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DEVELOPMENT OF AN INJECTABLE "MODIFIED MARBLING" SOLUTION FOR WHOLE MUSCLE BEEF CUTS

By

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ABSTRACT

DEVELOPMENT OF AN INJECTABLE "MODIFIED MARBLING" SOLUTION FOR WHOLE MUSCLE BEEF CUTS

By

Christine Suzanne Quinlan

Low quality beef cuts with <3% intramuscular fat are lower in tenderness, juiciness and flavor. Investigating non-meat ingredients that can mimic the properties of intramuscular fat and processing technologies that can incorporate this mixture into beef would be advantageous to the meat industry. The objectives of this research were to develop and determine the effects of the injection of a "modified marbling" solution containing sodium alginate (SA), iota carrageenan (IC) whey protein isolate (WPI) and modified food starch (MFS) on the quality attributes of USDA Select ribeye rolls.

In study 1, twenty-five ingredient combinations (ranging from 0.25 to 0.50% addition) of the four ingredients were formulated into 500 g solutions using a 2^4 central composite design. Solution pH, apparent viscosity and gel (24 h, 4 °C storage) objective color, water-holding capacity and strength were analyzed to determine the optimal solution. In study 2, the solution was modified and processing system parameters were determined on a multi-needle injector to incorporate the solution into whole muscle beef cuts. The solution was then injected into USDA Select ribeye rolls (5-7% pick-up). In studies 3 and 4, the properties of the "modified marbling" solution were verified in the meat by comparing the chemical and sensory attributes to control ribeye rolls (USDA Select, Low and Average Choice). The injected ribeye rolls were designated to 0, 14, 28, or 42 days of storage (1°C), weighed for ribeye purge and steaks (2.54 cm) were

fabricated on each storage day. A 7-day retail shelf life study (analysis of thiobarbituric acid reactive substances, color and percent purge) (study 3), Warner-Bratzler shear force and sensory evaluation (study 4) were conducted on the fabricated steaks.

The data from study 1 resulted in the following recommended levels of non-meat ingredients for the "modified marbling" solution: 0.4375% SA and IC and 0.375% WPI and MFS. In study 2, 3% beef tallow and 0.25% beef flavor were added to improve the hydrophobicity and flavor respectively of the "modified marbling" solution. Parameters were also set on an automatic, multi-needle injector to acquire the desired percent pick-up (5-7%) and "modified marbling" pattern. In study 3, the injected Select ribeye rolls had a significantly higher ($P<0.05$) ribeye purge than the Average Choice control. For TBARS values, the injected Select ribeye rolls were significantly higher ($P<0.05$) than the controls. There were no significant differences in color scores or steak purge between treatments in this study. In study 4, the injected ribeye rolls were higher ($P<0.05$) compared to the USDA Select control ribeye rolls in beef fat flavor, however a significant but small off-flavor was found ($P<0.05$) in the injected ribeye rolls. There were no differences between the injected and control ribeye rolls for shear force, sensory tenderness or juiciness.

The results indicate the viability of producing and injecting a "modified marbling" solution into whole muscle beef cuts. The solution also has the potential to improve lower quality beef cuts but more research is needed to improve the "modified marbling" properties. One possibility is that the amount of fat in the solution could be increased to achieve the benefits of flavor, hydrophobicity and to improve upon the tenderness, juiciness and marbling appearance of the injected whole muscle beef cuts.

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INTRODUCTION

Marbling or intramuscular fat is the fat within the lean cut surface of the *longissimus thoracis* muscle at the 12^{th} – 13^{th} rib interface of a beef carcass. The amount or degree of marbling is one of the primary factors for assigning a USDA beef carcass quality grade (USDA 1997) and has been shown to influence the palatability (tenderness, juiciness and flavor) of the final beef product. Smith and others (1984) reported minute, but statistically significant differences in meat palatability as the degree of marbling decreased from Moderately Abundant (USDA Prime) to Practically Devoid (USDA Standard). Also, Tatum and others (1980) found that rib steaks from High and Average Choice carcasses were juicier, more flavorful and more palatable than steaks from Low Select and High Standard carcasses.

Studies have been conducted to determine quality inconsistencies within the beef industry chain, from farm to retail. The results from the last National Beef Quality Audit (McKenna and others 2002) reported that the overall average scores for intramuscular fat and USDA beef carcass quality grades were Small^{06} (marbling score) and USDA Select^{79} (USDA Quality Grade) respectively. The fourth challenge in the "top ten quality challenges" identified from the audit was insufficient marbling since it was found that 45% of carcasses graded USDA Select (Slight degree of marbling), 53% graded USDA Choice (9% moderate, 26% modest and 65% small degree of marbling) and only 2% graded USDA Prime. The overall average scores for intramuscular fat and USDA beef carcass quality grades were below the expectations of the meat industry. These low scores can influence the consumer's purchasing decisions. When consumers are not

satisfied with the palatability of beef cuts, their intent to purchase beef may decrease and along with it the opportunity for the beef industry to generate revenue. Savell and others (1987) reported that beef packers demand beef carcasses that grade USDA Choice or higher due to substantial price discounts when carcasses grade less than USDA Choice.

In order to determine the amount of marbling necessary for acceptable palatability, Savell and Cross (1988) developed a "window of acceptability" for percent intramuscular fat of retail beef cuts. Broiling cuts (rib, loin, sirloin, etc.) containing 3-7% intramuscular fat are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability but at 3% intramuscular fat there is little room for error in cookery method or degree of doneness. Three percent intramuscular fat is associated with the minimum Slight degree of marbling, 5% is related to the midpoint of the Small amount of marbling and 7% is associated with the lower end of the Moderate amount of marbling. From the last National Beef Quality Audit, 45% of the carcasses had Slight degree of marbling or approximately 3% intramuscular fat and are at the lower edge of the "window of acceptability." This indicates an opportunity for improvement by increasing the amount of marbling in whole muscle beef cuts to ensure acceptable palatability.

Deposition of intramuscular fat is influenced by several pre-harvest factors such as breed, length of feeding, type of ration fed (concentrate vs. grass) and management but the palatability of whole muscle cuts fabricated from lower quality (less than USDA Choice) beef carcasses may be improved through post-harvest, innovative non-meat ingredient and processing technologies. Several different non-meat ingredients (salt, phosphate, gums, starches and non-meat proteins) and processing technologies (injection,

restructuring, mechanical tenderization, tumbling and mixing) have all ready been used to add value to lower quality meat products including whole muscle cuts. Development of a "modified marbling" solution that can be directly injected into lower quality whole muscle beef cuts at the level of 5-7% may enhance the overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

The hypothesis for this project was that a "modified marbling" solution manufactured with selected non-meat ingredients injected into less marbled beef ribeye rolls (USDA Select) would create a steak that is similar to USDA Choice beef steaks in tenderness, juiciness and flavor. To test this hypothesis, four separate studies were conducted. In study I, response surface methodology was utilized to determine the concentration of each ingredient (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) to be used in the development of the "modified marbling" solution. Study II was conducted to modify the solution, determine the processing system parameters and demonstrate that the "modified marbling" solution can be injected into whole muscle beef cuts. Study III and IV were done in order to verify the properties of the "modified marbling" solution in whole muscle beef cuts and to evaluate the shelf stability of the injected cuts. Ribeye rolls were injected with the solution, cut into steaks and the chemical (study III) and sensory (study IV) properties were compared to three controls (USDA Select, Low Choice and Average Choice).

This dissertation is formatted as five chapters. Chapter 1 is the review of literature. Chapters 2-5 are manuscript style chapters and Chapter 6 is followed by recommendations for future research. Finally, appendices are provided with step-by-step procedures for all protocols used in each study.

CHAPTER 1

LITERATURE REVIEW

1.1. Beef carcass quality

1.1.1. USDA beef carcass quality grades

USDA beef carcass quality grades are based on carcass maturity and the amount of marbling or intramuscular fat present on the exposed surface of the *longissimus thoracis* muscle at the 12^{th}-13^{th} rib interface (USDA 1997). There are eight quality grades: Prime, Choice, Select, Standard, Commercial, Utility, Cutter and Canner for steer and heifer carcasses. In order to determine the quality grade, the carcasses are split equally down the back into two sides and one side is partially separated into hindquarter and forequarter (ribbed between the 12^{th} and 13^{th} rib).

Maturity: Overall carcass maturity is composed of skeletal and lean maturity. The skeletal maturity is determined by the size, shape, and ossification of the bones and cartilages, especially of the split chine bones. Ossification occurs at an earlier stage of maturity in the split chine bones in the sacral vertebrae and at a later stage of maturity in the lumbar and thoracic vertebrae. The chine bones are also soft and very red in color in younger carcasses and hard and white in very mature carcasses. The size and shape of the rib bones are used as well when determining maturity. In younger carcasses, the rib bones only have a slight tendency toward flatness but in older carcasses, the rib bones are wide and flat (USDA 1997).

Lean maturity is determined by the color and texture of the lean flesh on the surface of the exposed ribeye separated between the 12^{th} and 13^{th} ribs. In a younger

carcass, the lean is very fine in texture and light, grayish red in color but as the carcass maturity increases, the texture of the lean becomes coarser and the color of the lean is a darker red. Skeletal and lean maturity is scored in percentages from A^0 (youngest) to E^{100} (oldest). The approximate ages corresponding to each maturity classification are: A: 9 to 30 months, B: 30 to 42 months, C: 42 to 72 months, D: 72 to 96 months and E: more than 96 months. Slightly more emphasis is placed on the skeletal maturity if the skeletal maturity is different from the lean maturity (USDA 1997).

Marbling: Marbling or the amount of intramuscular fat (fat found between the muscle bundles) is evaluated on the cut lean surface of the exposed ribeye muscle of beef carcasses separated between the 12^{th} and 13^{th} rib. The subjective assessment takes into account the amount, size, number and distribution of intramuscular fat deposits (Dubeski and others 1997). Marbling is the primary factor affecting quality grade. The degrees of marbling, in order of descending quantity, are abundant (Ab), moderately abundant (MAb), slightly abundant (SlAb), moderate (Md), modest (Mt), small (Sm), slight (Sl), traces (Tr) and practically devoid (PD) (Figure 1) (USDA 1997). Marbling can be scored as percentages, for example, if the amount of marbling is higher than the minimum small but less than the minimum modest, then the marbling level is between Sm^0 and Sm^{100}. If the amount of marbling is 50 percent of the way to modest, then the amount of marbling is Sm^{50}. Percentages should be no smaller than units of 10 (Romans and others 2001).

The relationship between maturity and marbling determines the carcass quality grade. Beef quality grades are commonly divided into thirds or halves. The most common divisions are: Prime (thirds), Choice (thirds), Select (halves), Standard (halves),

Commercial (thirds) and Utility (thirds). The symbols most commonly used for the divisions are: high (+), average (o) and low (-) (AMSA 2001).

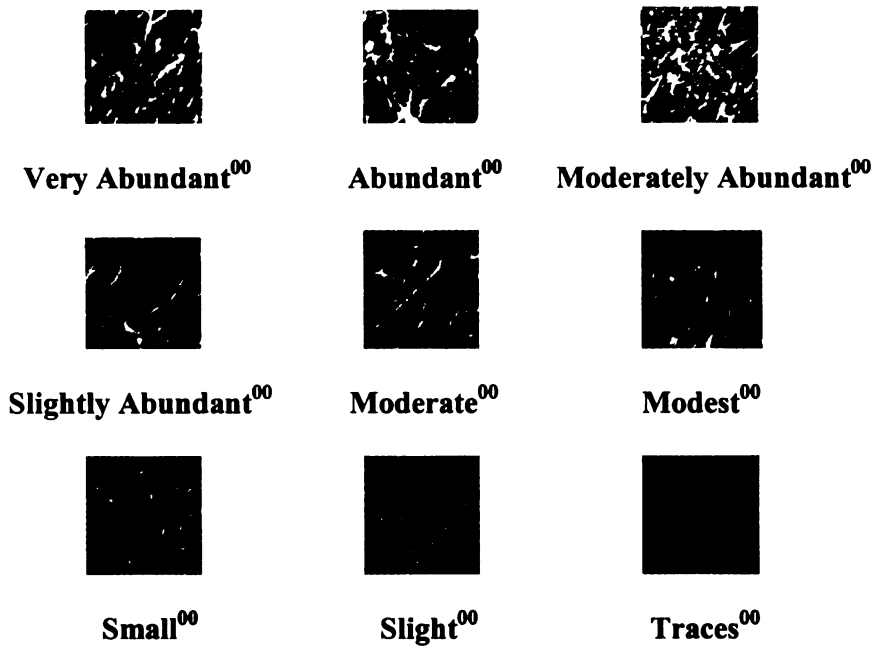

Figure 1.1. Lower limits of marbling degrees used in beef carcass quality grading. (Adapted from University of Nebraska Extension fact sheet by D Burson)

1.1.2. Growth and development of fat depositions

The growth and development of adipose tissue begins when it develops into lobes that are enclosed in a sheath of collagenous fibers. The adipose cells then begin to accumulate lipid (adipoblasts) and when the cell is filled with lipid it is known as an adipocyte (Aberle and others 2001). Adipose tissue masses can expand by hyperplasia (cell proliferation), hypertrophy (cell enlargemnent) or a combination of the two (Bjorntorp and Sjostrom 1971; Greenwood and Hirsch 1974; Stern and Greenwood 1974).

Adipoblasts develop at differing rates in different parts of the body. In young animals, deposits of fat usually develop in the visceral areas first and then deposit

beneath the skin (subcutaneous), between the muscles (intermuscular) and between muscle bundles (intramuscular) (Aberle and others 2001). Hood and Allen (1973) and Garbutt and others (1979) found that hyperplasia is completed in the perirenal and subcutaneous adipose tissues by the first year of age in cattle and further increases in adipose mass after one year of age is assumed to be mainly from cell enlargement. However, since intramuscular fat is the last to develop, these fat cells continue to develop in growing and adult animals because a smaller proportion of nutrient intake is required for growth of other tissues and a greater proportion is available for energy storage. Hood and Allen (1973) found that intramuscular adipose tissue grows by both hyperplasia and hypertrophy in steers at 14 months of age.

1.1.3. Types of fat depositions

The fat portion represents the greatest source of variation in muscle tissue when altering the proportion of fat to lean (Allen 1988).

Subcutaneous fat: Subcutaneous fat (external fat) or the fat beneath the skin of the carcass is useful for predicting total fat content of beef carcasses (Johnson and Vidyadaran 1981; McIntyre and Ryan 1983). Beef carcass subcutaneous fat depth is taken at the 12-13th rib site and is more actively mobilized than other fat depots (Butler-Hogg and others 1985). The amount of subcutaneous fat deposited is influenced by several factors including: breed (Kempster and others 1976; Truscott and others 1983), sex (Seideman and others 1982), age, carcass weight and season (Hopkins and others 1993).

The amount of intramuscular fat and quality grade have been shown to affect and predict beef palatability but still others have found that they provide little assurance that

the beef will be palatable. Therefore, studies have been conducted to determine if the amount of subcutaneous fat is a predictor of beef palatability. Dolezal and others (1982a) found progressive increases in palatability of cooked beef as the amount of subcutaneous fat increased from less than 2.53 mm up to 7.61 mm, but subcutaneous fat greater than 7.61 mm did not improve palatability. The mechanism by which the amount of subcutaneous fat or fattening improved palatability (tenderness) was that the increased thickness of subcutaneous fat caused carcasses to chill more slowly, which increased enzyme activity and decreased sacromere shortening thereby improving meat tenderness. In another study, it was shown that 6-10 mm of subcutaneous fat was sufficient to retard the postmortem chilling process to assure that beef from young cattle were tender (Smith and others 1976). Tatum and others (1982), however, reported that subcutaneous fat or fat thickness alone was not an effective measure of cooked beef palatability compared to intramuscular fat and would not be a suitable substitute.

It was found though, that the combination of subcutaneous fat and intramuscular fat was an important factor in the determination of beef palatability. In studies with young bulls, Riley and others (1983a, b) showed that the combination of subcutaneous fat thickness and intramuscular fat was important in ensuring that meat from young bulls would be adequately tender. They found that steaks from Standard and Select bulls and steers that had less than 7.6 mm fat thickness were significantly less palatable than steaks from Choice steers or Select bulls with at least 7.6 mm of fat thickness.

Intermuscular fat: Intermuscular fat or seam fat is the fat found between the muscles and it has been shown that retail beef cuts contain twice as much separable seam fat as separable subcutaneous fat (Savell and others 1991). Closer trimming of

subcutaneous fat has emphasized intermuscular fat deposits, becoming more evident during the fabrication of retail cuts. Retail cuts from the beef rib contain the highest percentage of seam fat compared to cuts from the chuck, loin and round (USDA 1990; Savell and others 1991) and there is a considerable amount of variation in the amount of seam fat from anterior to posterior end within the rib (Moore and others 1989). Wulf and others (1994) found larger amounts of seam fat in the 7^{th}, 8^{th} and 9^{th} rib bone sections.

Since consumers demand leaner beef in the retail case (Cross and others 1986) and because it is difficult to remove intermuscular fat without destroying the shape and integrity of the cut, studies have been conducted to identify factors that contribute to intermuscular fat deposition. Carcass traits connected to the USDA yield grade equation (hot carcass weight, % kidney, pelvic and heart fat, ribeye area and fat thickness) (USDA 1997) and USDA quality grade (marbling and maturity) have been found to be good predictors of seam fat. Jones and others (1990) showed that the amount of seam fat increased as the USDA yield grade and marbling score increased. They determined that carcasses with high yield grades should be avoided in order to decrease the amount of seam fat on trimmed retail cuts.

Intramuscular fat: The intramuscular fat (fat found between the muscle bundles) or marbling is the most variable component of fat deposition. Savell and others (1986) found that the amount of chemical fat in uncooked *longissimus lumborum* muscle of 518 beef carcasses varied from 10.42% in Moderately Abundant degree of marbling to 1.77% in Practically Devoid.

1.1.4. Factors affecting deposition of intramuscular fat

Carcasses vary in composition through genetic, nutritional, hormonal and management/environmental effects.

Breed: Breed can influence the amount of intramuscular fat since animals of different breeds grow and develop in a specific manner and produce carcasses with distinctive characteristics particular to that breed. Dubeski and others (1997) found that Angus breeds had superior marbling compared to Hereford and Hereford x Angus breeds and they also found that Holsteins were similar to Hereford x Angus with less intramuscular fat than Angus but more than Herefords. Dairy breeds including Holsteins, have less external fat than most beef breeds and the carcasses are superior in USDA yield grade compared to some beef breeds (Cole and others 1963; Young and others 1978; Nour and others 1983). Angus cattle have been found to deposit intramuscular fat at an earlier age and produce more than Herefords or Shorthorns (Kauffman and other 1968; Cramer and others 1973).

Nutrition: Nutrition can also affect the amount of intramuscular fat deposited given that nutrition dictates the rate of growth of the animal and the extent of development. The type of ration fed (concentrate vs. grass) can influence the degree of intramuscular fat since feeding a high concentrate diet produces a rapid animal growth rate, which increases the deposition of intramuscular fat. Vestergaard and others (2000) found that the intramuscular fat content of bulls fed a roughage based diet was 50% lower in the *semitendinosus and longissimus lumborum* and 30% lower in the *supraspinatus* compared to bulls fed a concentrate based diet. Reduced tenderness of meat from grass-fed steers has also been reported (Bowling and others 1978; Schroeder and others 1980).

This could be partly due to cold shortening because of the thin subcutaneous fat cover and the rapid growth rate of grain-finished steers, which reduces the effect of connective tissue on muscle toughness (Allingham and others 1998). Vestergaard and others (2000) showed that shear force values of the *semitendinosus* were 33% higher (less tender) for bulls fed the roughage based diet and sensory panel scores for tenderness, flavor and juiciness of the *longissimus lumborum* also were lower. An off-flavor, additionally, was almost solely detected in meat from roughage fed bulls.

Increasing the amount of time cattle are fed high-concentrate diets can also enhance the deposition of intramuscular fat in beef carcasses due to increased carcass maturity and fat deposition, decreased yield grade, and increased percentage of carcasses grading USDA Choice. Increasing feeding time from 100 to 160 days had a beneficial effect on flavor but did not affect juiciness, tenderness, or overall palatability (Tatum and others 1980). Dolezal and others (1982b) found that extending feeding time beyond 90 to 100 days did little to increase beef palatability. It was recommended that the minimum marbling requirement to grade USDA Choice could be met with no loss in palatability if cattle were fed a high-concentrate diet for at least 90 days prior to slaughter.

Hormone implants: The use of hormone implants can also affect the deposition of intramuscular fat. Anabolic implants have been used to improve growth rate and feed efficiency of cattle. They have also been shown to reduce fat thickness, percentage of internal fat, USDA yield grade, marbling and USDA quality grade while increasing carcass weight, carcass conformation and *longissimus thoracis* muscle area (Galbraith and others 1981; Rumsey 1982; Trenkle 1987). Estrogens increase protein deposition by increasing the concentration of somatotropin secreted from the anterior pituitary and

insulin secreted from the β-cells of the pancreas (Aberle and others 2001). Androgens have been shown to increase carcass protein content of cattle by stimulation of muscle protein synthesis (Muir 1985). Trenbolone acetate (TBA), a synthetic androgen, however, has been shown to decrease both the rate of protein synthesis and degradation but the rate of degradation is less than the rate of synthesis, so net muscle protein deposition is increased (Buttery and others 1978).

Even though the use of implants has been shown to be economically beneficial (Trenkle 1987), there are concerns regarding the possible harmful effects of implants on beef quality. Thonney and others (1991) compared the use of implants containing both an estrogenic and androgenic with a non-implanted control and showed that the implants reduced tenderness of ribeye steaks. Gerken and others (1995), however, found that the use of a single estrogenic, androgenic or a combination of an estrogenic and androgenic had little effect on the deposition of intramuscular fat or on beef tenderness. Apple and others (1991) found that steers implanted with an estrogenic tended to produce less tender rib steaks than non-implanted control steers and steers implanted with an androgenic or an estrogenic plus an androgenic produced rib steaks that were similar in tenderness to those produced by non-implanted control steers. They also found that 50% of the cattle implanted with an estrogenic plus an androgenic graded low Choice or higher. It has been shown that estrogenic and androgenic implants either alone or in combination tend to reduce marbling scores and quality grades compared to non-implanted controls (Trenkle 1990; Bartle and others 1992).

Environment: The environmental conditions an animal is raised in may also have an influence on the growth rate and body composition of the animal, which may

influence the deposition of intramuscular fat. Warm-blooded animals need to maintain a constant body temperature, so heat loss must be equal to heat production in order to maintain normal physiological processes. In any environmental condition that requires an animal to generate or dissipate heat, the efficiency of growth is reduced. Changes in carcass composition can result from changes in energy depending on the growth stage of the animal and may influence the deposition of intramuscular fat (Aberle and others 2001).

The management or housing system used in raising cattle has also been found to influence deposition of intramuscular fat and palatability of meat cuts. It has been found that loose housing compared to tie-stall housing increased shear force value by 25 to 35% in *longissimus dorsi* (Jensen and Oksama 1996). Vestergaard and others (2000) found that the intramuscular fat content of bulls loose housed was 50% lower in the *semitendinosus and longissimus lumborum* and 30% lower in the *supraspinatus* compared to tie-stall housed bulls. Also, the shear force value of the *semitendinosus* was 33% higher in loose housed bulls compared to tie-stall housed bulls.

1.1.5. Intramuscular fat effects on palatability

Several studies have been conducted to determine the effects in which degree of marbling and quality grade have on meat palatability. Tatum and others (1982) showed that marbling had a low but positive relationship on all beef palatability traits but also found that 90% of the time steaks with Slight or higher degrees of marbling were more desirable in tenderness, flavor and overall palatability. Smith and others (1984) reported minute, but statistically significant differences in meat palatability (juiciness, tenderness, flavor) as the degree of marbling decreased from Moderately Abundant (USDA Prime) to

Practically Devoid (USDA Standard) (Figure 1). Wheeler and others (1994) found that shear force tenderness ratings and juiciness ratings improved slightly and shear force variation decreased slightly as marbling increased in meat from *Bos taurus* and *Bos indicus* cattle.

A study conducted by Smith and others (1987) determined the relationship between USDA quality grades and beef palatability. They found that loin steaks from Prime carcasses were more palatable than steaks from Choice through Canner carcasses 85.7% of the time and more palatable than steaks from Choice through Standard carcasses in 69.0% of comparisons. Also *Longissimus thoracis* steaks from USDA High Choice carcasses tended to have higher tenderness, juiciness and beef flavor intensity ratings than those from USDA Low Select carcasses (Wheeler and others 1999a). Tatum and others (1980) found that rib steaks from High and Average Choice carcasses were juicier, more flavorful and more palatable than steaks from Low Select and High Standard carcasses.

Savell and Cross (1988) determined that the minimum fat percentage required for acceptable palatability of broiling cuts is 3% on an uncooked basis (minimum Slight degree of marbling, USDA Low Select). They came to this conclusion after studying research conducted over many years and found that steaks with less than 3% intramuscular fat (Practically Devoid and Traces) were tougher, drier and less flavorful when evaluated by both trained and consumer sensory panels. However, 3% intramuscular fat or Slight marbling provides little room for error in cookery method or degree of doneness to ensure palatability.

They also determined two other levels of intramuscular fat related to increased palatability. Approximately 5% (midpoint of Small degree of marbling) and 7% (low end of Moderate amount of marbling) were associated with hierarchical degrees in palatability. From these studies, Savell and Cross (1988) described a "window of acceptability" for percent intramuscular fat (marbling) of retail beef cuts. Beef cuts containing 3-7% intramuscular fat (marbling) are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability.

Beef palatability is a major concern because when consumers are not satisfied with the palatability of beef cuts their intent to purchase additional beef products may decrease. The opportunity for the beef industry to generate revenue also decreases. Savell and others (1987) reported that beef packers demand beef carcasses that grade USDA Choice. When carcasses grade less than USDA Choice, a substantial price discount usually has been paid.

1.2. Beef carcass quality improvement initiatives

1.2.1. National Beef Quality Audits

Studies have been conducted to determine quality inconsistencies within the beef industry chain, from farm to retail. The National Beef Quality Audit (NBQA)-1991 (Lorenzen and others 1993) established the first major benchmark and showed that the overall mean marbling score for beef cattle carcasses was a Small^{24} (USDA Low Choice). However, the overall mean USDA Quality Grade for carcasses utilized in this study was Select^{86} (USDA High Select), indicating that lower quality (less than USDA Choice) beef carcasses were being produced. The 1995 NBQA (Boleman and others 1998) measured

the progress regarding the quality, consistency, and competitiveness of beef since the initial 1991 NBQA. This study determined that the overall mean marbling score was a Small^{06} and the mean USDA quality grade was Select^{79}. It was shown that 48.2% of the carcasses had marbling scores that corresponded to USDA Choice and 46.5% had marbling scores that corresponded to USDA Select. There was a reduction in marbling since the 1991 audit and they felt that the industry should be concerned with the observed decrease in the proportion of carcasses with marbling scores that corresponded to USDA Prime and Choice quality grades.

