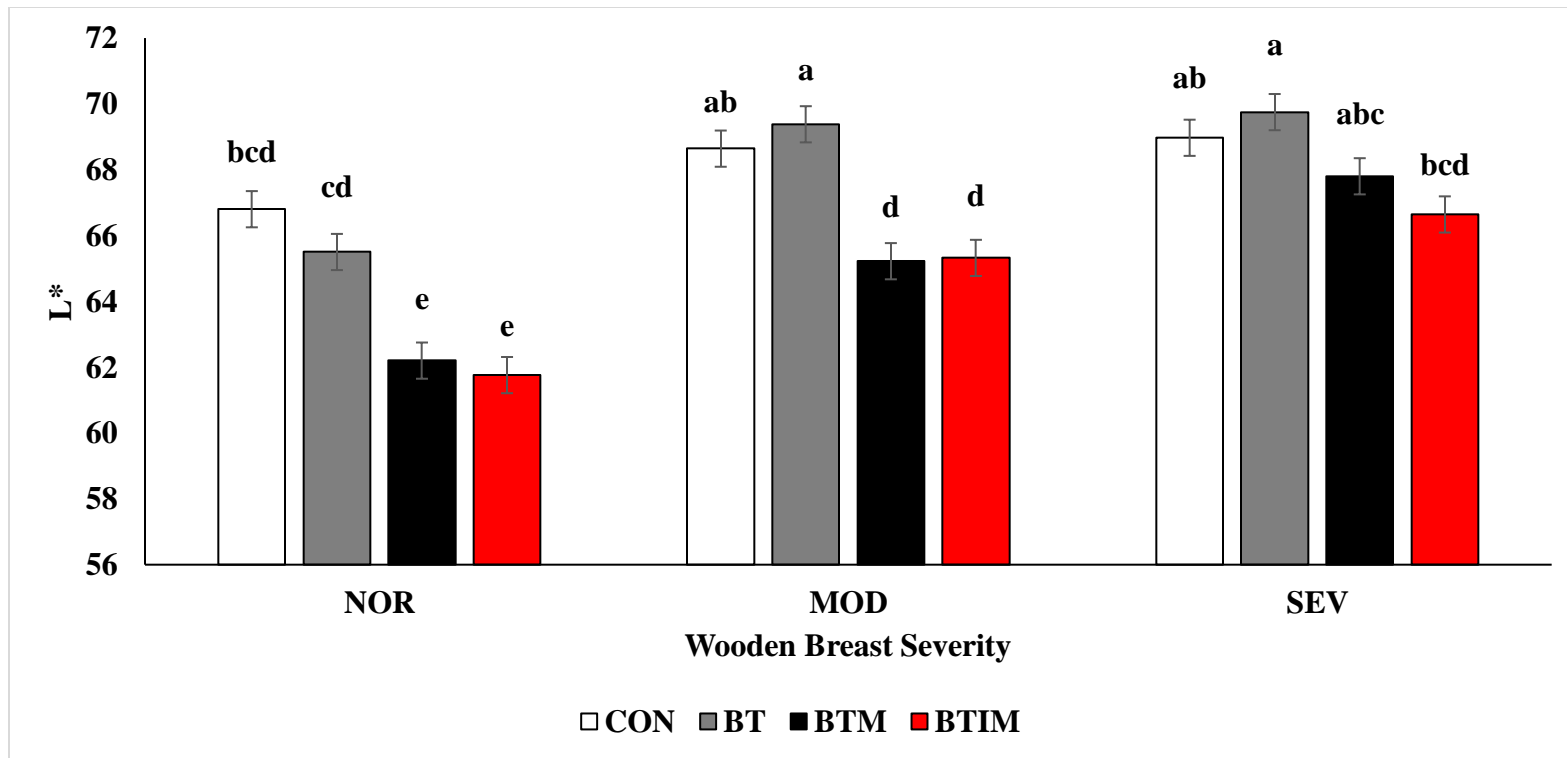
Table 4. Least Squares Means for the main effect of objective tenderness and texture for normal (NOR), moderate (MOD), and severe (SEV) wooden breast samples after stacked processing including not processed (CON), blade tenderization (BT), blade tenderization and vacuum tumbling (BTM), and blade tenderization plus multi-needle injection and vacuum tumbling (BTIM)