The most recent NBQA audit was conducted in 2000 (McKenna and others 2002). The purpose of this audit was to assess the current status of the quality and consistency of the U.S. fed steer and heifer population, to pinpoint inadequacies and shortfalls that the industry needs to improve upon and to track any progress made since the last audit (1995). The results from the 2000 audit found that the quality measured by marbling score and USDA quality grade appeared to be back to the level observed in the early 1990's.

1.2.2. Beef consumer satisfaction studies

The National Consumer Retail Beef Study (Savell and others 1987) was an industry-wide program supported by the government, beef producers, packers and retailers to identify the kind of beef products consumers prefer. The association between quality grade and taste appeal was looked at. Steaks from carcasses that varied in marbling were evaluated by 540 households and was the first nationwide study conducted to determine if consumers, rather than a trained sensory panel could detect differences in the palatability of beef steaks with different degrees of marbling. From the study, they

found that the degree of marbling in top loin steaks impacted palatability (juiciness, tenderness, flavor). The study also found that tenderness was the single most important factor affecting consumer perceptions of beef, but Neely and others (1998) found in a beef consumer satisfaction study that flavor could be as important as tenderness in determining consumer satisfaction.

1.3. Effects of fat on sensory attributes

Savell and others (1987, 1989) reported that the palatability of beef products affects consumers' purchasing decisions and numerous factors have been shown to affect beef palatability (tenderness, juiciness, flavor and overall acceptability) including the amount of intramuscular fat. Marbling has been reported to account for 5-10% variation in tenderness and 16% variation in juiciness (Blumer 1963; Pearson 1966; Parrish 1974; Jeremiah 1978).

1.3.1. Tenderness

Several theories have been postulated that explain how intramuscular fat contributes to muscle fiber tenderness. The lubrication theory states that intramuscular fat present in and around the muscle fibers lubricates the fibers and creates a more tender and juicy product that stimulates the sensation of tenderness. The bite theory states that within a bite size piece of meat, marbling decreases the bulk density of the meat by replacing protein with lipid. Since fat is much less resistant to shear force than protein, the decrease in bulk density is accompanied by an increase in real or apparent tenderness. The strain theory states that as marbling is deposited inside the walls of the perimysium

or endomysium, the connective tissue walls on the side of the deposit are thinned, decreasing their thickness and strength (Savell and Cross 1988).

Intramuscular fat has been shown to have a low to moderate relationship to tenderness in beef (Smith and Carpenter 1974). Wheeler and others (1994) reported that *Bos taurus* carcasses with Slight marbling exhibited higher shear force (less tender) values compared to beef carcasses with Small through Modest marbling scores. Carcasses with a Traces marbling score had a higher shear force value than those carcasses with Slight marbling scores. Davis and others (1979) investigated the tenderness variations that occurred among beef steaks from carcasses of the same USDA quality grade. The purpose of the study was to determine why some steaks are less palatable than others that are from the same USDA quality grade. Steaks from Choice, A maturity beef loins were used and the most tender steaks were found to have more intramuscular fat than the steaks found to be less tender. The percentages of intramuscular fat for steaks from four different tenderness groups of the Choice, A maturity beef loins were: very tender=7.6%, moderately tender=6.1%, slightly tender=5.6% and slightly tough=4.4%.

Moody (1976) concluded that the most important factors that affect meat tenderness are methods and/or rate of chilling and methods of cooking. Tenderness decreases as the degree of doneness increases (Cover and others 1962; Parrish and others 1973; Cross and others 1976) and 64% (Branson and others 1986) or 82% (NLSMB 1995) of beef consumers cook meat to a medium to very well degree of doneness. The negative effects of a higher degree of doneness on tenderness were much greater in less tender than in more tender *longissimus thoracis* steaks (Wheeler and others 1999b). It

has been hypothesized (Smith and Carpenter 1974; Savell and Cross 1988) that steaks from carcasses of lower quality grades are more affected by an elevated degree of doneness than are steaks of higher quality grades.

1.3.2. Juiciness

Juiciness is composed of the combined effects of initial fluid release and the sustained salivary flow from the stimulating effect of fat (Weir 1960). The initial fluid release gives the impression of wetness perceived during the first chews, which is produced by the rapid release of meat fluids. Sustained juiciness is the sensation of juiciness perceived during continued chewing created by the release of serum within the meat and partly by the stimulating effect of fat on salivary flow (Bratzler 1971). Sustained juiciness has been found to be related to intramuscular fat content (Pearson 1966). Intramuscular fat may affect product juiciness by enhancing the water-holding capacity of meat, lubricating the muscle fibers during cooking, increasing the tenderness of meat, simultaneously increasing the sensation of juiciness, and by stimulating salivary flow during mastication.

It has been shown that intramuscular fat has a low to moderate relationship to juiciness in beef (Smith and Carpenter 1974). Tatum and others (1980) found that rib steaks from High and Average Choice carcasses were juicier than steaks from Low Select and High Standard carcasses. Jones and others (1991) also found that intramuscular fat influenced juiciness in ribeye steaks. Steaks with Modest, Small and Slight amounts of marbling had higher mean trained sensory panel juiciness scores than those with Traces amount of marbling.

1.3.3. Flavor

Fat may affect flavor of meat products in two ways: 1) production of carbonyl compounds that are potent flavor contributors during fatty acid oxidation and 2) release of odoriferous compounds stored in fat during heating (Hornstein 1971). The species characteristic flavor tends to come from the lipid fraction of the meat when the volatile compounds are released from the fat or produced from triglyceride or phospholipid fractions (Hornstein and others 1960). The meaty flavor, however tends to be nonlipid in origin, but some amount of fat is necessary to give the full, rich beef taste.

Armbruster and others (1983) found that at Slight to Moderately Abundant degrees of marbling, roasts from Holstein cattle had better flavor which could be attributed to the higher concentration of water soluble constituents since they have more active muscle growth (Nour and others 1981). Conversely, at higher marbling scores and increased weight, the accumulation of more fat might have resulted in more flavorful roasts from Angus than from Holsteins. Branaman and others (1962) showed that roasts from beef type steers produced a more intense flavor in the lean than roasts from Holsteins but the flavor of fat was unaffected by breed.

Smith and Carpenter (1974) reported a low to moderate relationship of intramuscular fat to beef flavor. Armbruster and others (1983) found that marbling positively affected the flavor of rib roasts from Angus cattle. Wheeler and others (1999) also found that *longissimus thoracis* steaks cooked well done (80 °C) from Top Choice carcasses had higher beef flavor intensity ratings than those from Low Select carcasses.

1.4. Fat substitutes in meat products

In order for an ingredient to successfully replace or substitute fat it must mimic the taste, texture, and function of the fat it is replacing. The desired function of fat either flavor, lubrication, or heat transfer determines what properties developers of fat substitutes seek to achieve (Morrison 1990). Decreasing the fat content in meat products requires that product palatability – tenderness, juiciness, flavor and mouth-feel or texture be maintained and/or improved while maintaining economic value (Mandigo 1991). Functional properties of meat systems are primarily dependent on the interaction of the protein fraction with the other components. These interactions include: protein:water, protein:fat and protein:protein which determine the textural properties, yield, palatability, processing behavior and ultimately product value (Shand and Schmidt 1990).

Fat substitutes can be grouped into three general categories, protein-based, fatty acid-based and carbohydrate-based substitutes. Protein-based substitutes are ingredients derived from either plant or animal proteins. Plant-based protein additives include soy flour, soy protein concentrate, soy protein isolate, textured soy protein, corn germ meal, corn flour, oat flour, wheat flour and vital wheat gluten. Animal-based protein additives include nonfat dry milk, whey proteins, caseinates, blood plasma, and egg proteins (Mandigo 1991).

Fatty acid-based substitutes are fatty acids that have been chemically altered to provide fewer to no calories. Examples of fatty acid based substitutes include olestra (sucrose polyester), polydextrose, and esterfied propoxylated glycerols (Morrison 1990). These fat substitutes are used primarily in snack foods rather than in meat products.

Carbohydrate-based substitutes include starch derivatives and cellulose based derivatives. Starch based substitutes are mixtures of starch derivatives and water and are used to produce a variety of reduced fat foods. The mixtures do not have all the taste and functional properties of fat so they can only replace part of the fats and oils without a loss in quality (Morrison 1990). Cellulose based derivatives are gums and hydrocolloids. They have been used in a variety of food products to stabilize viscosity and emulsions, suspend particles and form gels.

In developing a fat substitute for intramuscular fat, developers may need to use a combination of ingredients that mimic the functional and organoleptic properties of fat. To create a "modified marbling" solution, special consideration must be given to gelation properties (to create "fat-like" particles resembling marbling), water retention, viscosity (to allow direct injection into whole muscle), color (lightness or L* values similar to fat) and melting point temperatures. Non-meat ingredients that may be suitable for development of a "modified marbling" solution include hydrocolloids, starches and whey proteins.

1.5. Alginate

Gums, also referred to as hydrocolloids, are long-chain, high-molecular weight polymers that dissolve or disperse in water. They create a thickening and sometimes a gelling effect. Used at low levels usually in the range of 0.1-0.5%, they dramatically increase viscosity and lead to emulsion stability (Glicksman 1982). A gum is not a single homogenous compound but rather a heterogeneous mixture of several different polysaccharides. Galactose is the most commonly repeated monomer (Towle 1973).

1.5.1. Background

Alginates are monovalent salts derived from brown seaweed. They are hydrophilic colloids and are widely used as thickeners, stabilizers and gelling agents in food and can be utilized as a fat replacement with a potential for textural modification due to gel formation (Mandigo 1991). Alginates are cold soluble and cold-setting and are heat and freeze thaw stable. Alginate is a linear copolymer composed of two monomeric units, D-mannuronate and L-guluronate linked together in a flexible chain by glycosidic linkages of 100-3000 units. The composition of alginate varies depending on the seaweed species and the part of the plant used.

1.5.2. Alginate manufacture

Alginate occurs in seaweed as a combination of calcium, magnesium, sodium, and potassium salts. The seaweed goes through more than 20 processing steps in order to produce alginate. The seaweed is milled and washed with water and acid and then the alginate is extracted with water and alkali and clarified. It is then filtered with a filter aid and precipitated with $CaCl_2$, which produces calcium alginate. The calcium alginate is then washed again with water and acid, which results in the final product (Draget 2000).

1.5.3. Functionality

Dissolving alginate in water causes the molecules to hydrate and the solution will gain viscosity. The alginate should be added slowly to the water and stirred vigorously to create a vortex. It should be added slowly into the vortex in order to avoid lumping and premixing alginate with another powder (sugar) or vegetable oil can help with dispersion. The dissolved molecules are not completely flexible since rotation around the glycosidic linkages can be hindered which results in a stiffened chain. Solutions with stiffened

molecules are highly viscous (FMC BioPolymer 2005). The viscosity of an alginate solution depends on both the concentration of the alginate and the number of monomer units in the chain (the more units in the chain, the higher the viscosity at similar concentrations). Alginate solutions have shear-thinning characteristics since the viscosity decreases with increasing shear rate (stirring speed) and the temperature also influences the effect of alginate to shear force (Draget 2000). As the temperature increases 1 °C, the viscosity of the solution drops approximately 2.5% (FMC BioPolymer 2005).

1.5.4. Gelation

The gelation of alginate requires polyvalent cations. Polyvalent cations, most commonly calcium, will react and cross-link with alginate polymers. As the polyvalent ion content of the solution is increased, thickening and gelation will occur (Pszczola 2003). The alginate needs to contain an adequate amount of guluronate monomers to react with the polyvalent cation. Regions of guluronate monomers in one alginate molecule can link to a similar region in another alginate molecule by polyvalent cations binding the alginate polymers together forming junction zones. This results in gelation of the solution (FMC BioPolymer 2005). An alginate gel can be considered part solution and part solid. When a heat stable alginate gel is formed, water is physically entrapped in the alginate matrix resulting in less released water (Onsoyen 1997) but the water molecules are free to migrate.

1.5.5. Applications of alginate in meat products

Alginate has been used as a non-meat ingredient in a variety of meat products. In a study by Ensor and others (1989), restructured ground turkey and turkey breast meat patties were formulated with a combination of sodium alginate (0-1.0%), calcium

carbonate (0-0.1875%) and lactate (0-0.6%) and compared to a no additive control. All of the restructured products with the sodium alginate/calcium carbonate binder had higher cook yields than the no additive control. Raharjo and others (1994) studied the quality characteristics of restructured steaks with veal trimmings or veal leg meat and sodium alginate/calcium lactate. The sodium alginate/calcium lactate used as a binder increased the binding force and sensory bind score, and decreased the cook loss when used at 0.4%. Berry (1997) found improvements in the acceptability of low fat ground beef patties by using alginate along with tapioca starch which greatly improved tenderness and juiciness, and also increased cooking yields. In another study, Devatkal and Mendiratta (2001) evaluated restructured pork rolls formulated with sodium alginate/calcium carbonate and found that the raw binding strength was significantly higher in the restructured pork rolls containing the sodium alginate/calcium carbonate.

1.6. Carrageenan

1.6.1. Background

Carrageenan is a generic term applied to a group of sulfated polygalactoses extracted from red seaweed. It is made of repeating galactose units and 3,6 anhydrogalactose (sulfated and nonsulfated) and are joined by alternating alpha 1-3 and beta 1-4 glycosidic linkages. Carrageenan functions as a gelling agent, stabilizer and thickener and is capable of forming viscous solutions at low concentrations in cold water with the viscosity dependent upon temperature, pH, concentration, and the type of carrageenan present (Wallingford and Labuza 1983). The solubility of carrageenan depends on the number and position of the ester sulfate groups on the repeating galactose

units. Higher levels of ester sulfate groups lower the solubility temperature of the carrageenan and produce a lower strength gel. There are three basic carrageenan types, kappa, iota and lambda, with each type differing in solubility and gelling properties.

Some but not all carrageenans exhibit cold solubility (Egbert and Huffman 1991). Kappa carrageenan is soluble in hot water and the addition of potassium ions increases the formation of a durable gel. The gel is strong, rigid and slightly opaque with normal usage levels between 0.02-2.0%. Iota carrageenan is soluble in hot water but sodium iota carrageenan is soluble in cold and hot water. The addition of calcium ions induces the formation of a durable, clear elastic gel that is freeze thaw stable and used at levels between 0.2 to 2.0%. Lambda carrageenan is partially soluble in cold water, fully soluble in hot water but does not form a gel. There is only random distribution of polymer chains and the solution ranges from low to high viscosity. The addition of cations has little effect on the viscosity and lambda carrageenan is normally used at levels between 0.1-1.0% (FMC BioPolymer 2005).

1.6.2. Carrageenan manufacture

In order to manufacture carrageenan, red seaweed is gathered, dried, baled, mechanically ground and sieved to eliminate impurities like sand and salt. The seaweed is then washed and extracted to separate the carrageenan from the extraneous plant fiber. The cellulosic material is removed by centrifuging the dissolved carrageenan mixture to eliminate the dense cellulosic particles and then filtered to remove the smaller particles. The solution is concentrated to accommodate the removal of water and then recovered by one of two different processing methods. For one method, the concentrated carrageenan is deposited into a solution of potassium chloride to raise the gelling temperature so the

filtrate will gel immediately. The gel is then frozen and compressed during thawing to remove excess water. In the second method, the concentrated carrageenan is precipitated in isopropyl alcohol. Since carrageenan is insoluble in alcohol, the filtrate turns into a coagulum of carrageenan, alcohol and water. The coagulum is compressed to remove the liquid and vacuum dried to remove the alcohol. Drying is done on a belt drier and the dried coagulum is ground (Imeson 2000).

1.6.3. Functionality

Carrageenan should be added slowly to the vortex of water produced by a high speed mixer. Carrageenan can be premixed with a dispersant like sugar or dispersed in liquid sugar, salt or glycerin to help with dissolving. It should be dispersed in cold water and then heated above its solubility temperature. The solubility temperature depends on the level of potassium and calcium ions present with the carrageenan but most carrageenans are heated to 77-79 °C for solubilization unless it is cold soluble. Cold soluble carrageenans should be dispersed in cold water by adding the carrageenan slowly to water with agitation.

The potassium or calcium ions are vital for effective gelation of the carrageenan solution. Increasing the level of ions improves the dispersion and strength of the gel. Carrageenan gels are thermally-reversible since the gels become fluid when heated above the gels' melting point temperature and resets into a gel when cooled with a minimal loss of gel strength (FMC BioPolymer 2005).

1.6.4. Interaction with non-meat ingredients

Iota carrageenan has been found to increase the viscosity of starch by as much as 10 times the viscosity of starch alone. Carrageenan can be useful in altering the textural,

mouth-feel and processing properties of a starch solution. The increase in viscosity allows reduction of the overall starch content by as much as 35-40% and improves the texture and flavor of the finished product.

Carrageenan also has the ability to interact with milk proteins. In milk proteins, there is a concentration of positive charges at peripheral locations on the casein micelle. This positive charge attracts the negatively charged sulfate groups on the carrageenan molecule to form linkages with the dispersed casein micelles. This reaction along with the normal water gelling capabilities of carrageenan can increase the gel strength by approximately 10-fold. (Imeson 2000).

1.6.5. Applications of carrageenan in meat products

Carrageenan has been used in a variety of meat product applications. Foegeding and Ramsey (1986) found that the addition of iota and kappa carrageenan in a low-fat meat batter resulted in increased water-holding capacity and firmness. In another study, Huffman and Egbert (1990) added 0.5% carrageenan to low fat beef patties (~9%) and compared them to all-beef patties with 10 or 20% fat. The carrageenan treatment was equal in sensory acceptability and beef flavor to the 20% fat beef pattie and more acceptable than the 10% fat beef pattie. Bater and others (1992) manufactured oven-roasted turkey breasts with a 70% added brine containing salt, phosphate, nonfat dry milk and various combinations of kappa carrageenan and starch. The incorporation or 0.5% kappa carrageenan increased yield, improved the visual appearance, sliceability, rigidity and decreased the expressible juice compared to the control product. In another study, Shand and others (1994) studied the effects of adding 0.5-1.0% kappa carrageenan to structured lean beef rolls. They found that the addition of kappa carrageenan increased

cook yield, improved the textural properties (bind, force to fracture, hardness) and reduced purge of vacuum packaged slices during storage. Rolls with 1.0% kappa carrageenan had the highest cook yield and highest force to fracture and hardness values. He and Sebranek (1996) added kappa carrageenan to frankfurters made with lean finely textured pork and beef. Kappa carrageenan at 0.5% reduced the cooking loss and increased firmness of the frankfurters.

1.7. Whey proteins

1.7.1. Background

The application of dairy proteins, especially whey protein concentrates, in the manufacture of reformed and restructured meat proteins has received much attention in recent years (Giese 1994). Whey protein, the by-product of cheese or rennet casein and acid casein manufacture, has been incorporated as water and fat binders and extenders and has the potential to improve cook yields and modify the textural characteristics of low-fat comminuted meat products (Comer and others 1986; Ellekjaer and others 1996; Keeton and others 1997). Whey protein products can be added to processed meat products at levels up to 3.5% in the finished product (USDA 1999) and are categorized on the basis of their composition, primarily based on their protein content (Huffman 1996). Products with an increased protein content are sold at higher prices. A variety of whey protein products are listed in Table 1.1.

Table 1.1. Types and composition of whey protein products (From Huffman, 1996).

Product name	Protein content (%)	Fat content (%)	Lactose content (%)	Ash content (%)
Whey powder	13	1	76	10
35% WPC[a]	34-35	4	53	8
50% WPC	53	5	35	7
80% WPC	80	4-7	7	4-7
Whey protein isolate	>90	1	1	3

[a] WPC: Whey protein concentrate

1.7.2. Whey protein manufacture

In order to manufacture whey protein, several processing steps are utilized including clarification, separation, pasteurization, crystallization, ultrafiltration/difiltration, ion exchange and drying (Mulvihill and Grufferty 1997). First, the liquid whey is recovered from the cheese or casein manufacturing and clarified to attain low levels of curd and prevent blocking of the heat exchanger. Clarification is completed by a combination of settling, screening and centrifugation. Next the fat is separated from the liquid whey by using a self-discharging separator and then the liquid whey is pasteurized immediately. Temperature and time for the pasteurization step are 72-75 °C and 15-20 s, respectively. After pasteurization, the liquid whey is evaporated under vacuum to increase the total solids to 40-60% and done at low temperatures (below 70 °C) to avoid denaturation of the proteins. Reverse osmosis can be applied before evaporation to increase the efficiency of the evaporator, which increases the solid content of the liquid whey to around 20%.

The next step is to crystallize the liquid whey to remove the lactose by using a crystallization tank seeded with finely ground α-lactose monohydrate or well-crystallized whey powder. After crystallization, the crystallized lactose can be separated from the liquid whey by centrifugation. The liquid whey is then ultrafiltrated/difiltrated to increase the protein content and then pasteurized again to reduce the number of microbes. The minerals in liquid whey can be removed by ion-exchange and after ultrafitration/difiltration and ion-exchange, whey proteins containing 95% protein can be produced (Huffman 1996). The final step in the production of whey proteins is drying. In order to produce non-caking, high solubility and more functional whey powders, a multi-stage drying process in usually used in the whey industry. Liquid whey is pre-crystallized before the first stage of drying where the pre-crystallized whey is spray-dried to achieve 5-8% moisture content. The final drying step and post-crystallization are done on a fluidized bed to produce whey powders with low bulk densities.

1.7.3. Functionality

The composition of whey protein products affects their functionality. During the spray drying process, the lactose protects the proteins from denaturation therefore whey protein products with low lactose contents tend to have a higher degree of denatured proteins. Also, residual fat from the milk in whey protein products can affect its foaming properties, so as a result, whey proteins with a lower fat content have superior foaming properties. The mineral content of the whey protein products is also important for the functionality. Calcium is the most important mineral and a high concentration of calcium can cause aggregation and gelation of the whey products during intense heating but at a neutral pH, calcium phosphate can increase the heat stability. No single whey product

has all the functionalities required so often whey products are combined to achieve the desired functionality (Jost 1993).

One of the key functional properties of whey proteins is their ability to form heat induced three dimensional gel structures with increased water-holding capacity to improve cook yield, potential texture modifying properties and improved sliceability (Morr 1979; Morr and Ha 1993). Whey protein products have also been found to aid in solubility by creating a smooth texture and reducing the gritty and powdery taste in meat products. The viscosity is increased with whey protein products by enhancing the body and texture through thickening and whey proteins also act as emulsifiers by forming stable fat/oil emulsions, preventing oiling-off and "fat caps" and acting as a meat protein replacement. Browning is also increased in meat products since whey protein products enhance the Maillard, non-enzymatic browning reaction and add color and visual appeal. Whey protein products add to the flavor and aroma by having little or no flavor of their own, being compatible with cooked meat flavors and with spice/seasoning blends. Whey protein products also improve the nutrition content since they have a superior amino acid profile and can serve as a source of calcium enrichment (Keaton 1999).

1.7.4. Applications of whey proteins in meat products

Several studies have been conducted in which the application of whey proteins in meat products was evaluated. Hemar and others (2002) tested the rheological properties of whey protein isolate. They added whey protein isolate to kappa-carrageenan mixtures in aqueous solution and observed no phase separation in the mixture. Whey proteins have also been shown to reduce cook loss and improve textural parameters. McCord and others (1998) added whey protein isolate to salt-soluble muscle protein and found that it

increased the water-holding capacity of the gels. In another study, Ensor and others (1987), showed that adding whey protein concentrate to knockwurst proved to be a good binder compared to soy protein isolate and calcium-reduced non-fat dried milk. Chen and Trout (1991) reported that adding whey proteins (2.0% whey protein concentrate) in restructured beef steaks decreased cooking loss. Whey proteins can also be used to improve emulsion stability in comminuted meat products. Hung and Zayas (1992) reported that compared to all beef frankfurters (20% fat), beef frankfurters containing 3.5% whey protein concentrate had increased water-holding capacity and decreased cooking loss.

Due to the ability of whey protein products to bind large amounts of water and fat, they are good candidates for fat replacers in meat products. El-Magoli and others (1996) added whey protein concentrate (0 to 4% addition) as a fat-replacer in low fat ground beef patties formulated to contain 11-22% fat. At the 4% level, whey protein concentrate served as a fat-replacer without sacrificing product palatability and flavor. Hughes and others (1998) added whey protein (3.0%) in low fat beef franks to reduce the fat content from 12 to 5%. Reduced fat (5%) beef franks containing whey protein concentrate had similar sensory attributes compared to those without whey protein concentrate and had higher hardness, adhesiveness, gumminess and chewiness values than the 12% fat beef franks.

1.8. Modified food starch

1.8.1. Background

Starches are polysaccharides that consist of repeating glucose units. Starch molecules have one of two molecular structures, a linear structure, known as amylose; and a branched structure known as amylopectin (Hegenbart 1996). Amylose and amylopectin associate through hydrogen bonding and arrange themselves radially in layers to form granules of starch. Granule size and shape can change greatly due to type of starch and degree of chemical modification (McCormick 1985). Many varieties of starches can be isolated from many different sources such as corn, potato, rice, tapioca and wheat. In addition, each type of starch differs in amylose and amylopectin content as well as granule size and structure. The amylose generally provides the gel strength and the amylopectin gives viscosity to solutions (Hegenbart 1996).

Properties of starches can be improved by modification through reactions at the hydroxyl groups. For modified food starches, only a few of the hydroxyl groups are modified. Ester or ether groups are attached at very low degrees of substitution (DS) to the hydroxyl groups. DS values are usually <0.1 and normally in the range 0.002-0.2, so on average there is one substituent on every approximately 500 D-glucopyranosyl units. Modification is completed in order for the starch to withstand various heat, shear and acid conditions associated with various processing methods to introduce specific functionalities. Small levels of derivatization can change the properties of starches dramatically (BeMiller and Whistler 1996).

1.8.2. Modified food starch manufacture

Modifications can be done by either a single process or by a combination of processes. A majority of modified food starches are crosslinked, which alters a starch by using chemicals that cause intermolecular covalent bonding. Chemicals that can be used are metaphosphates, phosphorus oxychloride, citric or adipic acid or epichlorohydrin. Starch chains linked together with crosslinkers reinforce the starch granule and reduce the rate and degree of granule swelling (McCormick 1985). Another process used to produce modified food starches is through the use of chemical reactions. Chemical reactions currently used to produce modified food starches are: esterification, etherification, acid modification, bleaching and oxidation. The modification process for potato starch cross-binds phosphorous groups and masks hydroxyl groups with acetyl groups (Skrede 1989), resulting in changed molecular properties and functionality (Howling 1980).

Pregelatinized or cold-soluble starch can be produced by using starch that has been cooked, dried and redissolved in cold water. The starch-water slurry flows between two nearly touching and counter rotating, steam-heated rolls. The starch slurry is gelatinized and pasted, coats the rolls and dries quickly. The dry film is scraped from the roll and ground (BeMiller and Whistler 1996). The resulting products are precooked starches and the process can be completed on both chemically modified and unmodified starches. Pregelatinized starches contain more ruptured and retrogradated granules in order to produce the starch paste. They are soluble in cold water and gel without cooking (McCormick 1985)

1.8.3. Functionality

Starch granules are insoluble in cold water unless they are pregelatinized but

when heated in water, they undergo gelatinization. Gelatinization is the disruption of molecular order within the starch granules. Leaching of amylose occurs during gelatinization and total gelatinization occurs over a range of temperatures with larger granules gelatinizing first. Continued heating in excess water results in further granule swelling, additional leaching of soluble components and eventually total disruption of granules which results in the formation of a starch paste (BeMiller and Whistler 1996).

A combination of modification processes enhances the functionality of starches. Changes in functionality include increased solubility, increased or decreased paste viscosity, increased freeze/thaw stability, enhancement of gel formation and gel strength, reduction in gel syneresis, improvement of interaction with other substances and modification of cooking characteristics.

1.8.4. Applications of modified food starch in meat products

Carbohydrates such as starches and flours have been used in the meat industry to improve cooking yields due to their ability to absorb large amounts of water (Keeton 1991) and have been shown to be effective in reducing purge of low fat/high added-water bologna (Claus and Hunt 1991; Dexter and others 1993) and other meat products. Starches also have the ability to improve the texture of meat products. Motzer and others (1998) studied the addition of modified food starch, kappa-carrageenan and isolated soy protein in different levels of PSE pork. They found that the modified food starch decreased bind strength and expressible moisture and increased yields in the 100% PSE treatment. Modified food starch improved the water retention of PSE pork in restructured products. In another study, Ioffe and others (2002) used different starches to study the textural stabilization of extruded beef jerky analogs. They found that a 5% addition of

modified starches stabilized the beef jerky analogs after one month of storage to a higher degree than the treatments containing only potato starch. Beggs and others (1997) evaluated turkey frankfurters with 2.379 to 6.621% modified cornstarch and 20.93 to 35.07% water. They found that for optimal sensory and physical attributes (internal color, compression, purge loss and pH) the best levels of modified corn starch and added water were 2.3 and 33.6% respectively.

1.9. Ingredient combination and interaction

Carbohydrate based fat substitutes or mixtures of gums, starches and/or proteins appear to offer the most cost effective means of replacing a significant portion of fat in meat products and duplicating the textural and sensory characteristics of animal fat (Keeton 1991). It seems that the addition of a single type of a substance is not enough to achieve this significant replacement but synergistic action between ingredients have been found to provide the fat replacement desired (Glicksman 1991).

The significance of the addition of more than one type of non-meat ingredient added to a meat product working in combination was seen in a study by Prabhu and Sebranek (1997). Eight treatments using kappa carrageenan at 0 or 1.5% and starch at 0, 2, 3.5, or 5% in hams were evaluated for cook yield, purge, color, texture and sensory attributes. The incorporation of carrageenan at 1.5% increased yield, decreased purge and had a lower sensory perception of juiciness. Increasing the amount of starch, however, increased the perception of juiciness. In another study, Suman and Sharma (2003) investigated the influence of different fat levels (6, 8, 10 or 20%) on the physico-chemical and sensory characteristics of low-fat ground buffalo meat patties prepared

using a combination of carrageenan (0.5%) and sodium alginate (0.1%). The cook yield and gain in height of the buffalo patties were significantly higher and the shear force values were significantly lower for patties at all low-fat levels compared to the control with 20% fat. Due to significantly higher sensory scores, the 10% fat level was chosen as the optimum for low fat ground buffalo meat patties even though at the 8% fat level the sensory rating was between good and very good. This was similar to what Lin and Keeton (1998) found when they formulated low-fat ground beef patties with both alginate and carrageenan. They were comparable to regular beef patties (20% fat level) in textural properties.

1.10. Value-added Technologies

Several different processing technologies have been used to improve product uniformity (color, texture), tenderness, juiciness and flavor in meat products. Swart (2000) defines value-added as processing steps or technologies that add to the end state of a product that make the improved product valued by customers. The goal of producing value-added products is to increase the overall acceptability of meat products by consumers. Injection, restructuring, mechanical tenderization, tumbling, mixing and use of ingredients such as salt, phosphate, gums, starches and non-meat proteins can improve a product's value. Meat products can be included in this category by implementing technologies ranging from a slight modification in packaging or creating a new name for an existing product to producing a restructured or reformed product.

1.10.1. Injection

Injection is used to distribute a brine or marinade into whole muscle meat and

poultry through needles that penetrate into the muscle and distribute the brine or marinade under pressure. Injection is used to improve juiciness, tenderness, and flavor of a meat product. Research has shown that injection is an ideal method to distribute non-meat ingredients such as salt, phosphate, nitrite, cure accelerators, sweeteners, seasonings, non-meat proteins, starches, gums, water, and preservatives in meat products.

The following studies show the effects in which injecting different non-meat ingredients into lower quality meat products have on tenderness, juiciness, and flavor of the final product. In a study by McGee and others (2003), USDA Select inside beef rounds were injected with a solution of sodium lactate, sodium tripolyphosphate and sodium chloride. Warner-Bratzler shear force, cooking loss and sensory characteristics were determined. The injected treatments were found to be more tender than the control products for both Warner-Bratzler shear force and consumer sensory panel scores. The injected treatments also had a lower cooking and re-heating loss compared to the controls. Lawrence and others (2003) used *semitendinosus and longissimus lumborum* muscles from USDA Select carcasses to study the effects of staged injection of calcium lactate followed by phosphate and salt on water binding and palatability scores. The injection of calcium lactate followed by phosphate and salt significantly increased the pump yield and decreased the expressible moisture values compared to the injection of calcium lactate only. The staged injection of calcium lactate followed by phosphate and salt significantly improved the myofibrillar and overall sensory tenderness scores of *longissimus lumborum* muscle compared to the non-marinated control.

In another study by Hashim and others (1999), bone-in chicken breast quarters were marinated with a lemon-pepper marinade by injection or immersion and honey (10,

20 and 30%) was substituted for water in the marinades. The injected chicken retained more marinade, lost less juice during roasting and had a lower shear force value than the immersed chicken. The addition of honey to the marinade of the injected chicken increased the honey flavor without affecting the appearance, aroma, and other flavor attributes or texture.

1.10.2. Restructuring

Restructured products are manufactured from muscle groups that are partially or completely comminuted and reformed into the same or different form. Restructuring uses three basic approaches: chunking and forming, flaking and forming, and tearing and forming (Pearson and Gillett 1996). A number of advantages occur by taking the muscles apart, physically manipulating them and reforming them into a specific shape. Restructured products have a texture that closely resembles intact meat cuts but are more economical to produce. They are produced from less tender muscles and meat trimmings that are cheaper raw materials compared to boneless intact meat cuts. Restructuring helps control accurate portion and composition of meat products, easier slicing and serving and more accurate predictions of yields (Akamittath and others 1990) but problems such as color instability and fat oxidation occur.

1.10.3. Mechanical Tenderization

Mechanical tenderization is a technology used to improve tenderness of meat products by destroying connective tissue and muscle fibers (Aberle and others 2001). It is very effective in improving the tenderness of meat from carcasses with large amounts of connective tissue (Pearson and Gillet 1996). Huffman (1981) and Booren and others (1981) found improvements in tenderness measured by compression and Kramer shear,

respectively, by blade tenderizing restructured pork chops, USDA Good (currently called Select) and Choice beef steaks, and restructured beef steaks respectively. The advantages of mechanical tenderization are that it improves tenderness of steaks and chops, creates more uniform tenderness within a product, and improves cost effectiveness and ease of implementation in a plant setting (Hayward and others 1980).

1.10.4. Challenges for value-added products

Processing technologies can increase the utilization of lower value muscles by applying processes to increase uniformity of color, texture and tenderness. However, a number of challenges occur when producing value-added products. Technologies such as marination by injection, restructuring, blade tenderization and vacuum packaging can be confusing to the consumer and cause problems with consumer acceptance. The lack of familiarity with the terminology printed on value-added labels such as "enhanced" creates confusion as to what has been added or done to the product. Consumers may wonder whether value-added products compare to traditional products in safety and wholesomeness.

Another challenge of value-added products is controlling and extending the shelf life (Sutton and others 1997). Lipid oxidation reduces the shelf life of meat products by causing rancidity. Rancidity is one of the most serious flavor problems in meat products (Pearson and Gillett 1996) and is accelerated when mechanical disruption of tissue occurs during production. Rancidity occurs when fats are oxidized, become free radicals and react with a number of pre-existing reactants. These products readily decompose into acids, aldehydes, alcohols, carbonyls, and ketones and some of these compounds can then

contribute to strong flavors or odors that contribute to the rancidity of the product (Schmidt 2000).

Controlling the development of off-flavors is another challenge associated with value-added products. "Fresh" flavor or flavors that are recognized as meat-type flavors by consumers are necessary for acceptance. Off-flavor development is a result of previously discussed lipid oxidation, warmed-over-flavor (Craig and others 1991), and the use of non-meat ingredients. Warmed-over-flavor describes the rapid development of undesirable flavors in cooked meat during refrigerated storage. Oxidation of phospholipids contributes to the development of this undesirable flavor (Hettiarachchy and Gnanasambandam 2000).

1.10.5. Benefits of value-added products

Although there appears to be several challenges associated with the development or manufacture of value-added products, there are several reasons to continue to develop them. One reason is that value-added products utilize lower value meat, which can be harder to market. There is little demand for lower value meat but if value-added technologies can be applied, the demand will increase and therefore raise the value. Another reason to manufacture value-added products is to improve the uniformity of existing products. Miller (2000) stated that value-added products allow for improvements in quality attributes by 1) having a more uniform color of the cut lean surface and possibly improving the species color or appearance, 2) improving the tenderness of a product line or improving tenderness uniformity within a product, 3) improving juiciness of a product line or improving juiciness uniformity within a product and 4) extending the shelf life of products.

Value-added products increase product variety or choices. Gums, starches and non-meat proteins can replace expensive animal protein (Keeton and others 1984) creating the opportunities to produce lower-cost extended products. This can become increasingly important when developing meat products that are economically competitive with other protein sources such as beans.

1.11. Summary of literature

The amount of marbling or intramuscular fat has been shown to influence the palatability (juiciness, tenderness, flavor) of beef cuts. Savell and Cross (1988) developed a "window of acceptability" for percent intramuscular fat or marbling of retail beef cuts. Beef cuts containing 3-7% intramuscular fat are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability so it is important to have at least 3% intramuscular fat in whole muscle beef cuts.

The amount of marbling in whole muscle meat cuts has been found to be below the expectations of the meat industry which can influence the consumer's purchasing decisions. When consumers are not satisfied with the palatability of beef cuts, their intent to purchase beef may decrease and along with it is the opportunity for the beef industry to generate revenue. The deposition of intramuscular fat or marbling is influenced by many factors such as breed, length of feeding, type of ration fed and management but it has been shown that there is plenty of room for improvement in the amount of marbling or intramuscular fat in whole muscle beef cuts in order to improve the palatability of the final beef product.

The palatability of whole muscle cuts fabricated from lower quality (less than

USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. Several non-meat ingredients (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) have been used as fat substitutes in a variety of processes meat products. Also, several different processing technologies have been used to add value to lower quality meat products including whole muscle cuts. The development of a "modified marbling" from selective non-meat ingredients that can mimic the properties of intramuscular fat and can be directly injected into lower quality whole muscle beef cuts may enhance its overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

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CHAPTER 2

DEVELOPMENT OF A "MODIFIED MARBLING" SOLUTION FOR WHOLE MUSCLE BEEF CUTS

Abstract

A "modified marbling" solution containing sodium alginate (SA), iota carrageenan (IC), whey protein isolate (WPI) and modified food starch (MFS) was developed to inject into low quality beef cuts to mimic the properties of intramuscular fat. Twenty-five ingredient combinations (ranging from 0.25 to 0.50% addition) of the four ingredients were formulated into 500 g solutions using a 2^4 central composite design. Solution pH and viscosity, and gel (24 h, 4 °C storage) objective color, water-holding capacity, water-holding capacity over time and gel strength were analyzed to determine the optimal solution. Higher levels of SA and IC increased ($P<0.05$) gel viscosity. SA increased ($P<0.05$) pH and gel L* (lightness) color value which was comparable to beef rib fat L* values (77.2 vs. 83.6). SA and IC significantly ($P<0.05$) affected water-holding capacity and SA, IC and MFS were significant ($P<0.05$) factors for water-holding capacity over time. All four ingredients significantly ($P<0.05$) affected gel strength. The recommended levels of non-meat ingredients for the solution were 0.4375% SA and IC and 0.375% WPI and MFS. Theses results indicate the feasibility of an injectable "modified marbling" solution.

Keywords: "modified marbling", solution, intramuscular fat, sodium alginate, iota carrageenan, whey protein isolate, modified food starch

Introduction

The palatability (tenderness, juiciness and flavor) of whole muscle cuts fabricated from low quality (less than USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. Several non-meat ingredients (salt, phosphate, gums, starches and non-meat proteins) and processing technologies (injection, restructuring, mechanical tenderization, tumbling and mixing) have already been used to add value to meat products. The development of a "modified marbling" solution from selective non-meat ingredients that can mimic the properties of intramuscular fat and can be directly injected into lower quality whole muscle beef cuts may enhance the overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

When decreasing or substituting the fat in meat products, the product palatability: tenderness, juiciness, flavor and mouth-feel or texture must be maintained or improved while maintaining economic value (Mandigo 1991). Functional properties of meat systems are primarily dependent on the interaction of the protein fraction with the other components. These interactions include: protein:water, protein:fat and protein:protein which determine the textural properties, yield, palatability, processing behavior and ultimately product value (Shand and Schmidt 1990).

In developing a fat substitute for intramuscular fat, a combination of ingredients may be needed in order to mimic the functional and organoleptic properties of intramuscular fat. Consideration must be given to gelation properties (to create "fat-like" particles resembling marbling), water retention, viscosity (to allow direct injection into whole muscle) and color (lightness or L* values similar to fat). Non-meat ingredients

chosen for the development of the "modified marbling" solution includes sodium alginate (SA), iota carrageenan (IC), whey protein isolate (WPI) and modified food starch (MFS).

Alginates have been used in a variety of meat products because they create a thickening and sometimes gelling effect (Glicksman 1982). Carrageenans function as gelling agents, stabilizers and thickeners and are capable of forming viscous solutions at low concentrations in cold water (Wallingford and Labuza 1983) so likewise have been used in a variety of meat product applications. Whey proteins have been incorporated as water and/or fat binders and extenders. They have the potential to improve cook yields and modify the textural characteristics of low-fat comminuted meat products (Comer and others 1986; Ellekjaer and others 1996; Keeton and others 1997). Modified food starches have been used in the meat industry to improve cooking yields due to their ability to absorb large amounts of water (Keeton 1991) and to improve the texture of meat products.

The objective of this study was to develop a "modified marbling" solution using these selected non-meat ingredients that mimic the properties of intramuscular fat to inject into lower quality, less marbled whole muscle beef cuts. To achieve this objective, response surface methodology was utilized to determine the concentration of each ingredient (SA, IC, WPI, MFS) to use in the development of the "modified marbling" solution.

Materials and Methods

Ingredient selection

To identify an optimal combination of non-meat ingredients that mimics the sensory and functional properties of marbling, a variety of non-meat ingredients (appendix 1) were selected that have specific quality or functional attributes (color, water binding and retention, gelling properties, viscosity, pH). Each ingredient was dispersed in water and allowed to gel. After subjectively observing the characteristics of the dispersions and gels (appendix 1), the non-meat ingredients selected for further evaluation were SA, IC, WPI and MFS.

Non-meat ingredients

The SA (Protanal RF 6650) and IC (RE 0804-01) were donated by FMC Bio Polymer (Princeton, NJ). The WPI (Alacen 895) was purchased from New Zealand Milk Products (Lot# 047U45283431314, Lenoyne, PA) and the MFS (PenPlus 47) was donated by Penford Food Ingredients Co. (Englewood, CO). The calcium sulfate dihydrate F.C.C. (CAS 10101-41-4) was purchased from Voigt Global Distribution LLC (Kansas City, MO) and the phosphate was a blend containing sodium tripolyphosphate and sodium polyphosphate (Brifisol 512) and purchased from BK Giulini (Simi Valley, CA). Phosphate and calcium sulfate were added to aid in the gelation of the sodium alginate.

Solution manufacture

The manufacture of the solutions was conducted by weighing and adding the ingredients to 946.4-ml lidded glass jars with the appropriate amount of water (appendix 2). The ingredients were added in the following order: phosphate, mixture of SA and vegetable oil (for hydration per recommendations of FMC Bio Polymer), IC, WPI, MFS and calcium sulfate solution for a mixing time of 2 min per ingredient. The solutions were mixed using a 4-blade mixing head: 2-blades perpendicular and 2-blades parallel to the shaft attached to a drill (Model 6220, S-B Power Tool Co., Chicago, IL). Solution pH and viscosity were determined and after 24 hr of refrigerated storage (4 °C), the gels were analyzed for color, water-holding capacity, water-holding capacity over time and gel strength.

Apparent viscosity and pH determination

Apparent viscosity of the solution was determined at 30 °C at speed setting 100 using a Brookfield viscometer (Model HBTD, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Measurements were converted to apparent viscosity readings using the following equation: $\eta = \frac{M\,k''}{\Omega}$ and recorded at an average shear rate of 30.5 $\frac{1.}{s}$

where

$M = \frac{\%\ \text{torque}}{100} \times 57{,}496\ \text{dyne cm}\ \frac{N\,M}{10^7\ \text{dyne cm}}$

$k'' = 61{,}220\ \frac{\text{rad}}{m^3}$

$\Omega = 10.5\ \frac{\text{rad}}{s}$

Solution pH was determined at 22 °C using an Accumet pH Meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA). The pH meter was calibrated with standard phosphate buffers pH 4.0 and pH 7.0.

Objective color evaluation

Objective color of the gel was determined by pouring the solution into a Petri dish, covering with a lid and allowing to gel at 4 °C for 24 hr. Color measurements were taken using a Minolta Chromameter CR-310 (Commission International D'Edairerage (CIE) L*a*b* Ramsey, NJ) with a 5.5 cm reading orifice. Before measuring, the chromameter was calibrated with a standard white tile and the measurements were taken using the multi-read function. Readings were taken of the exposed surface of each gel sample for L* (lightness), a* (redness), and b* (yellowness) values.

Determination of water-holding capacity and water-holding capacity over time

The water-holding capacity of the gel was determined by removing the gel from the jar after the solution was chilled at 4 °C for 24 hr. Approximately 10 g of sample was placed in a 50 ml polycarbonate tube and centrifuged at 4 °C at 40,000 *x g* for 30 min (Honikel and Hamm 1994). Tubes were removed from the centrifuge, supernatant poured off, and the gel and tube were weighed. Water-holding capacity was measured in triplicate and determined by the following formula:

$$\frac{\text{weight of gel after centrifugation}}{\text{weight of gel before centrifugation}} \times 100$$

The water-holding capacity over time of the gel was determined by removing the gel from the jar after the solution was chilled at 4 °C for 24 hr. The gel was cut into

approximately 2.5 x 2.5 x 1.3 cm samples. A piece of filter paper was laid inside a Petri dish and both were weighed. The sample cube was placed on the filter paper and the Petri dish, filter paper, and gel cube were weighed, covered and stored at 22 °C for 2 hr. The cube was removed from the filter paper and the filter paper and Petri dish were reweighed. This procedure was used to simulate temperature abuse conditions that may occur during transportation and storage of meat injected with the solution to determine how well the gel can retain water and structure under these conditions. Water-holding capacity over time was measured in triplicate and determined by the following formula:

$$\frac{\text{weight of gel after storage at 22 °C for 2 hr}}{\text{weight of gel before storage at 22 °C for 2 hr}} \times 100$$

Gel strength determination

The gel strength of the solution was analyzed on a TA-HD*i* texture analyzer utilizing a 5 kg load cell and a 1.3 cm diameter acrylic cylinder attachment 3.5 cm in height. Gels were analyzed in 50 ml polycarbonate tubes placed in a molded steel pipe on a heavy-duty platform to eliminate tube movement and variability during analysis. The acrylic probe penetrated the gel plug in the geometric center of the sample depressing the gel 1.2 cm before retracting. Peak force was recorded in grams with a crosshead speed of 1.7 mm/s. All samples were conducted in triplicate.

Experimental design and statistical analysis

Preliminary studies were conducted to determine the selection of ingredients to use for the "modified marbling" solution and to determine the concentration ranges of the selected ingredients. Based on the preliminary studies, twenty-five ingredient

combinations of the selected non-meat ingredients ranging from 0.25 to 0.5% addition were formulated into 500 g solutions using a central composite design with one treatment combination replicated six times to determine error degrees of freedom (appendix 9). Response surface methodology was used to determine the effect of the four non-meat ingredients (SA, IC, WPI, and MFS) on solution viscosity, pH, and gel objective color, water-holding capacity, water-holding capacity over time and gel strength.

The data were analyzed using the Proc GLM procedure of the Statistical Analysis System (SAS User's Guide, Version 8.2. Cary, NC: SAS Institute 2002) to determine which factors were significant ($P<0.05$) within the total model. Response surface regression (Proc RSREG) equations were run on those factors that were significant ($P<0.05$). Response surface graphs were generated and Proc IML was used to determine the predicted level of each ingredient.

Results and Discussion

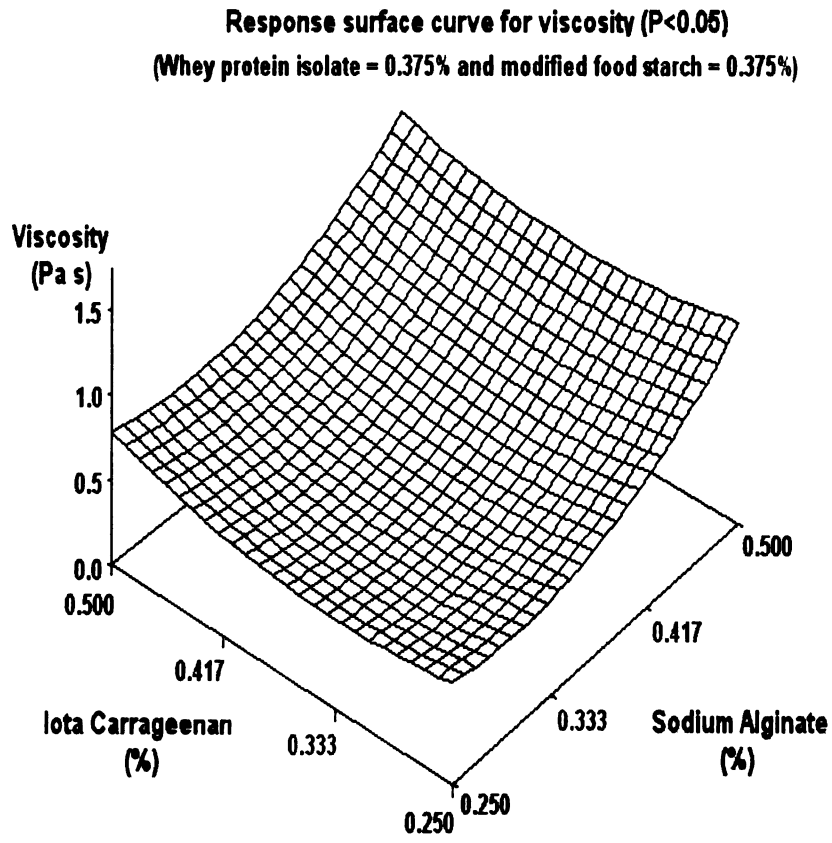
Apparent viscosity of the solutions

The apparent viscosity of the solutions was 0.7 Pa s at an average shear rate of 30.5 1/s and Proc GLM showed that the model was significant ($P<0.05$) so response surface regression was run. The total model was significant ($P<0.05$) as well as the linear ($P<0.05$) and quadratic ($P<0.05$) effects. SA and IC were significant ($P<0.05$) factors and the following parameters were significant ($P<0.05$): IC (linear), SA x SA, IC x IC (quadratic) and MFS x WPI (cross product). SA had the largest influence of all the ingredients on apparent viscosity (Figure 2.1.a) since apparent viscosity increased as the percentage of SA increased. The apparent viscosity also increased as the percentage of IC increased but the shape of the curve was not the same as with SA. This similar pattern was seen in a study conducted by Marcotte and others (2001) where the apparent viscosity of several food hydrocolloids was measured at three concentrations and four temperatures. They found that at higher gum concentrations there was an increase in apparent viscosity. Also, Rao and Kenny (1975) and Speers and Tung (1986) found that the effect of concentration on apparent viscosity of hydrocolloids is usually described by either an exponential or a power relationship. Observations by the researchers in our study found that the range of apparent viscosities of the solutions would be injectable.

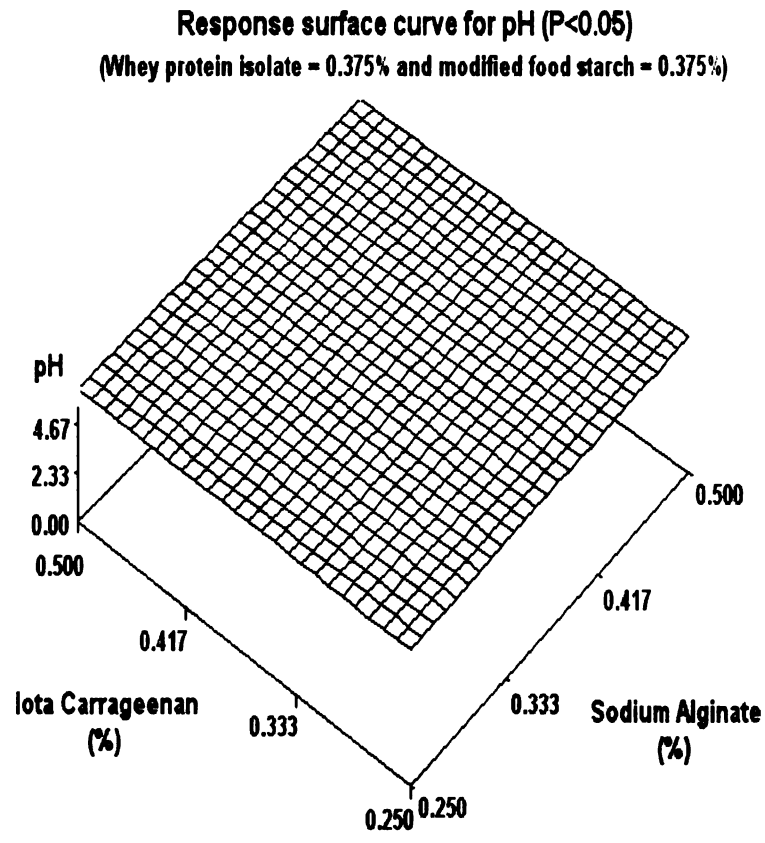
Since the WPI and MFS ingredients of the solution were not significant, they were held at the center point (0.375%) when creating the graphs and the graphs were based on the addition of SA and IC. This was consistent with the rest of the attributes analyzed and the graphs were created in the same manner for all the attributes. For the

Figure 2.1. Response surface curves for significant (P<0.05) total regression models for apparent viscosity at an average shear rate of 30.5 1/s and pH of "modified marbling" solutions.