Item	Wooden Breast Severity			SEM	P-value	
	NOR	MOD	SEV			
<i>Objective Tenderness</i>						
Multiblade Shear, N	33.53 <sup>c</sup>	42.97 <sup>b</sup>	57.64 <sup>a</sup>	2.58	<0.0001	
Total Energy, N.mm	170.04 <sup>c</sup>	246.16 <sup>b</sup>	312.92 <sup>a</sup>	22.13	<0.0001	
<i>Objective Texture</i> <sup>1</sup>						
Hardness, N	19.83 <sup>b</sup>	18.30 <sup>b</sup>	22.32 <sup>a</sup>	1.28	<0.0001	
Adhesion, N.sec	-0.06 <sup>b</sup>	-0.04 <sup>b</sup>	0.00 <sup>a</sup>	0.01	<0.0001	
Resiliency, %	35.81 <sup>b</sup>	36.91 <sup>b</sup>	42.05 <sup>a</sup>	1.01	<0.0001	
Cohesion	0.69 <sup>b</sup>	0.70 <sup>b</sup>	0.74 <sup>a</sup>	0.01	<0.0001	
Springiness, %	84.78 <sup>b</sup>	84.35 <sup>b</sup>	89.30 <sup>a</sup>	1.02	<0.0001	
Chewiness	11.71 <sup>b</sup>	10.66 <sup>b</sup>	14.69 <sup>a</sup>	1.15	<0.0001	
Item	Processing Treatment				SEM	P-value
	CON	BT	BTM	BTIM		
<i>Objective Tenderness</i>						
Multiblade Shear, N	45.38 <sup>a</sup>	48.29 <sup>a</sup>	44.08 <sup>a</sup>	41.11 <sup>b</sup>	2.71	0.0195
Total Energy, N.mm	235.65	256.57	250.23	229.71	22.73	0.2519
<i>Objective Texture</i> <sup>1</sup>						
Hardness, N	21.01 <sup>ab</sup>	21.35 <sup>a</sup>	18.99 <sup>b</sup>	19.25 <sup>b</sup>	1.33	0.0448
Adhesion, N.sec	-0.02	-0.04	-0.03	-0.04	0.01	0.6187
Resiliency, %	38.58 <sup>a</sup>	35.49 <sup>b</sup>	38.61 <sup>a</sup>	40.36 <sup>a</sup>	1.05	<0.0001
Cohesion	0.72 <sup>ab</sup>	0.69 <sup>b</sup>	0.70 <sup>b</sup>	0.73 <sup>a</sup>	0.01	0.0006
Springiness, %	85.57 <sup>ab</sup>	83.51 <sup>b</sup>	87.56 <sup>a</sup>	87.94 <sup>a</sup>	1.12	0.0048
Chewiness	12.98	12.38	11.71	12.34	1.18	0.3855