2.1.a.



2.1.b.



apparent viscosity of the solutions, the stationary point was a saddle point and the recommended use level of each ingredient was 0.305% SA, 0.338% IC and 0.375% WPI and MFS.

pH of the solutions

The mean pH of the solutions was 6.5 and Proc GLM showed that the model was significant ($P<0.05$). Response surface regression found that the total model was significant ($P<0.05$) along with the linear ($P<0.05$) effect and SA was significant as a factor ($P<0.05$). This was also seen in a study done by Devatkal and Mendiratta (2001) where they used calcium lactate with salt-phosphate and alginate-calcium gels in restructured pork rolls. They found that the pH was significantly ($P<0.05$) higher in pork rolls containing alginate and calcium. Means and Schmidt (1986) also saw a significantly ($P<0.05$) higher pH when evaluating structured beef steaks containing factorial treatments of SA and calcium carbonate compared to the control. Figure 2.1.b shows that the pH of the solutions increased when the percentage of SA increased and also when the percentage of IC increased. A pH in the range of fresh meat (5.5-5.9) would be the target for the solutions and 6.49 is just outside the range. The stationary point was a saddle point and the recommended use level of each ingredient for pH was 0.494% SA, 0.377% IC and 0.375% WPI and MFS.

Objective color of the gels

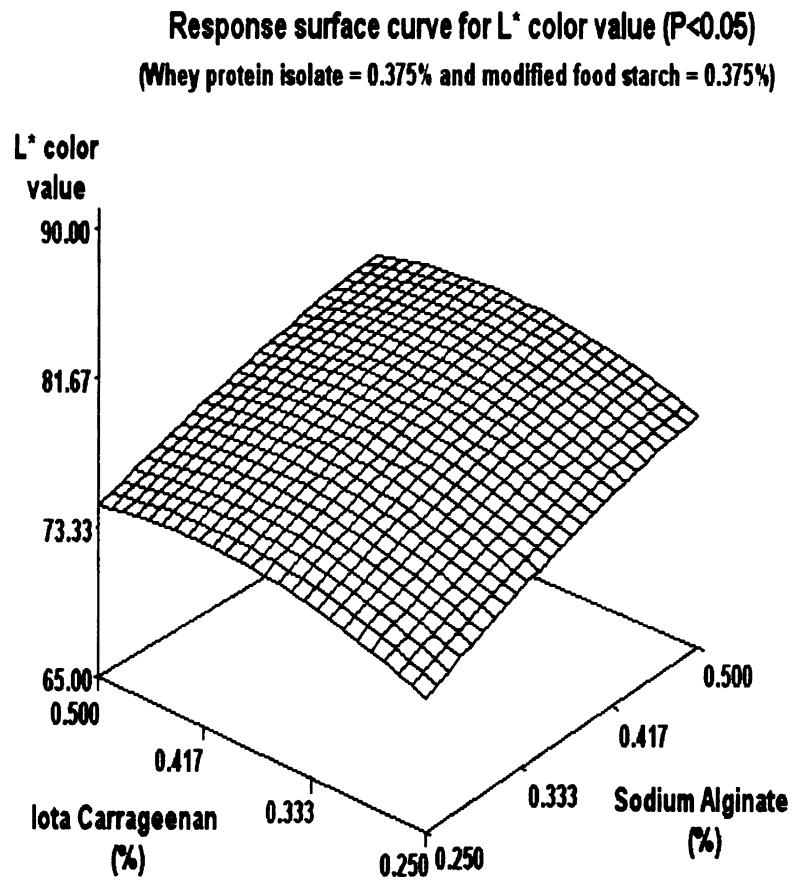
The L* color value of the gels had a mean of 77.2 and the model was significant ($P<0.05$). Response surface regression showed that the total model was significant ($P<0.05$) along with the linear ($P<0.05$) and cross product ($P<0.05$) effects. The factor SA was significant ($P<0.05$) as well as the parameter MFS x WPI (cross productP ($P<0.05$). Figure 2.2.a shows that the L* color value tended to increase as the percentage of SA increased. The L* color value also increased as the percentage of IC increased but only to approximately the center point (0.375%) and then it plateaued. The L* color values were also comparable to beef rib fat L* values (83.6). The stationary point was a saddle point and the recommended use levels of each ingredient for L* was 0.702% SA, 0.413% IC and 0.375% WPI and MFS.

The mean a* color value was –4.8 and the model was not significant ($P>0.05$). The response surface regression was not run.

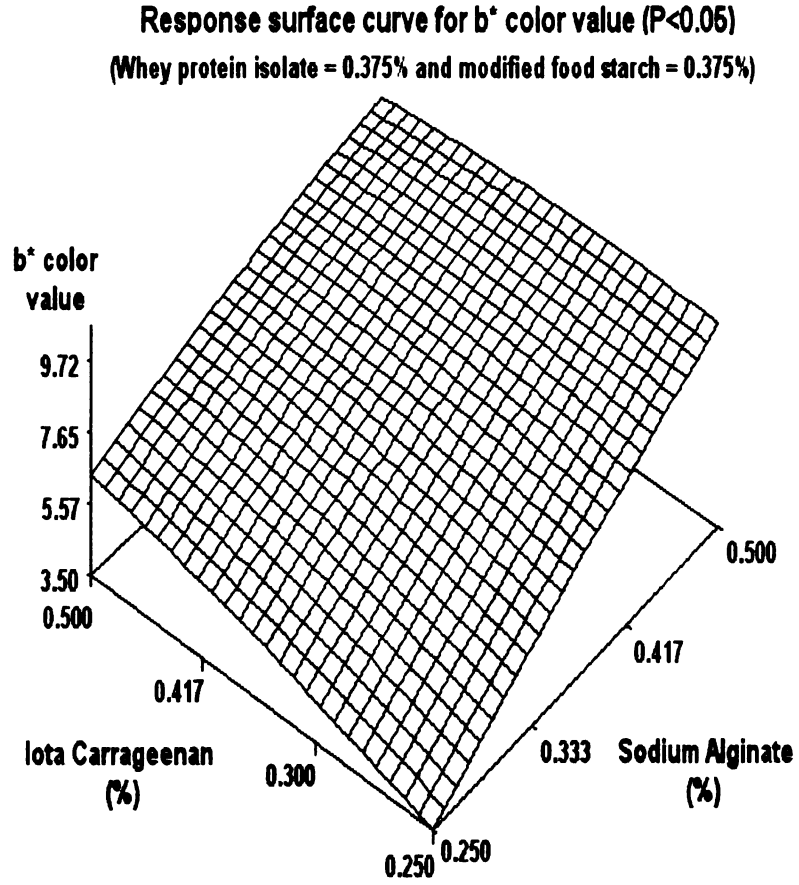
The b* color value of the solutions had a mean of 7.2 and the model was significant ($P<0.05$). The total model ($P<0.05$) and linear effect ($P<0.05$) were significant for response surface regression. SA was a significant ($P<0.05$) factor and figure 2.2.b showed that the b* color value increased as the percentage of SA increased and also increased as the percentage of IC increased but not to the extent as with the SA. The stationary point was a saddle point and the recommended level of each ingredient to use in a "modified marbling" solution for b* value was 0.023% SA, 1.081% IC and 0.375% WPI and MFS.

Figure 2.2. Response surface curves for significant (P<0.05) total regression models for L* and b* color values of "modified marbling" gels.

2.2.a.



2.2.b.



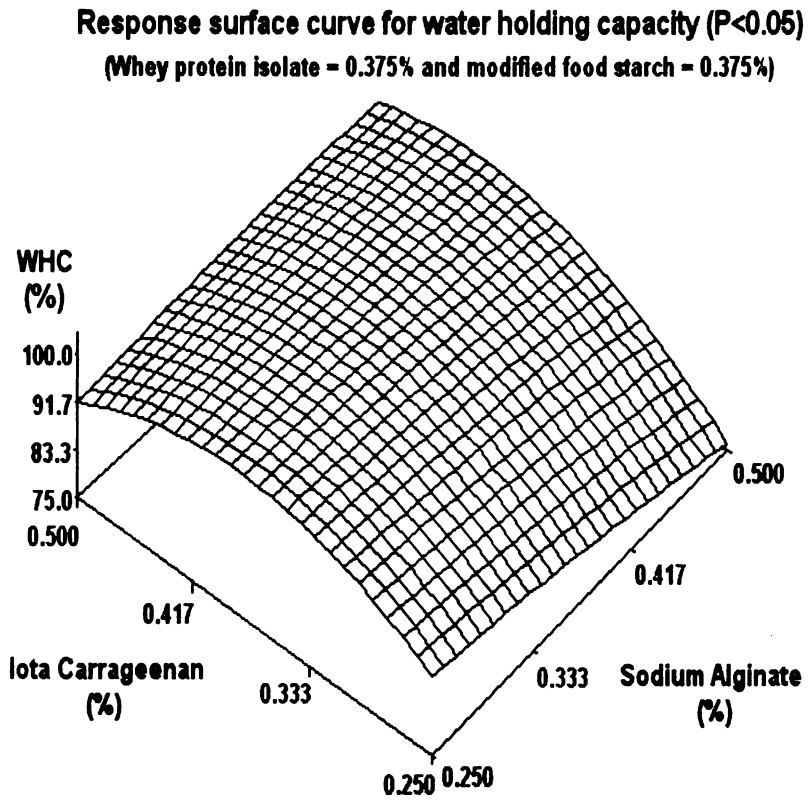
Water-holding capacity and water-holding capacity over time of the gels

The mean for the water-holding capacity of the gels was 98.0% and the model was significant ($P<0.05$). The response surface regression showed that the total model was significant ($P<0.05$) along with the linear ($P<0.05$) and quadratic ($P<0.05$) effects. The factors SA and IC were significant ($P<0.05$) along with the following parameters ($P<0.05$): IC (linear), IC x IC (quadratic) and IC x SA (cross product). From the response surface curve (Figure 2.3.a), the water-holding capacity decreased as the percentage of SA increased and increased as the percentage of IC increased to approximately the center point (0.375%) and then plateaued. This was also seen by Foegeding and Ramsey (1986) who found that the addition of iota and kappa carrageenan in low-fat meat batters resulted in an increased water-holding capacity. Also, Wallingford and Labuza (1983) found that carrageenan had very good water binding capacity when evaluating the water biding properties of nine food hydrocolloids in a low fat meat emulsion. The stationary point was a saddle point and the recommended use level of each ingredient for water-holding capacity was 0.281% SA, 0.391% IC and 0.375% WPI and MFS.

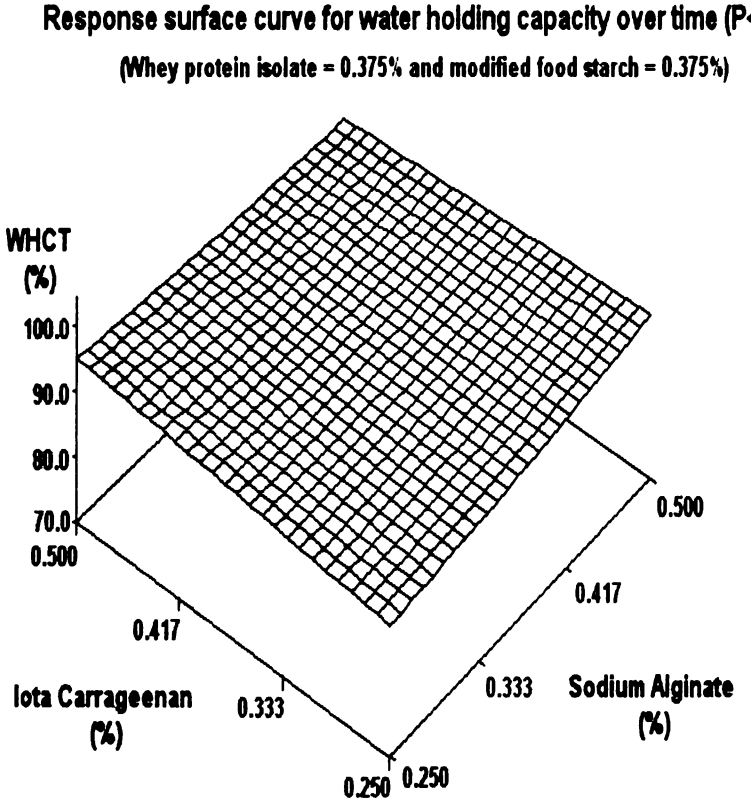
For water-holding capacity over time of the gels, the mean was 93.0% and the model was significant ($P<0.05$). The response surface regression showed that the total model was significant ($P<0.05$) as well as the linear ($P<0.05$) and cross product effects ($P<0.05$). The factors SA, IC and MFS were significant ($P<0.05$) along with the parameters ($P<0.05$) IC x SA and MFS x WPI (cross product). From Figure 2.3.b, the water-holding capacity over time increased proportionally as both the percentage of SA and IC increased. The water-holding capacity and water-holding capacity over time

Figure 2.3. Response surface curves for significant (P<0.05) total regression models for water-holding capacity and water-holding capacity over time of "modified marbling" gels.

2.3.a.



2.3.b.



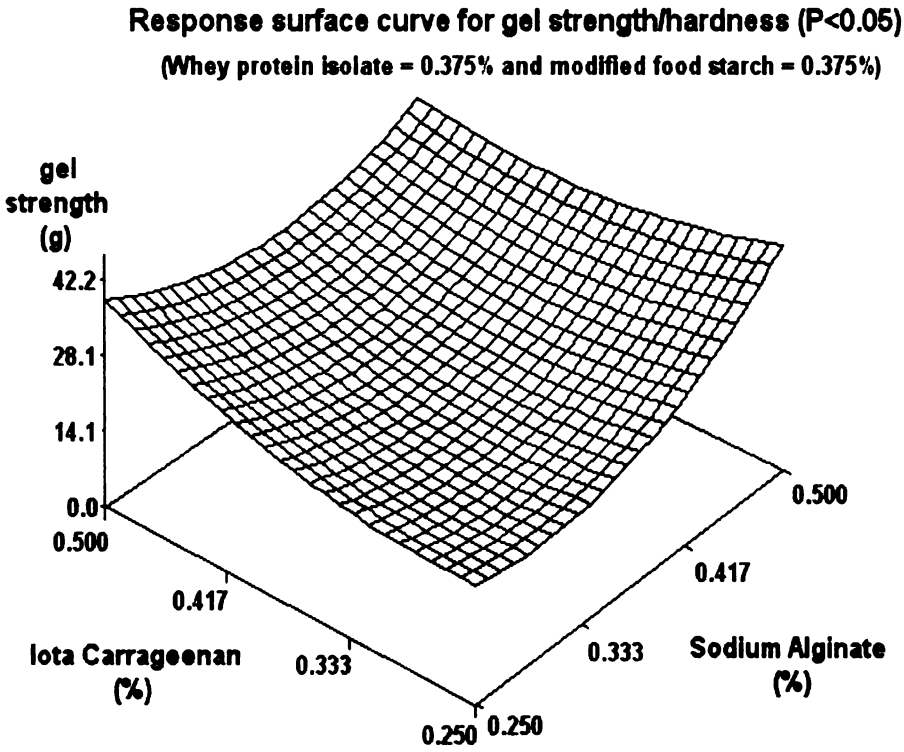
should be at least in the range of an average cooked product yield (75-85%) and both the water-holding capacity and water-holding capacity over time were above this range. The stationary point was a saddle point and the level of each ingredient to use in a "modified marbling" solution for water-holding capacity over time was 0.434% SA, 0.483% IC and 0.375% WPI and MFS.

Gel strength of the gels

The mean of the strength of the gels was 22.6 g and the model was significant ($P<0.05$). From the response surface regression, the total model ($P<0.05$), linear ($P<0.05$), quadratic ($P<0.05$) and cross product effects ($P<0.05$) were significant. The factors SA, IC, WPI and MFS were significant ($P<0.05$) as well as the following parameters ($P<0.05$): IC and WPI (linear), SA x SA and IC x IC (quadratic) and IC x SA and WPI x IC (cross product).

From the response surface curve (Figure 2.4), the gel strength increased as the percentage of SA increased and it also increased as the percentage of IC increased but the shape of the curve was not the same as with SA. This was also seen by Raharjo and others (1994) who studied the quality characteristics of restructured steaks with veal trimmings or veal leg meat and sodium alginate/calcium lactate and found that sodium alginate/calcium lactate used as a binder increased the binding force. Devatkal and Mendiratta (2001) found similar results when they evaluated restructured pork rolls formulated with sodium alginate/calcium carbonate and found that the raw binding strength was significantly higher in pork rolls containing sodium alginate/calcium carbonate. In another study, Shand and others (1994) studied the effects of adding

Figure 2.4. Response surface curve for significant (P<0.05) total regression models for gel strength of "modified marbling" gels.



0.5-1.0% kappa carrageenan to structured lean beef rolls. They found that the addition of kappa carrageenan improved the textural properties (bind, force to fracture, hardness).

The stationary point was at a minimum and the recommended use level of each ingredient for gel strength was 0.315% SA, 0.300% IC and 0.375% of WPI and MFS.

Development of the solution

After the use levels of each ingredient, for each attribute were made, the overall levels of ingredients recommended for the final solution were: 0.4375% SA and IC and 0.375% WPI and MFS. The solution was manufactured using these levels and observed by the researchers to be appropriate in order to give the "modified marbling" solution the desired functional properties (gelation, water retention, color). The solution was then scaled up for pilot plant use in a state of the art injector designed to handle solutions of this type for precise injection at a targeted additive level of 5-7%.

Conclusion

A "modified marbling" solution was developed from selected non-meat ingredients (SA, IC, WPI and MFS) and has the potential to be injected into whole muscle beef cuts. Higher levels of SA and IC increased gel viscosity. SA increased pH and gel L* (lightness) color value which was comparable to beef rib fat L* values. SA and IC significantly affected water-holding capacity and SA, IC and MFS were significant factors for water-holding capacity over time. All four ingredients significantly affected gel strength.

This study was designed to develop a "modified marbling" solution that mimicked intramuscular fat in appearance and functionality to inject into lower value whole muscle beef cuts. The results of this study demonstrate that a solution can be developed which looks like fat and is injectable. The findings of small-scale studies in the laboratory indicate the feasibility of further development of the solution for injection.

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CHAPTER 3

SCALE-UP OF A "MODIFIED MARBLING" SOLUTION FOR AN ON-LINE INJECTION PROCESSING SYSTEM FOR WHOLE MUSCLE BEEF CUTS

Abstract

A "modified marbling" solution containing sodium alginate (SA), iota carrageenan (IC), whey protein isolate (WPI) and modified food starch (MFS) was modified in order to prevent absorption of muscle myoglobin pigments into the solution and attempt to mimic the flavor of beef fat. In addition it was scaled to be used in an injection system designed for high volume processing. Beef tallow was tested at different levels (1-4%) and different types and levels (0.25-1.0%) of beef flavoring were evaluated. The processing system and parameters were determined and the solution was manufactured and injected and tumbled into the whole muscle beef cuts for a pilot plant study. Three percent beef tallow and 0.25% beef flavor were added to the "modified marbling" solution. Parameters were set on an automatic, multi-needle injector in order to acquire the desired percent pick-up (5-7%) and "modified marbling" pattern. The ribeye rolls designated to different storage days did not significantly differ in injection pick-up and tumbling loss measured immediately after injection and tumbling, respectively. The injection pick-up (9.75%) was significantly higher ($P<0.05$) for the injected Select but there was no significant difference between the three controls.

Keywords: "modified marbling", solution, injection, parameters

Introduction

Fat substitutes that are protein and carbohydrate-based are hydrophilic ingredients and since muscle myoglobin pigments are also hydrophilic, the pigments can absorb into the ingredients or the ingredients can absorb into the pigments. This was an unanticipated result of preliminary injection observational studies. All the non-meat ingredients used to manufacture the "modified marbling" solution (sodium alginate (SA), iota carrageenan (IC), whey protein isolate (WPI) and modified food starch (MFS) are protein or carbohydrate-based. The muscle myoglobin pigments tended to absorb into the solution causing color variation among the gelled particles (marbling) when injection was completed. A possible solution to the problem is to add a hydrophobic ingredient to the solution in order to prevent or slow down the absorption of the pigments.

Injection has been used to physically distribute a brine or marinade into whole muscle meat and poultry through needles that penetrate into the muscle and distribute the brine or marinade under pressure. Injection is used to improve juiciness, tenderness, and flavor of a meat product. Research has shown that injection is an ideal method to distribute non-meat ingredients such as salt, phosphate, nitrate, cure accelerators, sweeteners, seasonings, non-meat proteins, starches, gums, water, and preservatives in meat products.

In a study by McGee and others (2003), USDA Select inside beef rounds were injected with a solution of sodium lactate, sodium tripolyphosphate and sodium chloride. The injected treatments were found to be more tender than the control products for both Warner-Bratzler shear force and consumer sensory panel scores. The injected treatments also had a lower cooking and re-heating loss compared to the controls. In another study

by Hashim and others (1999), bone-in chicken breast quarters were marinated with a lemon-pepper marinade by injection or immersion and honey (10, 20 and 30%) was substituted for water in the marinades. The injected chicken retained more marinade, loss less juice during roasting and had a lower shear force value than the immersed chicken. The addition of honey to the marinade of the injected chicken increased the honey flavor without affecting the appearance, aroma, and other flavor attributes or texture.

The best processing technique to use in order to get the "modified marbling" solution into whole muscle beef cuts is injection. Injection should acquire the desired percent pick-up (5-7%) to achieve an acceptable "modified marbling" pattern.

The objectives of this study were to modify the solution, determine the processing system and parameters and demonstrate that a "modified marbling" solution can be injected into whole muscle beef cuts. To achieve this objective, beef tallow and beef flavor were added to the solution to prevent or slow down the absorption of the muscle myoglobin pigments into the injected marbling and to mimic the flavor of beef fat respectively. The processing system used to inject the solution into whole muscle beef cuts was determined, parameters were set, and the "modified marbling" solution was injected into the beef cuts in the pilot plant study.

Materials and Methods

Solution modification

Results summarized from preliminary injection observation studies using a hand-held injector indicated that muscle myoglobin pigments absorbed into the "modified marbling" solution causing color variation among the gelled particles. It was hypothesized that addition of a hydrophobic ingredient to the "modified marbling" solution was needed to prevent the absorption of the pigments so beef tallow (B4102, Proliant Meat Ingredients, Ames, IA) was tested at different levels (1-4%) in the solution.

In addition, informal tasting of the "modified marbling" solution by the researchers found the need for the addition of a beef flavor. Sensory evaluations were conducted in a discussion format with a trained sensory panel of six healthy panelists between ages twenty and sixty-five (four female and two male) using subcutaneous beef ribeye fat as a standard. They evaluated the beef fat flavor intensity and mouth coating/texture of the "modified marbling" solution. The "modified marbling" solution and beef ribeye fat were prepared by cooking the samples in 25-ml covered glass bottles in a water bath to an internal temperature of 71 °C (the endpoint cooking temperature of a steak.) The samples were transferred to Soufflé cups and served to the panelists. From the evaluations, changes were made to the flavor of the "modified marbling" solution by experimenting with different beef flavors (powder, solid, liquid) and adjusting the amounts (0.25-1.0%) in order to try to mimic the flavor of beef fat. Changes were made to attempt to mimic the mouth coating/texture of the "modified marbling" solution by adjusting ingredient levels and processing procedures. Modifications were made to the other ingredients as needed to accommodate the addition of beef tallow and flavoring.

Injection parameters

Several tests were conducted to determine the machine parameters to use with the injector (IMAX 520, Wolf-tec, Inc., Kingston, NY) in order to incorporate the "modified marbling" solution into whole muscle beef cuts. Ribeye roll sections were injected with the "modified marbling" solution, weighed to determine percent pick-up of the solution and the "modified marbling" pattern was observed. Changes were made to the injection parameters as needed to acquire the desired percent pick-up (5-7%) and "modified marbling" pattern.

The solution was injected into the ribeye rolls using an IMAX 520 injector (Wolf-tec, Inc., Kingston, NY) with a 378-L brine tank containing an external, stainless steel centrifugal pump. The injector contained two hundred 4-mm needles with four 1.5-mm exit holes. The method of injection was one-way and the pump pressure was 4.5-bar. The injector had a walking beam to transport the product at a speed of 39-strokes/min and the solution temperature set point was 35 °C.

Needle injection study

There was a concern that the penetration of the injection needles through the ribeye rolls could affect the tenderness of the steaks and bias the proposed study, which studied the effect of the "modified marbling" solution on tenderness. A study was conducted to compare Warner-Bratzler shear force of needle injected (without the "modified marbling" solution) and non-injected ribeye rolls. A total of ten ribeye rolls, 112A (2 USDA Average Choice, 2 USDA Low Choice, 3 USDA High Select and 3 USDA Low Select) were purchased at a local meat company (Popoff Quality Food

Service, Lansing, MI). The ribeye rolls were cut in half and the anterior end was run through the injector without solution and the posterior end was not run through the injector. The injector contained two hundred 4-mm size needles with two 2-mm holes and the walking beam speed was 30-strokes/min. Two steaks (2.5 cm) were cut from the middle of the ribeye half (opposite from the anterior or posterior end). This resulted in adjacent steaks being compared for treatment effects in each ribeye

Steaks were cooked on a Taylor clamshell grill (Model QS24 Taylor Co, Rockton, IL). The upper plate was set to 104 °C and the bottom plate at 102 °C with a 2.7-cm gap between plates. The temperature was monitored using a copper constantan thermocouple (0.051 cm diameter, 15.2 cm length; Omega Engineering Inc., Stamford, CT) inserted into the geometric center of the steak and cooked to an endpoint temperature of 71 °C. Steaks were stored at 4 °C for 24 hr and six 1.27-cm cores were taken parallel to the longitudinal axis of the fibers using a drill press-mounted corer. Cores were sheared perpendicular to the fibers using a Warner-Bratzler head on a TA-HD*i* Texture Analyzer (Texture Technologies Corp., Scotsdale, NY).

Raw materials and non-meat ingredients

Ribeye rolls (112A) were selected and purchased from a meat packing plant (Smithfield Beef Enterprise, Plainwell, MI). Beef carcasses were yield and quality graded to obtain USDA Select, USDA Low Choice and USDA Average Choice carcasses for this study. Selected carcasses were tagged for identification and followed through the fabrication line. The rib sections (6^{th}-12^{th} rib) were removed from both sides of the carcass, boned out to produce a boneless ribeye roll (IMPS 112A), and vacuum packaged.

Ribeye rolls were loaded into boxes and transported to the meat laboratory at Michigan State University. At Michigan State University, replicates were balanced within grade so that any differences within grades allocated to each treatment were minimized.

The SA, IC, WPI, MFS and calcium sulfate were purchased or donated from the same sources as in study 1 and the beef tallow (B4102) and the beef stock (B1304) were donated from Proliant Meat Ingredients (Aimes, IA)

Solution manufacture and analysis

Solutions (SA, IC, WPI and MFS) for the pilot plant study were manufactured at the Michigan State University meat laboratory. Batches of "modified marbling" solution (approximately 68 kg) for each of the four replicates were produced using a Rotostat mixer (Model 80XP63SS, Admix Inc., Londonderry, NH). Ingredients were weighed (appendix 11) and phosphate was added to half of the water and mixed for 2 min at 1500 rpm. The whey protein isolate and beef tallow were added next followed by the mixture of SA and vegetable oil (for hydration per recommendation of FMC BioPolymer) and then the remainder of the water in the solution. The Rotostat speed was increased to 2000 rpm. The beef flavor, IC, MFS and presolubilized calcium sulfate solution were added and the mixing speed was gradually increased to 3500 rpm. The total mixing time was 8 min or until the desired thickness was achieved.

The properties of the "modified marbling" solution were determined by measuring the solution viscosity and pH immediately after the solution was manufactured. The solution was stored for 24 hr at 4 °C to allow the solution to gel and

objective color, water-holding capacity, water-holding capacity over time, and gel strength/hardness were determined using the same procedures as in study 1.

Processing and injection procedures for subsequent studies

One treatment (injected USDA Select) and three controls (USDA Select control, USDA Low Choice control, and USDA Average Choice control) were evaluated. The USDA Select ribeye rolls were processed by taking four ribeye rolls for each replicate (one ribeye roll for each storage day: 0, 14, 28, and 42) and cutting each in half (anterior and posterior). For the USDA Low Choice and Average Choice controls, two ribeye rolls were used for each replicate and were cut in half. The ribeye halves were randomized across each storage day for each replicate and the Select ribeye halves were also randomized between the injected and control treatments as a completely randomized, balanced design (appendix 13 and 14).

The control ribeye rolls (USDA Select control, USDA Low Choice control, and USDA Average Choice control) were passed through the injector (IMAX 520 using the set parameters) without solution, weighed, tumbled for 1 min with a Roschermatic tumbler (Model MM 80, Colmatic Co., Long Island City, NY) and reweighed. This was done based on the results of the needle injection study. All control ribeye rolls were packaged in 30.5 x 61.0 cm vacuum packaged bags (Cryovac Sealed Air Co., Duncan, SC) and stored in boxes at 1 °C. The injected USDA Select ribeye rolls were weighed, injected with the "modified marbling" solution, weighed, tumbled for 1 min, and reweighed. Tumbling was conducted in order to better distribute the "modified marbling" solution within the ribeye rolls to achieve the desired marbling pattern.

Control ribeye rolls were also tumbled in order to keep consistency between the treated and control ribeye rolls. The ribeye rolls were injected with the needles penetrating into the non-fat side of the meat at a targeted 5-7 % injection, vacuum packaged and stored in the same manner as the controls. This process was replicated four times.

Experimental design

Modifications were made to the solution to address encountered problems. An automatic brine injector was then set-up with the appropriate parameters to inject 5-7% of the "modified marbling" solution into whole muscle beef cuts.

The injection verification study was analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS User's Guide, Version 8.2, Cary, NC: SAS Institute, Inc., 2002) to determine the effect of needle injecting ribeye rolls without the "modified marbling" solution on Warner-Bratzler shear force. Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure.

The experimental design used for the pilot plant study was a split plot design with treatment as the whole plot factor and storage day as the split plot factor. The effect of injecting the "modified marbling" solution into ribeye rolls on quality attributes (injection pick-up and tumbling loss) of the ribeye rolls was analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS User's Guide, Version 8.2, Cary, NC: SAS Institute, Inc., 2002). Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure.

Results and Discussion

Modification of the solution

Different levels of beef tallow (1-4%) were tested in the "modified marbling" solution in attempt to decrease the absorption of muscle myoglobin pigments into the solution. Three percent beef tallow was observed to be the most effective. Also, from sensory evaluations and follow-up testing in the lab, it was found that a powder beef flavor at 0.25% be added to the solution in order to attempt to mimic the flavor of beef fat. Due to the modifications made to the solution, the amount of whey protein isolate was increased from 0.375 to 1.5% to assist in emulsifying the beef tallow since whey proteins have been found to improve emulsion stability. Hung and Zayas (1992) reported that compared to all beef frankfurters (20% fat), beef frankfurters containing 3.5% whey protein concentrate had increased water-holding capacity and decreased cooking loss. The amount of sodium alginate was also increased from 0.4375 to 1.0% to strengthen the gel.

Needle injection study

A study was conducted to compare Warner-Bratzler shear force values of needle injected (without the "modified marbling" solution) and non-injected ribeye rolls to determine if the penetration of the injection needles of the IMAX 520 through the ribeye rolls would affect the tenderness of the steaks. There was no difference ($P>0.05$) between the needle injected (without the "modified marbling" solution) and non-injected ribeye rolls for Warner-Bratzler shear force values. The mean for the ribeye rolls needle injected was 3.18 kg and for the non-injected ribeye rolls was 3.50 kg. Even though the

difference between the needle injected and non-injected ribeye rolls was not significant (P=0.062), it was close to the predetermined level of significance (P<0.05) so all control ribeye rolls were needle injected without the "modified marbling" solution during the subsequent pilot plant study to prevent bias when evaluating tenderness.

Properties of "modified marbling" solution and gel

The properties of the "modified marbling" solution and gel from the pilot plant study are shown in table 3.1 for each replicate and were consistent across replicates. The viscosity of the solution was higher than in study 1 due to the need of ingredient addition based on problems encountered. Beef tallow was added to limit the absorption of the muscle myoglobin pigments into the "modified marbling" solution. The amount of WPI and SA was increased to accommodate for the addition of the beef tallow and the addition of these ingredients increased the viscosity of the solution. The pH of the solution and the L* value of the gel were similar to values encountered in study 1. The L* value measured for subcutaneous fat removed from the rib portion of a USDA Choice carcass was 83.6 which was a little higher but similar to the L* value of the gel.

The water-holding capacity and water-holding capacity over time were similar to the values optimized from study 1 but the gel strength was higher. The addition of ingredients to the solution to limit the absorption of the pigments produced a harder gel, which required a larger amount of force to penetrate it. The moisture, fat and protein percentages were similar to the ingredient formulation.

Table 3.1. Least square means for the properties of the "modified marbling" solution and gel injected into ribeye rolls.

	Replicate			
	1	2	3	4
Viscosity (Pa s)[a]	6.7	6.2	6.3	6.9
pH[b]	6.7	6.7	6.7	6.7
L*[c]	75.8	76.5	80.3	78.1
a*[d]	-4.1	-3.9	-3.8	-3.9
b*[e]	16.2	16.4	17.2	15.6
Water-holding capacity (%)[f]	85.9	94.5	91.3	97.2
Water-holding capacity over time (%)[g]	95.7	96.0	95.6	96.2
Gel strength (g)[h]	237.9	208.2	273.4	244.4
Moisture (%)	89.8	90.3	89.7	90.5
Fat (%)	2.4	1.5	2.0	1.4
Protein (%)	1.7	1.7	1.7	1.7

[a] Solution viscosity analyzed at 30 °C.

[b] Solution pH measured at 22 °C.

[c-e] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the gel using a Minolta Chromameter.

[f] Water-holding capacity of the gel at 4 °C, centrifuged for 30 min at 40,000 x g.

[g] Water-holding capacity of the gel after 2 h at 22 °C.

[h] Gel strength of gelled solutions measured in grams using a kg load cell and a 1.25 cm diameter acrylic probe.

Injection pick-up and tumbling loss of ribeye rolls for subsequent study

Table 3.2 shows the percent injection pick-up and tumbling loss for the injected and control ribeye rolls. The ribeye rolls designated to different storage days did not significantly differ in injection pick-up and tumbling loss measured immediately after injection and tumbling respectively.

The injection pick-up for the injected Select was significantly higher ($P<0.05$) than the controls but there was not a significant difference between the three controls. The controls had a small percentage loss of meat, which was due to small pieces of the ribeye roll coming off as they were passed through the injector. The average injection pick-up for the injected Select was a little higher then the targeted injection pick-up of 5-7%. The tumbling loss for the injected Select ribeye rolls were significantly higher ($P<0.05$) than the Select control and the Average Choice control ribeye rolls. The loss for the injected Select was higher than the Low Choice control but the difference was not significant.

Table 3.2. Least square means for injection pick-up and tumbling loss of injected and control ribeye rolls.

	Storage Days					Treatment				
						1	2	3	4	
Measurement	**0**	**14**	**28**	**42**	**SEM[c]**	**injected Select**	**control Select**	**control Low Choice**	**control Average Choice**	**SEM[c]**
Injection Pick-up (%)[d]	2.3[a]	2.3[a]	2.5[a]	2.4[a]	0.93	9.8[a]	-0.1[b]	-0.1[b]	-0.2[b]	1.83
Tumbling Loss (%)[e]	0.1[a]	0.1[a]	0.1[a]	0.2[a]	0.06	0.4[a]	0.1[b]	0.1[ab]	0.0[b]	0.07

[a-b] Means having different superscripts within same rows are significantly different ($p<0.05$).

[c] Standard error of the mean (SEM).

[d] Injection pick-up = the amount of solution picked-up for the injected Select or the amount of meat lost during the penetration of the injection needles through the controls.

[e] Tumbling loss = the amount of solution or meat lost during a 1 min tumbling cycle.

Conclusion

The "modified marbling" solution was modified to address problems encountered during plans to scale up for the pilot plant study and the parameters for the injection processing system were developed. Three percent beef tallow and 0.25% beef flavor were added to the "modified marbling" solution. Parameters were determined for an automatic, multi-needle injector in order to acquire the desired percent pick-up (5-7%) and "modified marbling" pattern for Select ribeye rolls. The ribeye rolls designated to different storage days did not significantly differ in injection pick-up and tumbling loss measured immediately after injection and tumbling respectively. The injection pick-up (9.75%) was significantly higher for the injected Select but there was not a significant difference between the three controls.

This study was designed to modify the "modified marbling" solution, determine the parameters for a specific injector and demonstrate that the "modified marbling" solution can be injected into whole muscle beef cuts. The results of this study indicate that the "modified marbling" solution could be successfully modified for a specific equipment piece and the solution could be successfully injected into whole muscle beef cuts. Further research will be needed to improve upon the "modified marbling" solution and injection parameters for implementation in an industrial application.

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CHAPTER 4

CHEMICAL AND PHYSICAL PROPERTIES OF WHOLE RIBEYE ROLLS INJECTED WITH THE "MODIFIED MARBLING" SOLUTION COMPARED TO NON-INJECTED CONTROLS

Abstract

Ribeye rolls (IMPS 112A) injected with the "modified marbling" solution (5-7% targeted pick-up) were compared to control ribeye rolls in chemical attributes. USDA Select, Low and Average Choice ribeye rolls were passed through an automatic brine injector without injecting solution (controls). Ribeye rolls were designated to 0, 14, 28, or 42 days of storage (1°C), weighed for ribeye purge and steaks (2.54 cm) were fabricated on each storage day. A 7-day retail shelf life study (analysis of TBARS, color and percent steak purge) was conducted on fabricated steaks from each treatment and proximate analysis was conducted. The injected Select had a significantly higher ($P<0.05$) ribeye purge than the Average Choice control. The injected Select had the highest percent moisture, lowest percent fat and lowest cooked product yield. For TBARS values, the injected Select was significantly higher ($P<0.05$) than all the controls. There also were no significant differences in color scores between treatments. The "modified marbling" solution has the potential to improve lower quality beef cuts but more research is needed to improve the "modified marbling" properties. One possibility is that the amount of fat in the solution could be increased to achieve the benefits of both the non-meat ingredients and the fat.

Keywords: intramuscular fat, "modified marbling", non-meat ingredients, solution, injectable

Introduction

The amount of marbling or intramuscular fat has been shown to influence the palatability (juiciness, tenderness, flavor) of beef cuts. Savell and Cross (1988) determined that the minimum fat percentage required for acceptable palatability of broiling cuts is 3% on an uncooked basis (minimum Slight degree of marbling, USDA Low Select). They came to this conclusion after studying research conducted over many years and found that steaks with less than 3% intramuscular fat (Practically Devoid and Traces) were tougher, drier and less flavorful. However, 3% intramuscular fat or Slight degree of marbling provides little room for error in cookery method or degree of doneness to ensure palatability. They also determined two other levels of intramuscular fat related to increased palatability. Approximately 5% (midpoint of Small degree of marbling) and 7% (low end of Moderate amount of marbling) were associated with hierarchical degrees in palatability. Beef cuts containing 3-7% intramuscular fat (marbling) are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability.

Studies have been conducted to determine quality inconsistencies within the beef industry chain, from farm to retail. The results from the last National Beef Quality Audit (McKenna and others 2002) reported that the overall average scores for intramuscular fat and USDA beef carcass quality grades were Small^{06} (marbling score) and USDA Select^{79} (USDA Quality Grade) respectively. The fourth listed challenge in the "top ten quality challenges" identified from the audit was insufficient marbling since it was found that 45% of carcasses graded USDA Select (Slight degree of marbling), 53% graded USDA Choice (9% moderate, 26% modest and 65% small degree of marbling) and only 2%

graded USDA Prime. Forty-five percent of the carcasses had Slight degree of marbling or approximately 3% intramuscular fat and are at the lower edge of the "window of acceptability." This indicates an opportunity for improvement by increasing the amount of marbling in whole muscle beef cuts to ensure acceptable palatability.

The palatability of whole muscle cuts fabricated from lower quality (less than USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. The development of a "modified marbling" solution from selective non-meat ingredients (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) that can mimic the properties of intramuscular fat and can be directly injected into lower quality whole muscle beef cuts may enhance its overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

The objective of this study was to verify the properties of the "modified marbling" solution in whole muscle beef cuts. To achieve this objective, USDA Select ribeye rolls were injected with the solution, cut into steaks and chemical attributes were compared to non-injected USDA Select, Low and Average Choice control ribeye steaks.

Materials and Methods

Processing procedures

After the solutions were manufactured and the ribeye rolls were injected as described in chapter 3, the day 0 ribeye rolls were allowed to equilibrate for 48 hr and then removed from the package and cut for analysis. Thus the ribeye roll controls for day 0 in this study were actually 48 hr after injection. Ribeye steaks were cut from the middle of the ribeye rolls (opposite from the anterior or posterior end) with a 1.3 cm steak was cut for pH, proximate composition, TBARS analysis and scanning electron microscopy. A 2.5 cm steak was then cut for the retail meat case shelf-life study and two more 2.5 cm steaks were cut and randomized for sensory evaluation and Warner-Bratzler shear force which was done in another study (chapter 5).

The steak designated for the retail meat case shelf-life study was weighed and placed on 12.7 x 20.3 cm foam trays. The trays were overwrapped with polyvinyl chloride film (PVC) (RMF-61 HY stretch meat film, Borden Chemical, North Andover, MA) with a water vapor transmission rate of 26g/254 sq. cm per 24 hr at 38 °C, 90% R. H. The oxygen transmission rate was 1,400cc/254 sq. cm per 24 hr at 23 °C and the carbon dioxide transmission rate was 13,400cc/254 sq. cm per 24 hr at 23 °C. The steaks were placed in a 1 °C retail meat case (Model SC-CMS35-6, Mc Cray Refrigerator Co., Inc., Philadelphia, PA). The retail meat case lighting produced a luminance of 122 lumens on the inside shelf of the meat case and 62 lumens on the outside glass surface of the meat case. The steaks were allowed to equilibrate inside of the meat case for 2 hr and objective and subjective color was evaluated on the steak through the PVC film. Color measurements were also taken on day 3, 5, and 7. On day 7 the PVC film was removed.

The steaks were reweighed for percent purge determination and a sample was taken for day 7 TBARS analysis.

On storage day 14, 28, and 42 the appropriate ribeye rolls were removed from the package, weighed for percent ribeye purge (loss of fluid) and the above steps were repeated for analysis.

Ribeye purge

Percent ribeye purge was determined on each storage day (14, 28, 42) after being stored in boxes at 1 °C. The ribeye rolls were weighed prior to storage and then removed from the package, blotted dry and reweighed. The percent ribeye purge was determined by the following equation:

$$\frac{\text{weight before storage} - \text{weight after storage}}{\text{weight before storage}} \times 100$$

Cooked product yield

On each storage day (0, 14, 28 and 42), steaks were evaluated for cooked product yield. Steaks were weighed and then cooked on a Taylor clamshell grill as described in chapter 4. Steaks were weighed after cooking and allowed to drip for 5 min. Percent cook yield was calculated as follows:

$$\frac{\text{cooked steak weight}}{\text{steak weight before cooking}} \times 100$$

Steak purge

Percent steak purge was determined on day 7 of the retail meat case study for each storage day (0, 14, 28 and 42) after being stored in a refrigerated (1 °C) retail display case on foam trays overwrapped with PVC film for 7 days. The steaks were weighed prior to storage and then removed from the foam trays, blotted dry and reweighed. The percent steak purge was determined by the following formula:

$$\frac{\text{weight before storage} - \text{weight after storage}}{\text{weight before storage}} \times 100$$

Thiobarbituric acid reactive substances (TBARS)

On day 0 and 7 of the retail meat case shelf-life study for each storage day (0, 14, 28 and 42), TBARS analysis was conducted as an indicator of oxidative rancidity. Four replicates were run for each sample according to methods established by Tarladigis and others (1960) and Zipser and others (1962) as modified by Rhee (1978).

Proximate composition

On each storage day (0, 14, 28 and 42), a 1.3 cm steak was cut from each ribeye half and one half of the steak was used for proximate composition. Samples were packed in Whirl-Pack™ bags (Fisher Scientific USA, Pittsburg, PA) and frozen at –10 °C for at least 24 hr before processing. Frozen samples were cut into small pieces and ground with dry ice into a fine powder using a Tekmar grinder (Tekmar Co, Cincinnati, OH), packed in opened Whirl-Pack™ bags, placed in the freezer (–10 °C) for at least 48 hr (to evaporate the dry ice), and sealed until further analysis.

Moisture, fat and protein contents of samples were determined according to AOAC (2000) methods 950.46B (oven drying), 991.36 (Soxhlet ether extraction), and 992.15 (combustion method, nitrogen measurement, Model FP-2000, LECO Co., St. Joseph, MI). Samples were analyzed in triplicate.

pH determination

On each storage day (0, 14, 28 and 42), a 1.3 cm steak was cut from each ribeye half and one half of the steak was used for pH analysis. Sample (1 ± 0.1 g) was collected in a 50-ml polycarbonate tube and 10-ml of deionized water was added. Samples were homogenized using a Polytron mixer (PT-35, Kinematica, AG, Switzerland). The pH of the homogenized samples was measured at room temperature (22 °C) using an Accumet pH meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA).

Melting point determination

The melting point of the "modified marbling" gel and beef subcutaneous ribeye fat was determined using differential scanning calorimetry (DSC) according to ASTM (1997) methods. Approximately 12 mg of sample was cut with a razor blade and placed in a DSC pan bottom (T40625, TA Instruments, New Castle, DE) and covered with a DSC pan lid (T40621, TA Instruments, New Castle, DE). The pan was placed in the DSC (2010, TA Instruments, New Castle, DE) along with the control pan. The experiments were conducted in triplicate at a heating rate of 10.1 °C per min from 20 °C to 80 °C for the beef ribeye fat and 20 °C to 150 °C for the "modified marbling" gel.

Objective and subjective color evaluation

On day 0, 3, 5, and 7 of the retail meat case study for each storage day, objective and subjective color measurements were taken. For objective color, a Minolta Chromameter CR-310 (Commission International D'Edairerage (CIE) L*a*b*, Ramsey, NJ) with a 5.5 cm reading orifice was used to measure L* (lightness), a* (redness), and b* (yellowness) values of ribeye steaks. Before measuring, the chromameter was calibrated with polyvinyl chloride film on a standard white tile and then one reading was taken of each steak.

For subjective color analysis, a color panel of four evaluators was used to determine lean color and marbling score of the ribeye steaks. All color evaluations were conducted under fluorescent lighting conditions. The lean color was determined on a 7-point scale adapted from a beef lean maturity scale where 1=extremely bright cherry-red and 7=extremely dark red (AMSA 2001). The marbling score was evaluated using beef marbling cards adapted from the official United States standard for grades of carcass beef (USDA 1997).

Scanning electron microscopy (SEM)

Electron micrographs were used to study the microstructure and to elucidate the relationship of the "modified marbling" solution and the muscle proteins. First the microstructure of the "modified marbling" gel was looked at to determine the structure of the non-meat ingredients before looking at them in the meat matrix. After the solution was allowed to gel for 24 hr at 4 °C, small pieces (1.0 x 2.0 x 2.0 mm) of the gel were cut and prefixed for 2 hr at 22 °C in 4.0% glutaldehyde solution buffered with 0.1 M sodium

phosphate pH 7.0. After the prefixation, the gels were postfixed at 4 °C overnight in 0.1% osmium tetraoxide solution. Fixed gels were then rinsed with 0.1 M sodium phosphate buffer (pH 7.0) and dehydrated in a graded series of ethanol (25, 50, 75 and 95%) for 20 min each followed by three 15 min changes in 100% ethanol. Dehydrated gels were dried using a carbon dioxide critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) and then mounted on stubs and coated with a 25-30 nm gold layer in an ion-sputter coater (Emscope laboratories Ltd., Ashford, Kent, UK).

Meat samples injected with the "modified marbling" solution and control samples were prepared by cutting 2.54 cm cubes and freezing in liquid nitrogen. The samples were placed between two fiberglass plates and pounded with a rubber mallet in order to produce sample pieces small enough to analyze. Samples were prefixed for 24 hr in 4% glutaldehyde buffered with 0.1 M sodium phosphate pH 7.0 at 4 °C. Samples were then rinsed thoroughly with 0.1 M sodium phosphate buffer (7.0) and dehydrated in a graded series of ethanol (25, 50, 75, 95%) for 20 min each, followed by three 15 min changes of 100% ethanol. After dehydration, samples were dried using a carbon dioxide critical point dryer, mounted on stubs and coated with a 25-30 nm gold layer in an ion-sputter coater. The microstructure of both the gels and meat samples was observed using a scanning electron microscope (JOEL, Model JSM-6400V, version 96-2, Tokyo, Japan) at a 15 mm working distance using an accelerating voltage of 12 KV.

Experimental design

The experimental design used was a split plot design with treatment as the whole plot factor and storage day as the split plot factor. The retail meat case shelf-life study

was a repeated measurement design. The effect of injecting the "modified marbling" solution into ribeye rolls on quality attributes (cooked product yield, purge loss, color, pH, proximate composition, and lipid oxidation) of the ribeye steaks was analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS User's Guide, Version 8.2, Cary, NC: SAS Institute, Inc., 2002). Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure.

Results and Discussion

Ribeye purge, cooked product yield, steak purge and TBARS values of ribeye rolls

The ribeye purge, cooked product yield, steak purge and TBARS values are shown in table 4.1. After the ribeye rolls went through the designated time of storage, the ribeye purge was measured and it significantly ($P<0.05$) increased (from 1.2 to 2.9%) as the length of storage increased. An increase in purge over storage time is not unusual and often expected. An increase in purge was also seen in a study by Goddard and others (1996) where a solution of 2% lactic acid and 2% acetic acid was sprayed on beef strip loins in order to improve the chemical, physical and microbial attributes. They found that the amount of purge significantly increased with storage. This was likely caused by the degradation of muscle proteins, possibly due to the pH nearing the isoelectric point of the protein allowing the bound water to be released as purge.

The cooked product yield and steak purge did not change across storage days. The TBARS values, however, significantly increased ($P<0.05$) as the length of storage increased from 0.4 to 0.9 mg malonaldehyde/kg of sample. This could be due to the treatment effect in which the injected ribeye rolls were more prone to lipid oxidation than the Low or Average Choice ribeye rolls and this effect was probably due to not using antioxidants in the injected Select treatment. The use of antioxidants to decrease lipid oxidation was seen in a study by St. Angelo and others (1991). They infused 0.3M calcium chloride and 1% sodium ascorbate or 0.25% maltol into freshly slaughtered lambs to look at the differences in tenderization and warmed-over flavor. They found that with storage, the lamb patties from the lambs infused with either maltol or sodium

Table 4.1. Least square means for ribeye purge, cooked product yield, steak purge and TBARS values of injected and control ribeye rolls.

	Storage Days					Treatment				
						1	2	3	4	
Measurement	**0**	**14**	**28**	**42**	**SEM[d]**	**injected Select**	**control Select**	**control Low Choice**	**control Average Choice**	**SEM[d]**
Ribeye Purge (%)[e]	-	1.2[b]	1.8[b]	2.9[a]	0.28	2.8[a]	2.1[ab]	1.8[ab]	1.3[b]	0.39
Cooked Product Yield (%)	77.8[a]	77.3[a]	78.3[a]	78.1[a]	0.66	75.7[b]	77.0[b]	78.4[ab]	80.3[a]	0.72
Steak Purge (%)[f]	2.0[a]	1.6[a]	1.6[a]	2.2[a]	0.22	2.1[a]	1.8[a]	1.6[a]	1.9[a]	0.22
TBARS[g]	0.4[c]	0.6[bc]	0.6[b]	0.9[a]	0.05	0.9[a]	0.6[b]	0.6[b]	0.4[b]	0.06

[a-c] Means having different superscripts within same rows are significantly different ($p<0.05$).

[d] Standard error of the mean (SEM).

[e] Ribeye purge = the amount of liquid lost during storage of ribeye rolls.

[f] Steak purge = the amount of liquid lost during 7 days of storage under PVC film in a retail meat case.

[g] Thiobarbituric acid reactive substances measured in mg malonaldehyde/kg sample.

ascorbate used as antioxidants in addition to the calcium chloride had significantly lower TBARS than the lamb patties from lambs infused with only the calcium chloride.

For ribeye purge, the injected Select was significantly higher ($P<0.05$) than the Average Choice control and higher but not significantly different than the Select control and the Low Choice control. The significant difference in ribeye purge for the injected Select was expected since there was an average of 9.75% solution added to the ribeye rolls. This was also seen in a study by Milligan and others (1997), where a solution of $CaCl_2$ was injected into USDA Standard beef inside rounds at 5%. They found that the purge was significantly greater for the $CaCl_2$ injected roasts than for the control roasts.