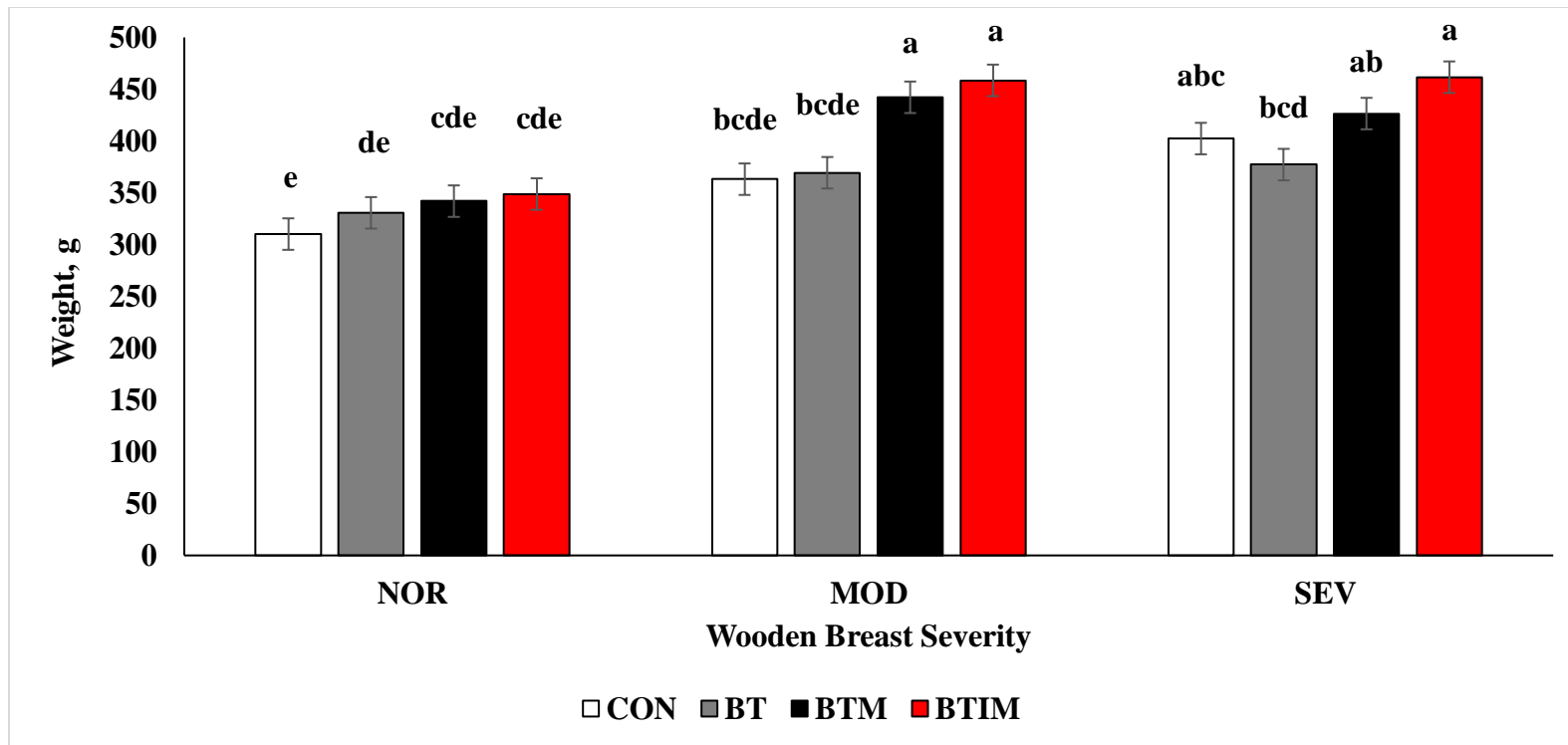
<sup>abc</sup>Means within a row with differing superscripts are different,  $P < 0.05$ .



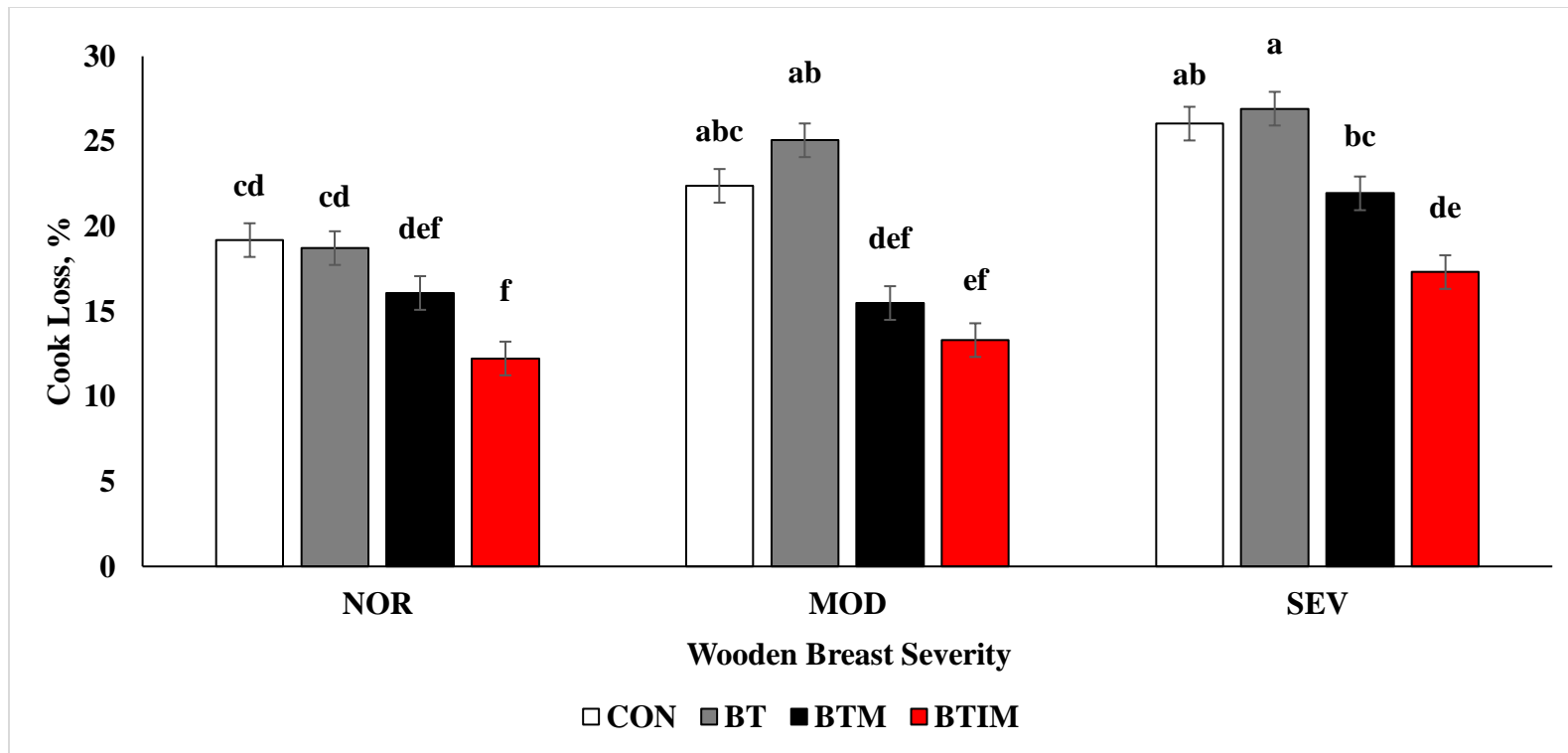
**Figure 1.** Examination of marinade retention in woody breasted fillets and the interaction of severity and treatment main effects. Bars without a common superscript differ ( $P < 0.05$ ).