When the steak was cooked, the injected Select had significantly lower ($P<0.05$) product yield than the Average Choice control and lower but not significantly different than the Select control and the Low Choice control. This difference would be expected since an average of 9.75% solution was added to the ribeye rolls. This was also seen in the study by Milligan and others (1997). In this study, control roasts had 3.9% less cooking loss than the roasts injected with the $CaCl_2$. There was no difference seen between the treatments for steak purge (steaks overwrapped with PVC film stored in the retail meat case for 7 days.) Even though an average of 9.75% "modified marbling" solution was added to the injected Select, it is speculated that the solution formed such a strong gel that little liquid came out causing there to be no difference in uncooked steak purge. Although the injected Select was significantly lower ($P<0.05$) in cooked product yield, the differences were not large.

For TBARS values across treatments, the injected Select was significantly higher ($P<0.05$) than all the controls. This was probably due to the addition of beef tallow,

which did not contain an antioxidant. Beef tallow without antioxidant was used to keep it consistent with the controls since an antioxidant was not added to any of the controls. Cannon and others (1995) studied the effect of vitamin E on lipid oxidation. Longissimus chops from pigs given either 100 mg vitamin E/kg diet or not supplemented with vitamin E were evaluated for lipid oxidation, microbial growth, sensory characteristics, cooking/storage losses and reheating losses. The TBARS values were significantly lower for the vitamin E supplemented chops than for the control chops.

Proximate composition and pH of ribeye rolls

Table 4.2 shows proximate composition and pH values of injected and control ribeye rolls. The moisture, fat and protein content differed among storage days but there was not a consistent increase or decrease. This difference could be due to the treatment effect in which the proximate composition of the injected Select was different from the Low Choice and Average Choice controls since all ribeye rolls (injected Select, Select, Low Choice and Average Choice controls) were analyzed together on each storage day. The pH of the ribeye rolls decreased from 5.6 to 5.1 as the length of storage increased. This was most likely due to bacteria growth by spoilage organisms since the pH sample taken was on the outer surface of the ribeye roll half. This was also seen in a study by Inglis and others (2004) where a meat-based entomophage diet either with or without antibacterial agents was analyzed for spoilage microorganisms over time. It was found that the pH of diets not containing antibacterial agents decreased rapidly over time and was due to an increase in spoilage bacteria.

Table 4.2. Least square means for proximate composition and pH of injected and control ribeye rolls.

	Storage Days					Treatment				
						1	2	3	4	
Composition	**0**	**14**	**28**	**42**	**SEM[d]**	**injected Select**	**control Select**	**control Low Choice**	**control Average Choice**	**SEM[d]**
Moisture (%)	70.8^{c}	74.8^{a}	73.2^{ab}	72.7^{b}	0.53	74.7^{a}	73.9^{a}	71.8^{b}	71.1^{b}	0.55
Fat (%)	7.0^{a}	3.5^{b}	3.5^{b}	3.7^{b}	0.41	3.4^{b}	3.5^{b}	4.7^{ab}	6.0^{a}	0.45
Protein (%)	22.9^{ab}	22.1^{b}	23.4^{a}	23.8^{a}	0.30	21.9^{b}	23.6^{a}	23.6^{a}	23.1^{ab}	0.31
pH	5.6^{a}	5.6^{a}	5.2^{b}	5.1^{c}	0.03	5.4^{a}	5.4^{a}	5.4^{a}	5.4^{a}	0.03

[a-c] Means having different superscripts within same rows are significantly different ($p<0.05$).

[d] Standard error of the mean (SEM).

The moisture content of the injected Select was significantly higher ($P<0.05$) than the Low Choice and Average Choice controls and higher but not significantly different than the Select control. For fat content, the Average Choice control was significantly higher ($P<0.05$) than the injected Select and the Low Choice control was higher but not significantly than the injected Select. Savell and others (1986) also found that the amount of chemical fat in uncooked *longissimus lumborum* muscle of beef carcasses varied across degrees of marbling from 10.42% in Moderately Abundant (USDA Prime quality grade) to 1.77% in Practically Devoid (USDA Standard quality grade). For protein content, the injected Select was significantly lower ($P<0.05$) than the Select control and the Low Choice control and lower but not significantly than the Average Choice control. There was no difference in pH across treatments.

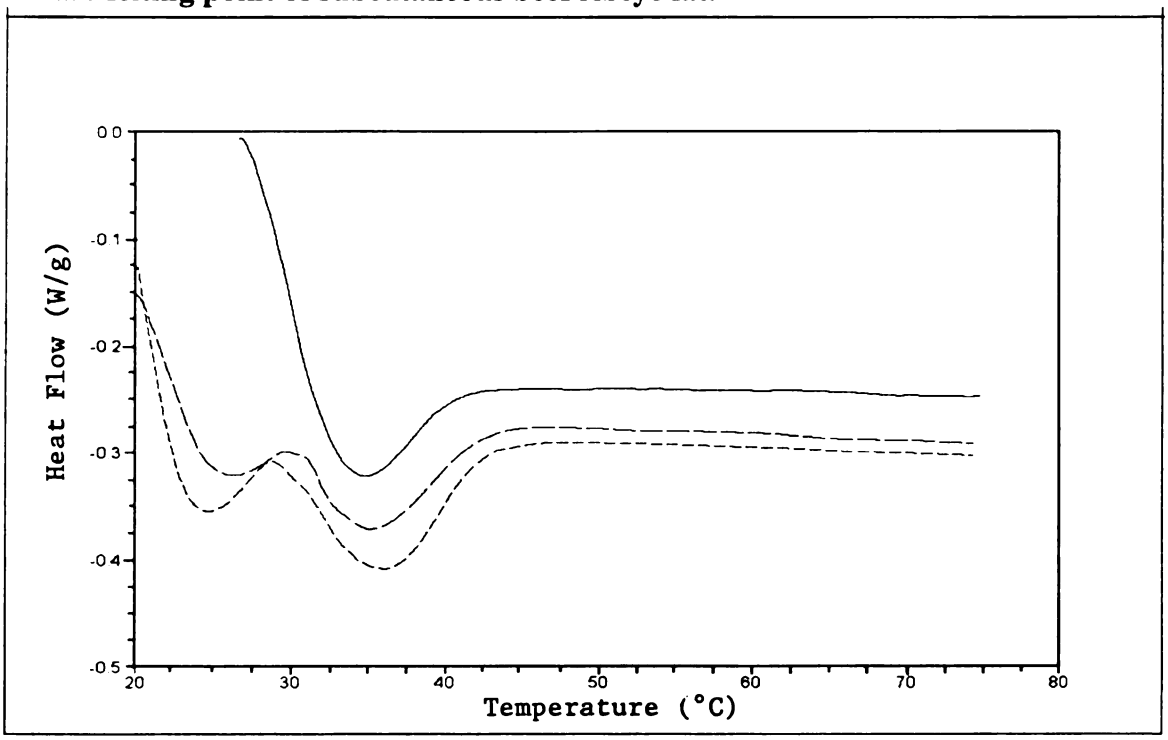
Endothermic peaks of beef ribeye fat and "modified marbling" gels

The average melting point of the three subcutaneous beef ribeye fat readings (Figure 4.1.a) was 35.6 °C, which is a little lower but close to the melting point recorded for beef tallow (40-48 °C) (Dugan 1987). It was also stated that beef fat melting point can vary depending on several conditions (breed, age sex and management style of the animal, type of fat, etc) thus the value of 35.6 °C is reasonable. The melting point temperature reported by Dugan (1987) was for beef tallow and not subcutaneous fat. Subcutaneous ribeye fat was used in this study.

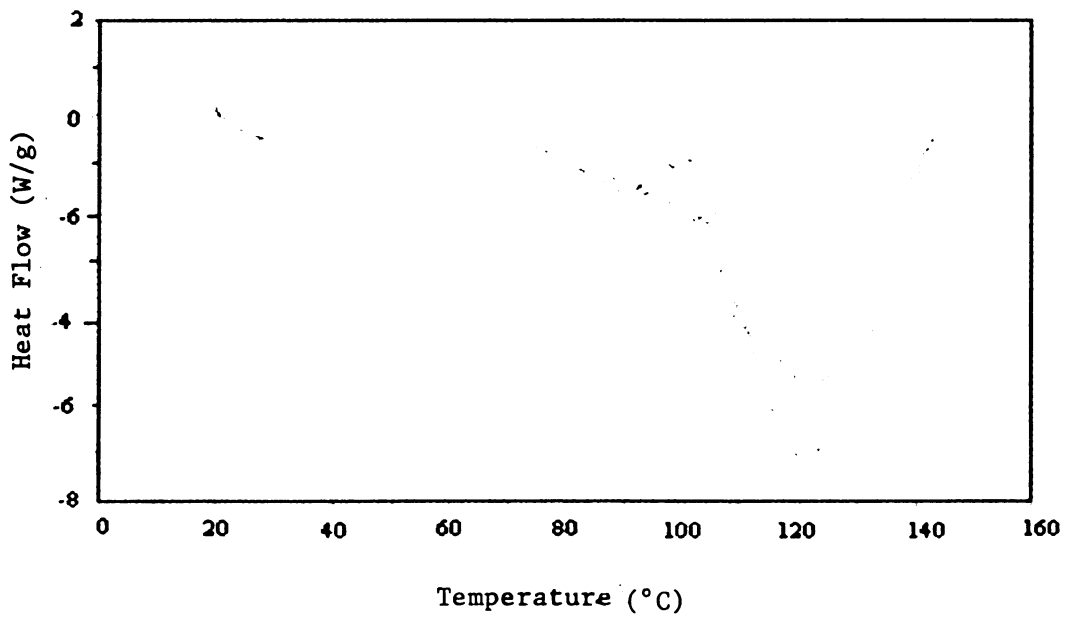
The average endothermic peak seen from the three readings for the "modified marbling" gel was 121.8 °C (Figure 4.1.b). Since the gel was approximately 90%

Figure 4.1. Endothermic peaks of beef ribeye fat and "modified marbling" gels.

4.1.a. Melting point of subcutaneous beef ribeye fat.



4.1.b. Endothermic peak of "modified marbling" gels.



moisture, it was most likely that the water in the solution vaporized at this temperature. This is a higher vaporization temperature than normally seen for water but the vaporization temperature increases when it is in a solution (Yan 2000). Since the gel did not melt and the water did not vaporize until it reached 121.8 °C, at 71 °C, the endpoint cooking temperature of a steak, the "modified marbling" was still a strong gel and this may have an effect on the sensory properties.

Objective and subjective color measurements of ribeye rolls

The objective and subjective color measurements are shown in table 4.3. Across storage days, there was no difference in L* values. The a* values for steaks became significantly ($P<0.05$) less red as the length of storage increased and the b* values became significantly ($P<0.05$) less yellow over time. It is reasonable that these changes could occur during storage. There were no differences in subjective color and marbling scores across storage days.

Across treatments, for L* value, the injected Select was higher than the Select control but this difference was not significant. This higher value of reflectance for the injected Select would probably be due to the amount of solution injected into the ribeye roll. There was no difference between treatments for a* and b* values. There was also no difference in subjective color scores across treatments. As far as marbling score, the Average Choice control and the Low Choice control were significantly higher than the injected Select and the Select control, which corresponds to the higher proximate fat content of the Average and Low Choice controls. There was no difference between the

Table 4.3. Least square means for objective and subjective color measurements of injected and control ribeye rolls.

	Storage Days					Treatment				
						1	2	3	4	
Measurement	**0**	**14**	**28**	**42**	**SEM[d]**	**injected Select**	**control Select**	**control Low Choice**	**control Average Choice**	**SEM[d]**
L*[e]	48.6[a]	47.3[a]	46.7[a]	48.3[a]	0.65	48.5[ab]	46.2[b]	47.4[ab]	48.8[a]	0.65
a*[f]	18.4[a]	18.6[a]	17.9[a]	15.1[b]	0.57	16.8[a]	18.4[a]	17.2[a]	17.5[a]	0.57
b*[g]	10.8[a]	10.7[a]	10.4[ab]	9.6[b]	0.30	10.4[a]	10.5[a]	10.1[a]	10.4[a]	0.30
Color Score[h]	2.8[a]	2.6[a]	2.7[a]	3.0[a]	0.13	2.8[a]	2.8[a]	2.8[a]	2.7[a]	0.16
Marbling Score[i]	Sl80[a]	Sl90[a]	Sl90[a]	Sl80[a]	0.91	Sl30[c]	Sl30[c]	Sl100[b]	Sm80[a]	1.00

[a-c] Means having different superscripts within same rows are significantly different ($p<0.05$).

[d] Standard error of the mean (SEM).

[e-g] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the steak using a Minolta Chromameter.

[h] Color scores were evaluated using a 7 point scale: 1=bright cherry red, 7=dark red

[i] Marbling score = evaluated using beef marbling cards adapted from the official United States standard for grades of carcass beef.

injected Select and Select control for marbling score. The "modified marbling" could not be seen in the injected Select ribeye steaks during retail display. However, during a preliminary study, where ribeye rolls were injected with the solution in order for the injector settings to be modified to acquire the marbling pattern desired, the "modified marbling" was visible. During the study though, there were problems with the injector not being designed to handle the solution viscosity so the mixing time of the solutions were decreased and used for all replicates. This may have altered the properties of the solution since the ingredients were not as thoroughly mixed as in preliminary studies and the "modified marbling" was not visible in the injected Select. This problem would easily be solvable by modifying the solution manufacturing procedures and processing parameters in order to produce visible "modified marbling". As the technology is adopted, there also may be an opportunity for injection equipment to be designed to handle higher viscosity injection solutions.

Objective and subjective color measurements and TBARS values of ribeye steaks in the retail meat case shelf-life study

The objective and subjective color measurements and TBARS values for the retail meat case shelf-life study are shown in tables 4.4 and 4.5. The standard errors of the mean (SEM) are different across retail days due to the repeated measurement design. The L* values were not significantly different throughout any of the retail days. The a* values significantly decreased or became less red ($P<0.05$) as the length of storage in the retail case increased during each storage period except for storage day 0. The b* values also significantly decreased or became less yellow ($P<0.05$) as the length of storage in the retail meat case increased. Jeremiah and Jones (1989) studied the effects of a 10 hr

Table 4.4. Least square means for objective and subjective color measurements and TBARS values over retail days for storage days 0 and 14 of injected and control ribeye steaks.

	Storage Days							
	0				14			
	Retail Days				Retail Days			
Measurement	**0**	**3**	**5**	**7**	**0**	**3**	**5**	**7**
L*[f]	48.5[a]	48.7[a]	48.6[a]	48.4[a]	46.6[c]	47.9[a]	47.3[bc]	47.5[ab]
SEM[e]	0.73	0.72	0.71	0.70	0.35	0.29	0.35	0.38
a*[g]	19.3[a]	18.5[a]	17.9[a]	17.7[a]	21.2[a]	19.1[b]	18.0[c]	16.0[d]
SEM	0.91	0.71	0.68	0.69	0.43	0.49	0.49	0.50
b*[h]	11.4[a]	10.8[b]	10.4[c]	10.5[c]	11.7[a]	10.8[b]	10.4[c]	9.7[d]
SEM	0.53	0.39	0.40	0.36	0.23	0.25	0.25	0.24
Color Score[i]	2.7[ab]	2.7[ab]	2.7[b]	2.9[a]	2.4[a]	2.6[a]	2.9[a]	2.7[a]
SEM	0.15	0.14	0.14	0.18	0.07	0.11	0.10	0.15
Marbling Score[j]	Sl100[a]	Sl80[b]	Sl80[b]	Sl80[b]	Sl90[a]	Sl90[a]	Sl90[a]	Sl90[a]
SEM	0.39	0.37	0.47	0.46	1.74	1.33	1.38	1.20
TBARS[k]	0.2[b]	-	-	0.6[a]	0.2[b]	-	-	0.9[a]
SEM	0.02			0.11	0.02			0.07

[a-d] Means having different superscripts within same rows are significantly different ($p<0.05$).

[e] Standard error of the mean (SEM).

[f-h] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the steak using a Minolta Chromameter.

[i] Color scores were evaluated using a 7 point scale: 1=bright cherry red, 7=dark red.

[j] Marbling score = evaluated using beef marbling cards adapted from the official United States standard for grades of carcass beef.

[k] Thiobarbituric acid reactive substances measured in mg malonaldehyde/kg sample.

Table 4.5. Least square means for objective and subjective color measurements and TBARS values over retail days for storage days 28 and 42 of injected and control ribeye steaks.

	Storage Day							
	28				42			
	Retail Day				Retail Day			
	0	3	5	7	0	3	5	7
L*[f]	46.3[a]	46.9[a]	46.9[a]	46.5[a]	48.3[a]	48.1[a]	48.3[a]	48.6[a]
SEM[e]	0.72	0.69	0.80	0.75	0.44	0.50	0.48	0.51
a*[g]	21.8[a]	18.2[b]	16.7[c]	14.9[d]	19.5[a]	16.1[b]	13.7[c]	11.1[d]
SEM	0.57	0.55	0.58	0.63	0.50	0.50	0.64	0.80
b*[h]	11.9[a]	10.4[b]	9.8[bc]	9.4[c]	10.9[a]	9.7[b]	9.1[c]	8.6[d]
SEM	0.35	0.30	0.26	0.28	0.28	0.25	0.26	0.22
Color Score[i]	2.3[c]	2.7[b]	2.8[ab]	3.1[a]	2.5[c]	2.8[c]	3.3[b]	3.4[a]
SEM	0.11	0.15	0.12	0.16	0.13	0.11	0.09	0.09
Marbling Score[j]	SI90[a]	SI90[a]	SI100[a]	SI80[a]	SI80[a]	SI70[a]	SI90[a]	SI90[a]
SEM	1.09	0.72	0.87	0.68	1.00	0.88	0.95	1.05
TBARS[k]	0.2[b]	-	-	1.1[a]	0.3[b]	-	-	1.5[a]
SEM	0.02			0.11	0.02			0.08

[a-d] Means having different superscripts within same rows are significantly different ($p<0.05$).

[e] Standard error of the mean (SEM).

[f-h] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the steak using a Minolta Chromameter.

[i] Color scores were evaluated using a 7 point scale: 1=bright cherry red, 7=dark red.

[j] Marbling score = evaluated using beef marbling cards adapted from the official United States standard for grades of carcass beef.

[k] Thiobarbituric acid reactive substances measured in mg malonaldehyde/kg sample.

water spray during chilling on pork carcasses. At 24 hr postmortem, the loins were removed and cut into 4 equal sized portions and vacuum packaged. One portion from each loin was randomly assigned to each storage day (0, 14, 28 and 42). Upon removal from storage, one chop was removed from the center of each portion, wrapped in oxygen permeable film and placed in a retail display case. They found that the retail display reduced the redness of all chops and reduced the yellowness of the chops stored for extended periods. This could be due to the lighting in the retail case, which may discolor the meat (Kraft and Ayres, 1954) or due to certain species of aerobic bacteria, which have been shown to discolor meat by reducing the oxygen tension to the meat surface (Robach and Costilow, 1962).

The subjective color scores significantly increased or became darker brown ($P<0.05$) with time in the retail case for each storage day except for storage day 14. The marbling scores were significantly different ($P<0.05$) across retail days for storage day 0 but there was no significant difference in the retail case for the other three storage days. The TBARS values were significantly higher ($P<0.05$) on retail day 7 than on retail day 0 for each storage day. This could be due to the treatment effect in which the injected ribeye rolls were more prone to lipid oxidation than the Low or Average Choice ribeye rolls and this effect was probably due to not using antioxidants as previously described. There was a significant ($P<0.05$) interaction between treatment and retail day for both a* and TBARS values. For a* value, this was probably a random interaction but for TBARS values, this interaction is reasonable since there was a significant ($P<0.05$) difference between retail days for each storage day and also a significant ($P<0.05$) difference between treatments.

Scanning electron microscopy (SEM)

The images from the scanning electron microscopy analysis are shown in Figure 4.2. In the "modified marbling" solution (a), the non-meat ingredients tended to interact with each other (b) in order to form a strong gel with the functional properties needed to mimic the appearance of intramuscular fat. Once clear images were seen of the "modified marbling" solution alone, the identification of the solution within the ribeye roll (injected USDA Select) was attempted and images of the USDA Select control were used to help identify the "modified marbling" solution injected in the meat. The solution tended to lie within the meat proteins (c) and did not seem to interact with the meat proteins (d). This is in agreement with the differential scanning calorimetry (DSC) analysis. It was found that the water in the gel did not vaporize until it reached 121.8 °C so the solution probably formed a strong gel. Since it was such a strong gel, it makes sense that the gel would lie within the meat proteins and not interact with them.

Figure 4.3. Scanning electron microscopy images.

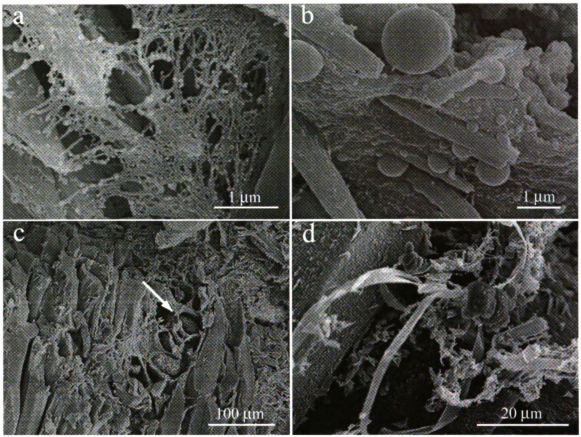


Figure 4.2. Scanning electron microscopy images. a) Image of the "modified marbling" solution and b) close-up image of the solution. c) Image of "modified marbling" solution in the ribeye roll (injected USDA Select) and d) close-up image of the solution in the ribeye roll.

Conclusions

The developed "modified marbling" solution injected into whole muscle beef cuts has potential for future applications. The injected Select had a significantly higher ($P<0.05$) ribeye purge than the Average Choice. This significant difference in ribeye purge would be expected since there was an average of 9.75% solution added to the ribeye rolls. The injected Select had the highest percent moisture, lowest percent fat and lowest cooked product yield. For TBARS values, the injected Select was significantly higher ($P<0.05$) than all the controls, which is most likely due to the use of beef tallow without antioxidants. There also were no significant differences in color scores between treatments even with the amount of "modified marbling" solution in the injected Select.

This study was designed to verify the "modified marbling" solution in whole muscle beef cuts by comparing the chemical attributes to controls. The results of this study indicate that this innovative ingredient and processing technology has the potential to improve lower quality beef cuts but more research is needed to improve the "modified marbling" properties. One possibility is that the amount of fat in the solution could be increased to achieve the benefits of flavor and hydrophobicity and to improve upon the marbling appearance of the injected whole muscle beef cuts.

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CHAPTER 5

SENSORY PROPERTIES OF WHOLE RIBEYE ROLLS INJECTED WITH THE "MODIFIED MARBLING" SOLUTION COMPARED TO NON-INJECTED CONTROLS

Abstract

Ribeye rolls (IMPS 112A) injected with the developed "modified marbling" solution (5-7% targeted pick-up) were compared to control ribeye rolls in sensory attributes. USDA Select, Low and Average Choice ribeye rolls were passed through the automatic brine injector without injecting solution (controls). Ribeye rolls were designated to 0, 14, 28, or 42 days of storage (1°C) and steaks (2.54 cm) were fabricated on each storage day. Warner-Bratzler shear force and trained sensory evaluation were conducted on fabricated steaks from each treatment and control. The injected ribeye rolls were higher ($P<0.05$) in beef fat flavor compared to the USDA Select control. However a slight off-flavor was found ($P<0.05$) in the injected ribeye rolls. There were no differences between the injected and control ribeye rolls for Warner-Bratzler shear force, sensory tenderness or juiciness. This innovative ingredient and processing technology has the potential to improve lower quality beef but more research is needed to improve the "modified marbling" properties. One possibility is that the amount of fat in the solution could be increased to achieve the benefits of both the non-meat ingredients and fat.

Keywords: intramuscular fat, "modified marbling", non-meat ingredients, solution, injectable

Introduction

Palatability (juiciness, tenderness, flavor) of beef cuts has been shown to be influenced by the amount of marbling or intramuscular fat. Tatum and others (1982) showed that marbling has a low but positive relationship on all beef palatability traits and also found that 90% of the time steaks with Slight or higher degrees of marbling were more desirable in tenderness, flavor and overall palatability. *Longissimus thoracis* steaks from USDA High Choice carcasses tended to have higher tenderness, juiciness and beef flavor intensity ratings than those from USDA Low Select carcasses (Wheeler and others 1999a).

Beef palatability is a major concern because when consumers are not satisfied with the palatability of beef cuts their intent to purchase additional beef products may decrease. The opportunity for the beef industry to generate revenue also decreases. Savell and others (1987) reported that beef packers demand beef carcasses that grade USDA Choice. When carcasses grade less than USDA Choice, a substantial price discount usually has been paid. Savell and Cross (1988) found that beef cuts containing 3-7% intramuscular fat (marbling) are perceived by consumers to be acceptable in tenderness, juiciness, flavor and overall palatability.

The deposition of intramuscular fat or marbling is influenced by many factors such as breed, length of feeding, type of ration fed and management but it has been shown that there is plenty of room for improvement in the amount of marbling or intramuscular fat in order to enhance the palatability of the final beef product. The palatability of whole muscle cuts fabricated from lower quality (less than USDA Choice) beef carcasses may be improved through innovative non-meat ingredient and processing technologies. Several different processing technologies have all ready been used to add

value to lower quality meat products including whole muscle cuts. The development of a "modified marbling" solution from selective non-meat ingredients (sodium alginate, iota carrageenan, whey protein isolate and modified food starch) that can mimic the properties of intramuscular fat and can be directly injected into lower quality whole muscle beef cuts may enhance its overall palatability by mimicking the organoleptic properties of fat and having an appearance similar to that of marbling.

The objective of this study was to verify the properties of the "modified marbling" solution in whole muscle beef cuts. To achieve this objective, USDA Select ribeye rolls were injected with the solution, cut into steaks and sensory attributes were compared to non-injected USDA Select, Low and Average Choice control ribeye steaks.

Materials and Methods

Processing Procedures

After the "modified marbling" solutions were manufactured and the ribeye rolls were injected as described in chapter 3, they were processed as described in chapter 4.

Warner-Bratzler shear force

On each storage day, steaks were evaluated for Warner-Bratzler shear force. Steaks were cooked on a Taylor clamshell grill as described in chapter 4. Steaks were stored at 4 °C for 24 hr and six 1.3 cm cores were taken parallel to the longitudinal axis of the fibers using a drill press- mounted corer. Cores were sheared perpendicular to the fibers using a Warner-Bratzler head on a TA-HD*i* Texture Analyzer (Texture Technologies Corp., Scotsdale, NY).