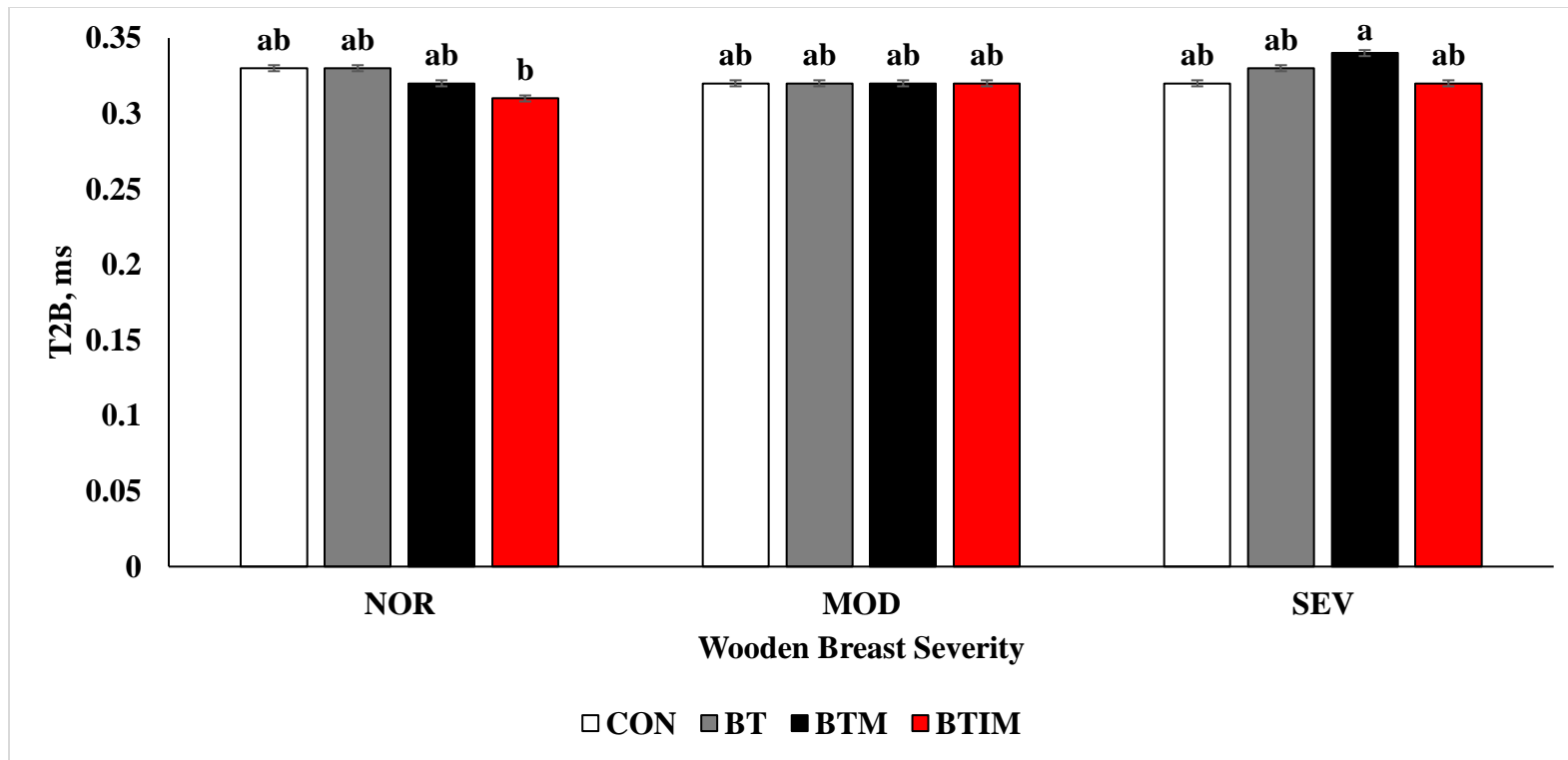
**Figure 2.** Examination of L\* or brightness in post processed woody breasted fillets and the interaction of severity and treatment main effects. Bars without a common superscript differ ( $P < 0.05$ )



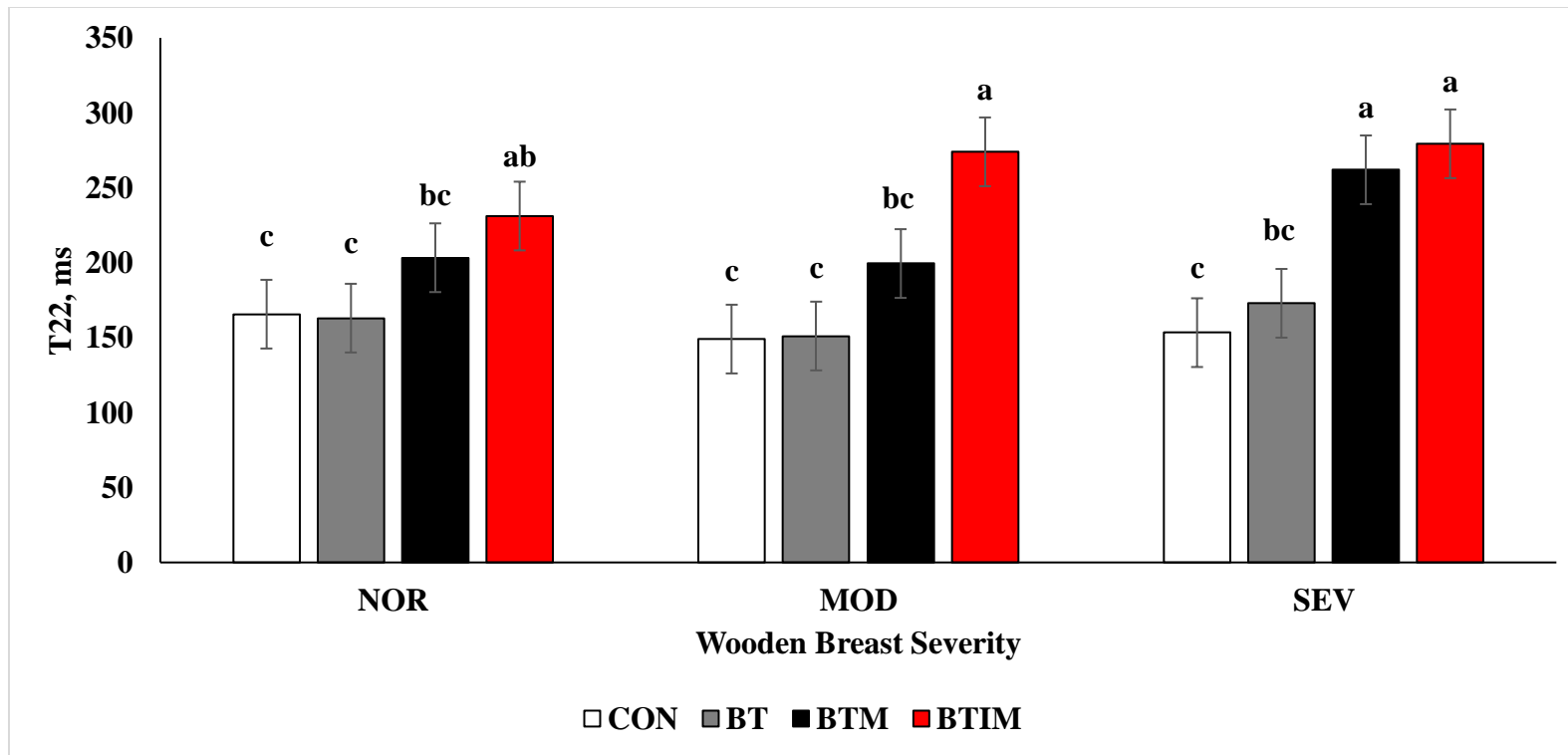
**Figure 3.** Examination of cooked weight in woody breasted fillets and the interaction of severity and treatment main effects. Bars without a common superscript differ ( $P < 0.05$ )



**Figure 4.** Examination of cook loss (%) in woody breasted fillets and the interaction of severity and treatment main effects. Cook loss was calculated as ((post-treatment weight – pre-cook weight)/ post-treatment weight). Bars without a common superscript differ ( $P < 0.05$ ).



**Figure 5.** Examination of T2B, bound water relaxation times, in woody breasted fillets and the interaction of severity and treatment main effects. Bars without a common superscript differ ( $P < 0.05$ ).



**Figure 6.** Examination of T22, extra-myofibrillar water relaxation times, in woody breasted fillets and the interaction of severity and treatment main effects. Bars without a common superscript differ ( $P < 0.05$ ).

## CHAPTER 4

### CONCLUSIONS

This research shows that with multiple forms of further processing it may be possible to remediate and potentially correct some of the textural issues noted within the woody breast myopathy. Some technological attributes like cook loss and marinade retention were improved with the addition of multiple processing methods. These results may be due to increased fiber disruption allowing for deeper and more effective marinade penetration. Additionally, it was possible to remediate moderately affect fillets to have similar textural attributes as unaffected fillets. Notably, it was still difficult to correct the textural attributes noted within SEV fillets, so in-house sorting may be necessary to effectively select fillets that can undergo remediation. This study should be followed by additional sensory studies to investigate whether consumers can detect differences between the varying severities and processing treatments to ensure that products would be considered acceptable subjectively. Further research should be conducted to investigate the remediation of woody breasted chicken.