Sensory evaluation

Sensory attributes of ribeye steaks were determined by a trained sensory panel on each storage day. Six healthy panelists between twenty and sixty-five (four female and two male) were trained according to AMSA (1995) and Meilgaard and others (1991). All panelists had experience in sensory evaluation and were previously trained to evaluate various meat products. Before product evaluation, three training sessions were held to familiarize the panelists with the attributes and evaluation procedures. An 8 point hedonic scale was used to measure 8 sensory attributes: juiciness, muscle fiber tenderness, connective tissue, overall tenderness, off-flavor intensity, beef broth flavor intensity, beef fat flavor intensity, and mouth coating. For juiciness, 1=extremely dry and 8=extremely juicy and for muscle fiber tenderness and overall tenderness, 1=extremely

tough and 8=extremely tender. For connective tissue, 1=abundant and 8=none and for beef broth and beef fat flavor intensity, 1=extremely bland and 8=extremely intense. For off-flavor intensity and mouth coating, 1=none and 8=abundant.

Training for beef broth and beef fat flavor intensity was conducted by using beef fat and hamburgers made from ground beef with different percentages of fat (80/20, 85/15, and 90/10) and mouth coating was established by using set references (corn starch=2, ground potato=4 and toothpaste=6, Meilgaard and others, 1991). Juiciness, muscle fiber tenderness, overall tenderness, connective tissue, and off-flavor intensity were well established attributes evaluated on a regular basis for whole muscle meat products using the same trained sensory panel.

Sensory evaluations were conducted in a climate controlled sensory evaluation room with partitioned booths and incandescent lights. The order of sample preparation was randomized within each session to minimize positional bias and a 3 digit random code was used to label the samples. Steaks were cooked on a Taylor clamshell grill as described in chapter 4 and sample preparation included cutting 1.3 cm cubes from the center portion of each steak and two cubes were placed in 2 oz. Soufflé cups and covered with a lid. Soufflé cups were placed in a 2 quart Pyrex® bowl with a lid and the bowl was covered with warm towels to insulate the bowl and keep the samples warm. The insulated bowl was placed in a cooler and transported to the sensory evaluation room. Each sample was served to the panelists in their booths. Expectorant cups were provided to prevent taste fatigue and distilled, de-ionized water, unsalted soda crackers and apple juice were used to clean the palate between samples. The panelists were standardized each day by evaluating a warm-up sample and discussing the results. Sixteen samples

were evaluated on each day and the day was divided into two sessions with a 15 min break between each session.

Cooking study

A cooking study was conducted on ribeye steaks and attributes were evaluated by a sensory panel in order to determine whether the lack of differences seen between the injected and control ribeye steaks for juiciness and tenderness was due to the cookery method and end-point temperature used. Four USDA Select ribeye rolls (112A) were purchased from a local meat company (Popoff Quality Food Service, Lansing, MI). The ribeye rolls were cut in half and two 2.5 cm steaks were cut from the middle (opposite of the shoulder and loin end) of each half. The four steaks from each ribeye roll were randomized to four treatments: clamshell grill (71 °C), clamshell grill (77 °C), Farberware® grill (Model 455ND, Kidde Inc., Bronx, NY) (71 °C), and Farberware® grill (77 °C). The steaks cooked on the clamshell grill were done using the procedure described in chapter 4. The steaks cooked on the Farberware® grill (104.7 °C surface temperature) were laid on the surface of the grill and the temperature of the steak was monitored using a copper constantan thermocouple (0.051-cm diameter, 15.2 cm length; Omega Engineering Inc., Stamford, CT) inserted into the geometric center of the steak. The steaks were cooked to 40 °C and then turned and cooked to the final desired temperature.

Sample preparation was conducted in the same manner as previously described. Juiciness, muscle fiber tenderness, overall tenderness, and connective tissue were evaluated using the same scale and testing procedures. The methods of cookery and

endpoint temperatures used were chosen since they resembled consumer's choice of cookery and desired endpoint temperatures used in their homes.

Experimental design

The experimental design used was a split plot design with treatment as the whole plot factor and storage day as the split plot factor. The effect of injecting the "modified marbling" solution into ribeye rolls on quality attributes (shear force and sensory attributes) of the ribeye steaks was analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS User's Guide, Version 8.2, Cary, NC: SAS Institute, Inc., 2002). Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure.

Results and Discussion

Warner-Bratzler shear force and sensory attribute values of ribeye rolls

The Warner-Bratzler shear force and sensory attribute values for the ribeye rolls are shown in Table 5.1. The Warner-Bratzler shear force values did not differ across storage days. The juiciness values decreased from 5.3 to 4.9 and the overall tenderness values decreased from 5.8 to 5.7 as the length of storage increased but the decreases were small and not significantly different. The juiciness values were similar to the percent ribeye purge presented in chapter 4, which also decreased as the length of storage increased so since there was not as much liquid within the ribeye rolls, the perceived juiciness was lower. There were not any differences in muscle fiber tenderness and the amount of connective tissue over storage days.

The off-flavor intensity became significantly ($P<0.05$) higher from 1.1 to 1.3 as the length of storage increased (Table 5.1). However, these differences in values were small. This corresponded to the TBARS values (chapter 4) during storage time, which could be due to the treatment effect in which the injected ribeye rolls were more prone to lipid oxidation than the Low or Average Choice ribeye rolls and this effect was probably due to not using antioxidants in the injected Select treatment. Because the differences were small, these results may occur during storage at 1 °C in a vacuum package. There was no difference in mouth coating across storage days. The beef flavor intensity decreased from 4.3 to 3.9 and beef fat flavor intensity also decreased from 3.8 to 3.5 as the length of storage increased but the decrease was not significantly different and the values were again small in scale. The beef flavor added to the solution may have decreased in intensity over time (42 days).

Table 5.1. Least square means for Warner-Bratzler shear force and sensory attribute values of injected and control ribeye rolls.

	Storage Days					Treatment				
						1	2	3	4	
Attribute[c]	**0**	**14**	**28**	**42**	**SEM**[d]	**injected Select**	**control Select**	**control Low Choice**	**control Average Choice**	**SEM**[d]
Warner-Bratzler Shear Force (kg)	3.3^{a}	2.8^{a}	3.0^{a}	3.1^{a}	0.16	3.1^{a}	3.3^{a}	2.7^{a}	3.1^{a}	0.16
Juiciness	5.3^{ab}	5.5^{a}	5.4^{a}	4.9^{b}	0.23	5.3^{a}	5.2^{a}	5.3^{a}	5.4^{a}	0.23
Muscle Fiber Tenderness	5.8^{a}	6.1^{a}	5.9^{a}	5.6^{a}	0.17	5.9^{a}	5.7^{a}	6.0^{a}	5.7^{a}	0.17
Connective Tissue	6.4^{a}	6.5^{a}	6.3^{a}	6.4^{a}	0.11	6.4^{a}	6.5^{a}	6.5^{a}	6.2^{a}	0.11
Overall Tenderness	5.8^{ab}	6.1^{a}	6.0^{ab}	5.7^{b}	0.14	5.9^{a}	5.8^{a}	6.0^{a}	5.8^{a}	0.14
Beef Flavor	4.3^{a}	4.2^{ab}	3.9^{b}	3.9^{ab}	0.14	4.2^{ab}	4.0^{ab}	3.9^{b}	4.3^{a}	0.14
Beef Fat Flavor	3.8^{ab}	3.7^{ab}	3.9^{a}	3.5^{b}	0.18	4.0^{a}	3.3^{b}	3.7^{a}	3.8^{a}	0.18
Off-flavor	1.1^{b}	1.2^{ab}	1.2^{ab}	1.3^{a}	0.09	1.4^{a}	1.2^{b}	1.1^{b}	1.1^{b}	0.09
Mouth coating	3.3^{a}	3.0^{a}	2.9^{a}	3.1^{a}	0.28	3.3^{a}	2.9^{a}	3.0^{a}	3.1^{a}	0.27

[a-b] Means having different superscripts within same rows are significantly different ($p<0.05$).

[c] Sensory attributes were evaluated using 8 point hedonic scale.
1=extremely dry/tough/abundant connective tissue/bland beef and beef fat flavor/non-off-flavor, mouth coating.
8=extremely juicy/tender/no connective tissue/intense beef and beef fat flavor/abundant off-flavor, mouth coating.

[d] Standard error of the mean (SEM).

Across treatments, there were no differences in Warner-Bratzler shear force, or juiciness, muscle fiber tenderness, amount of connective tissue and overall tenderness measured by sensory analysis. The "modified marbling" had neither a positive or negative effect on these attributes. The lack of change across treatments for tenderness values may have been due to the method of cookery and the end-point temperature used. The clamshell grill cooking method may be less abrasive on the steaks than open grill cookery methods, which most consumers use in their home. The end-point temperature of 71 °C did not show a difference but if a higher end-point temperature (77 °C) would have been used, perhaps a difference may have been detected. This temperature end-point may be more what the consumers would choose when cooking steaks in their homes. Also, the lack of difference for tenderness across treatments could have been attributed to passing all control ribeye rolls through the injector (single pass without solution). Even though the controls were passed through the injector to minimize bias when evaluating tenderness, this could have had a contrary effect and caused the tenderness values of the injected and control ribeye rolls to be similar. It was reported in chapter 3 that the injector needles did not affect tenderness. However, the level of significance for this conclusion was P=0.062.

The lack of difference seen in juiciness values was probably due to the ability of the solution to form a strong gel so that little liquid was released. The solution did not actually melt when the steak was cooked. Analysis using differential scanning calorimetry (DSC) found that the water in the solution vaporized and not until it reached 121.2 °C (chapter 4), which is far beyond the endpoint cooking temperature (71 °C) used when cooking steaks. When the steaks were cooked, the solution did not melt and release

water but probably held together as a strong gel. This is a possibility of why there was no difference seen in juiciness between the injected and control ribeye steaks.

There also was no difference among treatments for mouth coating. The "modified marbling" did not affect the mouth coating attribute. For beef flavor intensity, the injected Select was higher but not significantly different than the Select control and the Low Choice control. For the beef fat flavor intensity, the injected Select was significantly higher ($P<0.05$) than the Select control and higher but not significantly different than the Low Choice and Average Choice controls. Thus the addition of the "modified marbling" solution did not have an unfavorable effect on these attributes but even had a positive effect on the beef fat flavor intensity. This was probably due to the addition of beef flavor to the solution. The injected Select was also significantly ($P<0.05$) higher than the controls in off-flavor intensity, which corresponds to the TBARS values (chapter 4) and was probably due to not adding antioxidants to the "modified marbling" solution.

Cooking study of ribeye steaks

To further verify whether the lack of differences seen among treatments for juiciness and sensory and Warner-Bratzler shear force tenderness values was due to the cookery method and end-point temperature used, a cooking study was conducted on USDA Select ribeye rolls. This possibility was stated when discussing the absence of tenderness change previously observed. There were no differences seen for juiciness, muscle fiber tenderness, amount of connective tissue or overall tenderness between the

Table 5.2. Least square means for sensory attribute values of USDA Select ribeye steaks cooked by different cooking methods to different endpoint temperatures.

	Treatment				
	1	2	3	4	
	Clamshell Grill	Farberware® Grill	71° C	77° C	
Attribute[c]					SEM[d]
Juiciness	5.1[a]	5.7[a]	5.6[a]	5.2[a]	0.29
Muscle Fiber Tenderness	5.6[a]	5.8[a]	5.9[a]	5.6[a]	0.24
Connective Tissue	6.3[a]	6.5[a]	6.5[a]	6.3[a]	0.16
Overall Tenderness	5.7[a]	5.9[a]	6.0[a]	5.6[a]	0.20

[a-b] Means having different superscripts within same rows are significantly different ($p<0.05$).

[c] Sensory attributes were evaluated using 8 point hedonic scale.
1=extremely dry/tough/abundant connective tissue/bland beef and beef fat flavor/non-off-flavor, mouth coating.
8=extremely juicy/tender/no connective tissue/intense beef and beef fat flavor/abundant off-flavor, mouth coating.

[d] Standard error of the mean (SEM).

two cookery methods (clamshell grill and Farberware® grill) or the two end-point temperatures (71 °C and 77 °C) (Table 5.2).

The sensory scores for all four attributes were lower but not significantly different at 77 °C (well done) compared to 71 °C (medium). This observation was also made by Wulf and others (1996). They studied the effects of animal age, marbling score, calpastatin activity, subprimal cut, calcium injection and degree of doneness on the palatability of steaks from Limousin steers. They found that the degree of doneness had a significant effect ($P<0.05$) on taste panel tenderness and juiciness scores. The steaks were less tender and juicy as the degree of doneness increased. It also has been shown that palatability of meat cooked by dry methods is influenced more by temperature than by marbling. Cooking losses are increased as end-point temperature increases and the greater cooking losses decrease meat juiciness. High cooking losses along with protein hardening and toughening (induced by high cooking temperatured (72-74 °C)) reduces meat tenderness (Aberle and others 2001).

The sensory scores for all four attributes were lower for the clamshell grill than the Farberware® grill but not significantly. This showed that there was not a protective effect with the clamshell grill as previously stated. The clamshell grill may still have been less abrasive to some extent but the steaks grilled on the Farberware® grill, probably had higher flavor intensity than the steaks grilled on the clamshell grill. This is most likely due to the open grilling and the stronger flavor intensity may also lead to the higher juiciness scores.

Conclusions

The "modified marbling" solution injected into whole muscle beef cuts has potential for future applications. The injected Select ribeye rolls were higher in beef fat flavor compared to the USDA Select control, which was probably due to the addition of beef flavor. The "modified marbling" addition had no effect on other traits. There was however, a slight off-flavor found in the injected Select ribeyes rolls, which is most likely due to the use of beef tallow without antioxidants. There was no significant difference between the injected and control ribeye rolls in Warner-Bratzler shear force, sensory tenderness or sensory juiciness. The similar tenderness values may be attributed to passing all the control ribeye rolls through the injector (single pass without solution) to minimize bias when evaluating tenderness. Similar juiciness values may be the result of the ability of the solution to form a strong gel so that little liquid was released.

This study was designed to verify the "modified marbling" solution in whole muscle beef cuts by comparing the sensory attributes to controls. The results of this study indicate that even though there is potential for the "modified marbling" solution to improve low quality whole muscle beef cuts additional research is needed. One possibility is to increase the amount and type of fat used in the solution to achieve the benefits of flavor, hydrophobicity as well as to improve upon the tenderness and juiciness of the injected whole muscle beef cuts.

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Recommendations for future research

The main goal of this study was to develop a "modified marbling" solution from non-meat ingredients that mimicked the function and appearance of intramuscular fat in order to provide a fat substitute for whole muscle beef cuts. In study 1, non-meat ingredients were used for the "modified marbling" solution since it was thought that they would provide easy mixing and injecting of the solution and in addition give a health benefit to the consumer. Due to encountered problems of muscle pigments being absorbed into the hydrophilic "modified marbling" solution, a small amount (3.0%) of fat (beef tallow) was added in study 2. Future research should optimize the amount of fat used in the solution to achieve the benefits of flavor and hydrophobicity and to hopefully improve upon the tenderness, juiciness and marbling appearance of the injected whole muscle beef cuts.

Continued research is being conducted in this area by focusing on developing the "modified marbling" solutions from different combinations of lipids. The mixing and injecting techniques used are similar to those utilized in this study and due to the choice of lipids, the solution is liquid enough to inject and solidifies once in the meat. This research is looking at using only lipids to develop the "modified marbling" solution but there may be possibilities to utilize both lipids and the non-meat ingredients used in this study to gain the benefits of both. Future research is needed to determine the percentages of lipids and non-meat ingredients to use to obtain the optimal solution in both function and appearance.

When developing the solution, all functional properties should be looked at before incorporating it into the meat. In study 3 it was determined that the solution did not melt

but that the water in the solution vaporized. The water did not vaporize until it reached 121.2 °C, which is far beyond the endpoint cooking temperature of steaks (71 °C) and is one possibility of why there was no difference seen in juiciness between the injected and control ribeye steaks. At the endpoint cooking temperature of the steaks injected with the solution, the "modified marbling' solution was probably still a strong gel and not in liquid form which should have increased the juiciness.

The results from study 4 indicate that even though the sensory tenderness, Warner-Bratzler shear force and sensory juiciness of whole muscle beef cuts injected with the "modified marbling" solution developed from non-meat ingredients (SA, IC, WPI and MFS) were not significantly improved from the controls, there is potential for this solution. The fact that the "modified marbling" solution was significantly higher in beef fat flavor as measured by a trained sensory panel (probably attributed to the addition of beef flavoring) gives the solution promise. Also, the similar tenderness and juiciness values when comparing the injected and non-injected ribeyes may be attributed to passing all the control ribeye rolls through the injector (single pass without solution) to minimize bias when evaluating tenderness. The fact that there was just "no difference" found between the injected ribeye rolls and the controls and not a significant inferior effect gives potential to the solution.

Another opportunity for future research includes using the "modified marbling" solution as a carrier for beneficial ingredients. Possibilities include anti-microbials, antioxidants, and different marinades/flavors. These ingredients could be mixed into the solution and injected into meat cuts. This study was a new product development project

and from this project several technologies and applications were discovered, which provided a strong beginning to this area of research.

APPENDICES

Appendix 1: Ingredients evaluated for "modified marbling" solution

Ingredient	Observation
Modified food starch (dry blend)	Very thin solution, completely separated out after gelling
Konjac	Tan color
Soy protein isolate	Tan color, strong aroma
Methylcellulose	Gels when solution is heated not cooled
Kappa Carrageenan	Dark tan color, thin gel
Iota Carrageenan	**Light tan color, thick gel**
Modified pre-gelatinized food starch	**White color**
Whey Protein Isolate	**Translucent color, disperses well in solution**
Sodium Alginate	**Light tan color, thick gel**

Appendix 2: Bench top "modified marbling" solution formulation

Day 1

Treatment	1	2	3	4	5	6	7	8	9	10
Sodium Tripolyphosphate (g)	0.16	0.16	0.16	0.16	0.22	0.22	0.22	0.22	0.19	0.19
Sodium Alginate (g)	1.56	1.56	1.56	1.56	2.19	2.19	2.19	2.19	1.88	1.88
Vegetable oil (g)	3.12	3.12	3.12	3.12	4.38	4.38	4.38	4.38	3.76	3.76
Iota Carrageenan (g)	1.56	1.56	2.19	2.19	1.56	1.56	2.19	2.19	1.88	1.88
Whey Protein Isolate (g)	1.56	2.19	1.56	2.19	1.56	2.19	1.56	2.19	1.88	1.88
Modified Food Starch (g)	2.19	1.56	1.56	2.19	1.56	2.19	2.19	1.56	1.88	1.88
Calcium Sulfate (g)	3.12	3.12	3.12	3.12	4.38	4.38	4.38	4.38	3.76	3.76
Water for Calcium Sulfate solution (g)	15.60	15.60	15.60	15.60	21.90	21.90	21.90	21.90	18.80	18.80
Water (g)	471.13	471.13	471.13	469.87	462.25	460.99	460.99	460.99	465.97	465.97
Total (g)	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00

Day 2

Treatment	11	12	13	14	15	16	17	18	19	20
Sodium Tripolyphosphate (g)	0.16	0.16	0.16	0.16	0.22	0.22	0.22	0.22	0.19	0.19
Sodium Alginate (g)	1.56	1.56	1.56	1.56	2.19	2.19	2.19	2.19	1.88	1.88
Vegetable oil (g)	3.12	3.12	3.12	3.12	4.38	4.38	4.38	4.38	3.76	3.76
Iota Carrageenan (g)	1.56	1.56	2.19	2.19	1.56	1.56	2.19	2.19	1.88	1.88
Whey Protein Isolate (g)	1.56	2.19	1.56	2.19	1.56	2.19	1.56	2.19	1.88	1.88
Modified Food Starch (g)	1.56	2.19	2.19	1.56	2.19	1.56	1.56	2.19	1.88	1.88
Calcium Sulfate (g)	3.12	3.12	3.12	3.12	4.38	4.38	4.38	4.38	3.76	3.76
Water for Calcium Sulfate solution (g)	15.60	15.60	15.60	15.60	21.90	21.90	21.90	21.90	18.80	18.80
Water (g)	471.76	470.50	470.50	470.50	461.62	461.62	461.62	460.36	465.97	465.97
Total (g)	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00

Day 3

Treatment	21	22	23	24	25	26	27	28	29	30
Sodium Tripolyphosphate (g)	0.13	0.25	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Sodium Alginate (g)	1.25	2.50	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88
Vegetable oil (g)	2.50	5.00	3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76
Iota Carrageenan (g)	1.88	1.88	1.25	2.50	1.88	1.88	1.88	1.88	1.88	1.88
Whey Protein Isolate (g)	1.88	1.88	1.88	1.88	1.25	2.50	1.88	1.88	1.88	1.88
Modified Food Starch (g)	1.88	1.88	1.88	1.88	1.88	1.88	1.25	2.50	1.88	1.88
Calcium Sulfate (g)	2.50	5.00	3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76
Water for Calcium Sulfate solution (g)	12.50	25.00	18.80	18.80	18.80	18.80	18.80	18.80	18.80	18.80
Water (g)	475.48	456.61	466.60	465.35	466.60	465.35	466.60	465.35	465.97	465.97
Total (g)	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00

Appendix 3: Bench top "modified marbling" solution manufacturing procedures

1. Add the appropriate amount of water (22 °C) to 946.4-ml lidded glass jars.
2. Add sodium tripolyphosphate.
3. Mix with 4-blade mixing head: 2-blades perpendicular to the shaft and 2-blades parallel to the shaft attached to a drill (Model 6220, S-B Power Tool Co., Chicago, IL). Mix for two min.
4. Add the sodium alginate mixed with the vegetable oil for hydration and mix for 2 min.
5. Add the iota carrageenan and mix for 2 min.
6. Add whey protein isolate and mix for 2 min.
7. Add modified food starch and mix for 2 min.
8. Mix calcium sulfate with water, add to the mixture and mix for 2 min.
9. Repeat steps for each solution.

Appendix 4: Viscosity determination

Viscometer: Brookfield viscometer (Model HBTD, Brookfield Engineering Laboratories, Inc., Stoughton, MA)

1. Turn water bath on and turn knob to desired temperature reading (30 °C).
2. Turn power button of Brookfield viscometer to on.
3. Turn speed dial on the side of the viscometer to the desired speed (100).
4. Fill the metal tube with 18.6-g of solution.
5. Insert the tube into the viscometer from the bottom and then twist to lock the tube into place.
6. Plug the cord of the tube into the temperature recorder.
7. Insert the desired spindle into viscometer by setting into the tube of solution and then twisting into place.
8. Make sure the viscometer reads 0.0. If it does not, then turn the zero knob until it does.
9. When the temperature recorder reads the desired temperature, turn the motor button to on.
10. Wait for the viscosity reading to become constant and record reading.
11. Turn off motor button.
12. Unscrew spindle and take out metal tube.
13. Clean out metal tube.
14. Repeat as necessary.

Appendix 5: Objective color measurements (CIE L^*, a^* and b^* values)

Color meter: Minolta Chromameter CR-310 (Commission D'Edairerage (CIE) L*a*b*, Ramsey, NJ)

Calibration:

1. Turn power switch to on.
2. Press Calibrate.
3. If the displayed color space in not Yxy, press Color Space Select repeatedly to change to Yxy color space.
4. Check that indexes are set as desired by pressing Index Set and use the scroll key to advance through the indexes.
5. Use the arrow keys and Y/N to change settings is necessary.
6. Set the "Multi Cal" index to "N".
7. Set the calibration channel to 00.
8. Set the "Multi Measure" to "Y".
9. Place the tip of the measuring head flat against the surface of the white calibration plate.
10. Press the measuring head's measuring button.
11. After 5 s, "CAL" in the display will be replaced by "End".
12. Calibration is now completed.

Sample measurement:

1. Press Color Space Select to set desired color space (L*, a*, b*).
2. Place tip of measuring head flat against the specimen surface.
3. Press the measuring head's measuring button and measured data will be displayed.
4. Use Kimwipes to clean measuring orifice between measurements.

Appendix 6: Water-holding capacity determination

1. Weigh a 50-ml polycarbonate tube.
2. After the solution has gelled for 24 h at 4 °C, remove the gel from the jar by cutting around the edge of the jar.
3. Cut the gel into small pieces.
4. Place approximately 10-g of sample into polycarbonate tube and record weight.
5. Place tubes in appropriate centrifuge rotor and place in centrifuge.
6. Centrifuge at 4 °C at 40,000 *x g* for 30 min.
7. Remove tubes from centrifuge.
8. Pour off supernate.
9. Weigh tube and gel.
10. Subtract weight of tube in order to determine the weight of the centrifuged gel.
11. Water-holding capacity is determined by the following formula:

$$\text{Water-holding capacity} = \frac{\text{weight of gel after centrifugation}}{\text{weight of gel before centrifugation}} \times 100$$

Appendix 7: Water-holding capacity over time determination

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1. Lay a piece of filter paper inside the bottom of a petri dish and weigh the filter paper and petri dish together.

2. After the solution has gelled for 24 h at 4 °C, remove the gel from the jar by cutting around the edge of the jar.

3. Cut the gel into approximately 2.5 x 2.5 x 1.3-cm pieces.

4. Place the sample cube on the filter paper and weigh the petri dish, filter paper and gel cube.

5. Cover with the petri dish top.

6. Store at 22 °C for 2 h.

7. Remove the cube from the filter paper by scraping away all gel particles.

8. Weigh the filter paper and petri dish.

9. Water-holding capacity over time is determined by the following formula:

$$\text{Water-holding capacity over time} = \frac{\text{weight of gel after storage at 22 °C for 2 hr}}{\text{weight of gel before exposure to elevated temp}} \times 100$$

Appendix 8: TA-HD*i* gel strength settings

Texture Analyzer:	TA-HD*i* Texture Analyzer Texture Technologies Corporation, Scarsdale, NY
Software:	Texture Expert Version: 1.22

TA-HD*i* Settings:

Test Mode:	Measure Force in Compression
Option:	Return to Start
Pre-Test Speed:	5.0-mm/s
Test Speed:	1.7-mm/s
Post-Test Speed:	10-mm/s
Pre-Travel Distance:	51.0-mm
Compression Distance:	12-mm
Trigger Type:	Return
Data Acquisition Rate:	200 pps
Attachment/Accessory:	TA-10; 13-mm AOAC acrylic cylinder, 35-mm tall
	5-kg load cell
	TA-90; Heavy duty platform

Appendix 9: Ingredient combinations of non-meat ingredients using central composite design

Treatment	Non-meat Ingredients[a]			
	Sodium Alginate	Iota Carrageenan	Whey Protein Isolate	Modified Food Starch
1	0.3125[a]	0.3125	0.3125	0.4375
2	0.3125	0.3125	0.4375	0.3125
3	0.3125	0.4375	0.3125	0.3125
4	0.3025	0.4375	0.4375	0.4375
5	0.4375	0.3125	0.3125	0.3125
6	0.4375	0.3125	0.4375	0.4375
7	0.4375	0.4375	0.3125	0.4375
8	0.4375	0.4375	0.4375	0.3125
9	0.3125	0.3125	0.3125	0.3125
10	0.3125	0.3125	0.4375	0.4375
11	0.3125	0.4375	0.3125	0.4375
12	0.3125	0.4375	0.4375	0.3125
13	0.4375	0.3125	0.3125	0.4375
14	0.4375	0.3125	0.4375	0.3125
15	0.4375	0.4375	0.3125	0.3125
16	0.4375	0.4375	0.4375	0.4375
17	0.2500	0.3750	0.3750	0.3750
18	0.5000	0.3750	0.3750	0.3750
19	0.3750	0.2500	0.3750	0.3750
20	0.3750	0.5000	0.3750	0.3750
21	0.3750	0.3750	0.2500	0.3750
22	0.3750	0.3750	0.5000	0.3750
23	0.3750	0.3750	0.3750	0.2500
24	0.3750	0.3750	0.3750	0.5000
25	0.3750	0.3750	0.3750	0.3750

[a] = all ingredient values indicate percentage of ingredient used in formulation.

Appendix 10: Least square means for viscosity, pH, objective color, water-holding capacity, water-holding capacity over time and gel strength/hardness of the "modified marbling" solutions and gels.

	Non-meat Ingredients[a]				Measurements							
Treatment	SA	IC	WPI	MFS	Viscosity[b]	pH[c]	L*[d]	a*[e]	b*[f]	WHC[g]	WHCT[h]	Gel strength[i]
1	0.3125	0.3125	0.3125	0.4375	0.7	6.25	71.97	-4.54	3.75	97.0	90.4	20.4
2	0.3125	0.3125	0.4375	0.3125	0.5	6.38	75.50	-4.63	3.99	98.8	91.7	17.9
3	0.3125	0.4375	0.3125	0.3125	0.5	6.47	71.49	-4.58	4.29	99.6	92.2	23.3
4	0.3025	0.4375	0.4375	0.4375	0.7	6.33	74.89	-4.79	6.64	99.7	93.4	24.5
5	0.4375	0.3125	0.3125	0.3125	0.8	6.54	77.83	-4.68	7.96	94.3	92.3	27.5
6	0.4375	0.3125	0.4375	0.4375	0.7	6.49	75.54	-4.64	7.55	96.0	92.8	10.4
7	0.4375	0.4375	0.3125	0.4375	0.9	6.60	80.50	-4.76	8.34	99.7	93.6	14.5
8	0.4375	0.4375	0.4375	0.3125	0.9	6.65	78.27	-4.71	8.47	99.9	94.2	15.1
9	0.3125	0.3125	0.3125	0.3125	0.4	6.49	76.91	-4.93	6.54	99.6	92.3	18.6
10	0.3125	0.3125	0.4375	0.4375	0.4	6.45	73.24	-4.86	6.23	99.6	91.6	18.1
11	0.3125	0.4375	0.3125	0.4375	0.5	6.49	77.26	-5.06	7.05	99.8	94.8	23.5
12	0.3125	0.4375	0.4375	0.3125	0.5	6.44	80.21	-5.01	7.23	99.7	94.3	23.2

[a] Non-meat ingredients: SA = sodium alginate, IC = iota carrageenan, WPI = whey protein isolate, MFS = modified food starch.

[b] Solution viscosity analyzed at 30 °C.

[c] Solution pH measured at 22 °C.

[d-f] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the gel using a Minolto Chromameter.

[g] Water-holding capacity of the gel at 4 °C, centrifuged for 30 min at 40,000 *x g*.

[h] Water-holding capacity over time of the gel after 2 hr at 22 °C.

[i] Gel hardness of gelled solutions measured using a 5-kg load cell and a 1.25 cm diameter acrylic probe.

	Non-meat Ingredients[a]				Measurements							
Treatment	SA	IC	WPI	MFS	Viscosity[b]	pH[c]	L*[d]	a*[e]	b*[f]	WHC[g]	WHCT[h]	Gel strength[i]
13	0.4375	0.3125	0.3125	0.4375	0.8	6.57	78.88	-4.87	8.16	95.6	93.9	29.1
14	0.4375	0.3125	0.4375	0.3125	0.8	6.55	76.74	-4.72	8.15	94.1	95.3	28.6
15	0.4375	0.4375	0.3125	0.3125	0.8	6.60	77.12	-4.71	8.23	99.7	93.9	31.1
16	0.4375	0.4375	0.4375	0.4375	0.8	6.54	79.01	-4.80	8.64	99.9	93.8	32.9
17	0.2500	0.3750	0.3750	0.3750	0.5	6.34	74.64	-4.97	5.68	99.7	92.1	19.3
18	0.5000	0.3750	0.3750	0.3750	1.1	6.57	81.29	-5.00	9.37	94.9	94.2	40.3
19	0.3750	0.2500	0.3750	0.3750	0.6	6.55	76.61	-4.85	7.01	78.1	92.2	20.6
20	0.3750	0.5000	0.3750	0.3750	0.8	6.47	77.36	-4.79	8.06	99.2	93.0	31.2
21	0.3750	0.3750	0.2500	0.3750	0.6	6.56	77.02	-4.79	7.34	99.9	93.0	25.3
22	0.3750	0.3750	0.5000	0.3750	0.5	6.47	80.74	-5.13	7.65	99.2	93.4	22.5
23	0.3750	0.3750	0.3750	0.2500	0.6	6.52	78.06	-4.98	7.66	99.3	93.6	23.7
24	0.3750	0.3750	0.3750	0.5000	0.6	6.49	79.34	-5.06	7.43	99.0	92.4	22.8
SEM[j]					0.1	0.03	0.80	0.07	0.11	0.6	0.6	1.8
25	0.3750	0.3750	0.3750	0.3750	0.6	6.52	77.50	-4.78	7.42	99.7	92.8	18.9
SEM[k]					0.0	0.01	0.33	0.03	0.05	0.2	0.2	0.7

[a] Non-meat ingredients: SA = sodium alginate, IC = iota carrageenan, WPI = whey protein isolate, MFS = modified food starch.

[b] Solution viscosity analyzed at 30 °C.

[c] Solution pH measured at 22 °C.

[d-f] Color (Commission International De L'Eclairage) (CIE); reflectance (L*), redness (a*), yellowness (b*) on the surface of the gel using a Minolto Chromameter.

[g] Water-holding capacity of the gel at 4 °C, centrifuged for 30 min at 40,000 *x g*.

[h] Water-holding capacity over time of the gel after 2 hr at 22 °C.

[i] Gel hardness of gelled solutions measured using a 5-kg load cell and a 1.25 cm diameter acrylic probe.

[j] SEM = Standard error of the mean for treatment combinations 1-24.

[k] SEM = Standard error of the mean for replicated treatment combination.

Appendix 11: Commercial batch "modified marbling" solution formulation

Ingredient	%	g	lb
Sodium alginate	1.0	681.0	1.5
Sodium Tripolyphosphate		68.1	
Vegetable oil		1362	3.0
Calcium Sulfate		1362	3.0
Water		6810	15.0
Iota carrageenan	0.4375	297.9	
Whey Protein Isolate	1.5	1021.5	2.25
Modified Food Starch	0.375	255.3	
Beef Tallow	3.0	2043	4.5
Beef Flavor	0.25	170.3	
Water		54028.9	119.0/59.5
Total		**68100**	**150**

Appendix 12: Commercial batch "modified marbling" solution manufacturing procedures

Temperature of water = approx. 18°C
Temperature of beef tallow = approx. 7.0°C

1. Add tripolyphosphate to half the water and mix for 2 min at 1500 rpm using a Rotostat mixer (Model 80XP63SS, Admix Inc., Londonderry, NH).

2. Add whey protein isolate and beef tallow and continue mixing at 1500 rpm.

3. Add sodium alginate hydrated in the vegetable oil.

4. Add the other half of the water and increase the speed to 2000 rpm.

5. Add the beef flavor, then the iota carrageenan and the modified food starch.

6. Mix the calcium sulfate in the water and homogenize to eliminate large particles, add to the solution and increase speed to 3500 rpm.

7. Raise mixer head.

8. Total mixing time - 8 min

Appendix 13: Randomization of USDA Select ribeye rolls

Replicate 1

Day 0		Day 14		Day 28		Day 42	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
inj S[a]	cont S[b]	cont S	inj S	inj S	cont S	cont S	inj S

Replicate 2

Day 0		Day 14		Day 28		Day 42	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
cont S	inj S	inj S	cont S	cont S	inj S	inj S	cont S

Replicate 3

Day 0		Day 14		Day 28		Day 42	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
inj S	cont S	cont S	inj S	inj S	cont S	cont S	inj S

Replicate 4

Day 0		Day 14		Day 28		Day 42	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
cont S	inj S	inj S	cont S	cont S	inj S	inj S	cont S

[a] = injected USDA Select

[b] = control USDA Select

Appendix 14: Randomization of USDA Low Choice and Average Choice ribeye rolls

Replicate 1

USDA Low Choice Control				USDA Average Choice Control			
Ribeye 1		Ribeye 2		Ribeye 1		Ribeye 2	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Day 14	Day 0	Day 42	Day 28	Day 0	Day 14	Day 28	Day 42

Replicate 2

USDA Low Choice Control				USDA Average Choice Control			
Ribeye 1		Ribeye 2		Ribeye 1		Ribeye 2	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Day 0	Day 14	Day 28	Day 42	Day 14	Day 0	Day 42	Day 28

Replicate 3

USDA Low Choice Control				USDA Average Choice Control			
Ribeye 1		Ribeye 2		Ribeye 1		Ribeye 2	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Day 14	Day 0	Day 42	Day 28	Day 0	Day 14	Day 28	Day 42

Replicate 4

USDA Low Choice Control				USDA Average Choice Control			
Ribeye 1		Ribeye 2		Ribeye 1		Ribeye 2	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Day 0	Day 14	Day 28	Day 42	Day 14	Day 0	Day 42	Day 28

Appendix 15: Determination of ribeye purge

1. Weigh ribeye rolls and place in vacuum package bag.
2. Vacuum package and store at 1 °C for designated time of storage.
3. After designated time of storage, remove ribeye rolls from vacuum package bag.
4. Blot ribeye rolls dry and reweigh.
5. Percent purge loss is determined using the following calculation:

$$\% \text{ purge loss} = \frac{\text{weight before storage} - \text{weight after storage}}{\text{weight before storage}} \times 100$$

Appendix 16: Cooked product yield determination

1. Weigh steak before cooking.
2. After cooking to 71°C, allow steak to drip for 5 min on a metal rack.
3. Weigh cooked steaks.
4. Percent cook yield is determined by the following calculation:

$$\% \text{ cook yield} = \frac{\text{Weight of cooked steak}}{\text{Weight of uncooked steak}} \times 100$$

Appendix 17: Determination of steak purge in retail meat display case

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1. Weigh steaks on day 0 of each retail meat case study.
2. Place steaks on foam trays and overwrap with PVC film.
3. After 7 days of storage (1 °C) in the retail meat case, remove steaks from the foam trays.
4. Blot steaks dry and reweigh.
5. Percent purge loss is determined using the following calculation:

$$\% \text{ purge loss} = \frac{\text{weight before storage} - \text{weight after storage}}{\text{weight before storage}} \times 100$$

Appendix 18: Thiobarbituric acid reactive substances (TBARS) determination

Rhee, KS. 1978. Minimization of further lipid peroxidation in the destillation 2-thiobarbutiric acid test of fish and meat. J Food Sci 43:1776-1778.

Tarladigis, GG, Wats, BM, Younthan, MT, Dugan, L Jr. 1960. J Am Oil Chem 37:44-48.

Zipser, MW, Watts, BM. 1962. Lipid oxidation (TBA) methods. Food Technol 16(7):102.

Reagents:

1. TBA Reagent

   Prepare the amount of TBA Reagent needed for your samples according to the table below:

Thiobarbituric Acid	Total Vol. Water and Acid
1.4416 g	500 ml
0.7208 g	250 ml
0.5766 g	200 ml
0.2883 g	100 ml
0.1442 g	50 ml

   Dissolve the Thiobarbituric Acid (Eastman Organic Chemicals) in the distilled water, about 2/3 the total volume. Place flask in sonic cleaner (several minutes) and shake occasionally until TBA is dissolved. Allow reagent to come to room temperature then bring to volume. Store in cooler, may be kept for 2 days.

2. HCl Solution:

   Make volume as needed; 1:2, HCl : H_2O (v/v).

3. Antifoam (Thomas®, Swedeboro, NJ):

   The use of antifoam may not be necessary depending on the product. Fish and egg require antifoam while poultry does not.

Procedures:

1. Add 10 g of diced sample to 100 ml plastic bottle containing 50 ml distilled water plus 10 µl antioxidant solution (Tenox 5 – food grade BHA+BHT).

2. Homogenize sample using Polytron mixer (PT-35, Kinematica, AG, Switzerland) on speed setting 4 for 1 minute (Homogenized samples can be held in cooler if needed).

3. Into 500 ml extraction flasks, add glass beads (Fisher Scientific, Pittsburgh, PA), homogenized meat sample, 2.5 ml HCl solution, and if necessary antifoam.

   Note: total volume is 50 ml + 2.5 ml + 47.5 ml = 100 ml

4. Turn on condenser water and place graduated cylinders under spouts.

5. Connect extraction flasks to distilling tubes.

6. Turn heat control knobs to HI.

7. Distill and collect 50 ml of the distillate.

8. Transfer distillate to 50 ml centrifuge tubes, cap and hold in refrigerator for TBA reaction (can be held for 18 hours).

9. Invert each test tube containing the 50 ml distillate and pipette 5 ml into each of 2 tubes labeled "A" and "B". Prepare 2 blanks by pipetting 5 ml distilled water into both tubes labeled "A" and "B".

10. Add 5 ml of TBA Reagent into each tube containing 5 ml of sample and into both blanks. Thoroughly mix each tube using Vortex mixer (American Scientific Products, McGaw Park, IL).

11. Turn water bath on 100° C.

12. Place tubes in test tube rack and immerse into boiling water bath (model 9510 PolyScience, Sorvall Co., Niles, IL) for 30 minutes.

13. Turn on plate reader.

14. When the tubes are done heating in the water bath cool them in ice for at least 10 minutes.

15. Mix each test tube with sample for 10 seconds using Vortex mixer (American Scientific Products, McGaw Park, IL).

16. Pipette 200ul into well on plate (done in duplicate).

17. Place plate in plate reader and set up plate reader. Click on plate reader on computer, click on experiment 1. Go to "set up" and set the appropriate wavelength and chose end point analysis. Go to "template" and set up blank and unknowns according to plate. (Wavelength to 530 for fresh meat) Read samples within 1 hour.

18. Convert % T to optical density and multiply by the constant 7.8 (7.6 for poultry) to convert to mg malonaldehyde/1000 g of sample, i.e. TBA Number.

Absorbance is converted to mg malonaldehyde (MDA) /kg sample (TBARS value) using the following equation:

$$TBARS = A_{532nm} \times K \text{ (mg MDA/kg sample)}$$

Where K = (conc. in moles/5 ml of distillate $\times$ M.W.MDA $\times$ 10^7 $\times$ 100) / (Absorbance $\times$ wt. of sample $\times$ % recovery)

K is distillation constant and equal to 7.8 in this lab.

Appendix 19: Proximate composition determination

Sample Preparation (modified from section 983.18 Meat and Meat Products)

1. Section frozen meat into very small (<1 cm squares) pieces. This can be accomplished by smashing samples with a hammer to decrease size of sample for ease of grinding.
2. Add sample to Tekmar grinders (Tekmar Co, Cincinnati, OH) filling grinding chamber half full.
3. Then add dry ice to fill up chamber.
4. Grind 2 to 3 minutes using Tekmar grinder (Tekmar Co, Cincinnati, OH) until sample is ground into a fine powder. It may be necessary to stop in the middle of grinding and stir the sample for uniform grinding.
5. Transfer finely ground powder to labeled whirl pack bags. Loosely close bag so that dry ice can evaporate and dissipate. This takes about 2 days. Place in freezer immediately to prevent melting of powder.

Moisture content (oven drying method, AOAC method 950.46B, 2000)

1. Place a medium weigh boat on scale and zero. This is to keep the scale clean. Add folded filter paper labeled with sample ID. Record the weight then tare the scale.

2. Add 2 grams (± .03g) of thoroughly mixed sample to the paper. Once desired weight is reached record weight and fold over top. Place flat on tray. Do all samples in triplicate. Do not stack samples on tray. This will hinder the drying process.

3. Once tray is full, place in drying oven set at 100°C for 20 - 24 hours.

4. After drying, place samples using latex gloves or tongs in desiccators to cool completely before weighing. Once cool, weigh samples and record. This is your final weight for moisture and your initial weight for fat analysis. Use the following formula to determine the percent moisture in your samples:

$$\text{Moisture (\%)} = \frac{\text{wet sample wt.} - \text{dry sample wt.}}{\text{wet sample wt.}} \times 100$$

Fat content (Soxhlet ether extraction, AOAC method 991.36, 2000)

1. Take samples from moisture analysis and place in extraction tubes. Make sure that all the samples are below the level where the ether drains off (curved glass on outside of tube).

2. Add petroleum ether to clean boiling flasks until about ¾ full. Add 2 to 3 glass beads as a boiling aid.

3. Connect the extraction flask to the boiling flask and Soxhlet apparatus. Place parafilm on the joint. Mount both to the condensing units on top of extraction flasks using parafilm around joint.

4. Turn on condensing water so it runs at a steady stream.

5. Set Rheostats on 4.5 and run for 24 hours.

6. Place ether soaked samples onto a tray in a hood for 10 min to allow ether to dissipate.

7. Place samples in drying oven for 5 to 10 min to remove any possible moisture then place in desiccators for 1/2 hour to cool.

8. Weigh and record the weight of the samples. Calculate fat on wet basis with the following equation:

$$\text{Fat (\%)} = \frac{\text{dry sample wt.} - \text{extracted sample wt.}}{\text{wet sample wt.}} \times 100$$

Protein content (combustion method, AOAC method 992.15, 2000)

1. Weigh out approximately 1 gram of powdered meat into the tarred ceramic boat with nickel liner. Write the weight and sample ID on the side of the boat with pencil.

2. After weighing out samples, dry for 24 hours in the drying oven at 100°C. This removes moisture that can cause internal malfunctions with the Leco Protein Analyzer. Do not reweigh samples. Enter wet weight into computer.

Procedures for the LECO FP 2000 Nitrogen Analyzer

1. Open valves completely on oxygen, helium and compressed air tanks. Make sure tanks have adequate levels of gas (gauge should read >100psi) and that the pressure out of the tanks are set at 40 psi.

2. Press escape on upper left hand corner of touch screen until "front panel" comes up and then press it. On right hand side of screen a section labeled "analysis gas" can be found. Push the "on" button to turn gases on to the machine. Check to see that your furnace temperature is 1050°F (located on left part of screen).

3. Wait about 5 min for all gases to equilibrate then start your leak tests. Press escape from the front panel located in upper left corner. A screen with several icons will appear. Press "maintenance". This will bring up helium leak test, combustion leak test and ballast leak test icons. Press the helium leak test, if it passes move onto the combustion leak test. Run a ballast test only if there is a leak in the combustion system. Once finished, start running blanks.

4. Run several air blanks through to purge the system. To do this escape from the "maintenance" section and push the "analyze" icon. On the bottom of the screen you will see several commands. Push "select ID code". Move the highlighted line using the arrows to "blanks". Then push exit on bottom and push manual weight. This will bring up a touch screen with 0.2000000 on it. Push the enter button at least 10 times to bring up 10 rows of 0.20000. Then push analyze. The machine will run through these ten samples. Numbers should come down to about <0.2000% protein. Wait until several blanks have approximately the same protein content. Then run EDTA samples.

5. Weigh approx. 1.0 g EDTA samples out in the ceramic boats and write the weight on the side in pencil (at least four decimal places).

6. Push "select ID code" on the bottom of the screen. Move the highlighted line using the arrows to "edta". Select "manual weight" and put your weight into the machine pressing enter after each entry. Once weights are entered, press analyze. Follow the directions on the touch screen. Push your first sample into the chamber about one half inch so the door doesn't catch the boat. Push okay on the screen when it asks you to place your sample in the chamber. The next message will tell you to wait because the system is purging. Then the machine will then tell you to push the boat into the chamber. The machine will combust and analyze the sample in approximately 4-5 minutes.

7. Run 5-6 EDTA samples (approximately 1.0g) to verify machine is operating properly. EDTA is 59.9% protein. The samples should come out to be 59.9% +/- 0.2%.

8. If there is more variation in the percentages than 0.2%, DRIFT the samples to equilibrate the percentages. To drift, press escape until you reach the front panel.

Press Calibrate, then press Drift Correction. A new screen will pop up; press Carbon and then OK. Select the closest samples from the list of results by using the up/down arrows and "Include Result" button at the bottom of the screen. Pressing the "Include Result" will highlight the result and use it to recalibrate the machine. Once weights are all selected, choose "Process Results" at the bottom of the screen. Another screen will pop up asking if you would like to save the new calibration. Choose "Yes."

9. After the Drift Correction, escape to the front panel and choose Analyze. Continue running EDTA samples to ensure that the machine is working properly (59.9% +/- 0.2%). Once it is functioning properly, you may run your test samples. Push "select ID code" on the bottom of the screen. Move the highlighted line using the arrows to .

Appendix 20: pH determination

1. Homogenize 1 ± 0.1 g diced sample with 50 ml of deionized water in a 50 ml polycarbonate tube with Polytron mixer (PT-35, Kinematica, AG, Switzerland) set on speed setting 4 for two 10 s intervals. Rinse and blot dry Polytron bit between each sample.

1. Measure pH using an Accumet Scientific pH meter calibrated using buffers 4.0 and 7.0.

3. Rinse pH meter probe with distilled, deionized water between sample readings.

Appendix 21: Melting point determination

Differential Scanning Calorimetry (DSC): DSC (2010, TA Instruments, New Castle, DE)

Computer set-up

1. Turn on nitrogen gas to 50 psi.
2. Click on TA Instrument control.
3. Click on procedure.
4. Click on edit.
5. Double click on segment.
6. For beef ribeye fat, ramp from 20 °C to 80 °C and for "modified marbling" gel, ramp from 20 °C to 150 °C.
7. Click ok.
8. Click on summary.
9. Type in sample name (beef fat or solution).
10. Type in sample weight (approx. 12 mg).
11. Click on book and scroll arrow to 3½ floppy disk.
12. Insert 3½ floppy disk into computer.
13. Click apply.

Sample preparation

1. Use a razor blade and cut very small pieces of sample.
2. Place DSC pan bottom (T40625, TA Instruments, New Castle, DE) on scale and tare.
3. Place approx. 12 mg of sample in pan bottom using a tweezers and record weight.
4. Place DSC pan lid (T40621, TA Instruments, New Castle, DE) over pan bottom using a tweezers.
5. Place pan in crimping die (TA Instruments, New Castle, DE) and press lever down.

6. Remove sample pan.

7. Prepare a control pan by placing a pan lid over an empty pan bottom and denting in the denting apparatus.

Running the DSC

1. Take off the glass lid and gold cylinder from the DSC.

2. Place metal cylinder container on DSC.

3. Pour liquid nitrogen into cylinder and wait for temperature to drop to approx. 15 °C.

4. Once temperature has dropped, take off metal cylinder.

5. Open lid on DSC and place the control pan on the right slot and the sample pan on the left slot.

6. Place lid on the DSC along with the gold cylinder and the glass lid.

7. Press the green run arrow on the top left of the screen.

8. Once the sample has been run, remove the glass lid, gold cylinder and lid and remove the sample pan.

9. The control pan can stay the same for all samples.

10. Repeat as necessary.

Appendix 22: Scanning electron microscopy determination

Operating instructions for JSM 6400V provided by the Center for Electron Optics

Scanning electron microscope: JEOL scanning electron microscope, Model JSM-6400V, version 96-2, Tokyo, Japan

Caution: 1. Never change the accelerating voltage with the filament saturated. Change the accelerating voltage only when the filament is desaturated to the preheat value.

2. Never turn the filament knob faster than the directions describe.

3. At the 8 mm working distance nothing should extend more than 2 mm above the top of the sample hold.

Analysis procedures:

Start-up

1. Turn up the brightness on CRTs 1 and 2

2. Check the vacuum (10^{-7} range).

3. Check the heat/preheat light (on). This is located on the box to the right of the SEM.

Sample insertion

1. Working distance 39, X-25, Y-35, Tilt-0, Rotation-0.

2. Place samples in the sample holder, adjust height, attach holder to the sample insertion rod.

3. Pull the rod back into the spring clip. Place rod on the port, press the red button, and wait till the light goes out. Do not wait. After the light goes out, the port is no longer pumped. If you wait, the vacuum will decrease to dangerously low levels.

4. Turn the flat on the knob to the front and pull it to the right.

5. Push the rod in, place sample holder on the rail, unscrew the rod, and pull it out.

6. Push the knob to the left then turn the flat up.

7. Push the red button, wait till the vacuum is gone, and remove the rod.

Sample removal

1. First desaturate filament and turn off the accelerating voltage

2. Working distance 39, X-25, Y-35, Tilt-0, Rotation 0.

3. Pull rod back into spring clip. Place the rod on the port, press the red button, and wait till the light goes out.

4. Turn the flat on the knob to the front and pull it to the right.

5. Push the rod in, screw the rod into the holder, then pull the rod all the way out.

6. Push the knob to the left then turn the flat up.

7. Push the red button, wait till the vacuum is gone, and remove the rod.

Shut-down

1. Desaturate filament, and sample should be removed.

2. Turn down the brightness of CRTs 1 and 2.

3. Turn off the hanging lamp.

Appendix 23: TA-HD*i* Warner-Bratzler shear force settings

Texture Analyzer:	TA-HD*i* Texture Analyzer Texture Technologies Corporation, Scarsdale, NY
Software:	Texture Expert Version: 1.22

TA-HD*i* Settings:

Test Mode:	Measure Force in Compression
Option:	Return to Start
Pre-Test Speed:	5.00 mm/s
Test Speed:	3.30 mm/s
Post-Test Speed:	10 mm/s
Distance:	35.0 mm
Trigger	
Type:	Return
Distance:	0.5 mm
Stop plot at:	Trigger Return
Auto tare:	(selected)
Attachment/Accessory:	50 kg load cell
	TA-90; Heavy duty platform

Appendix 24: TA-HD*i* texture analyzer calibration and analysis procedures

Calibration Procedure:

Machine Calibration

1. Turn the texture analyzer (TA) on. The power button is located on the bottom right side toward the front.

2. Log on to texture analyzer program on computer (Texture Expert Analyzer) found on computer desktop.

3. Turn TA key to the "run" position.

4. Remove any attachments or platforms that are present on the TA.

5. Attach calibration weight hanger attachment and weight hanger.

6. Turn TA key to machine configuration.

7. Press "ENT (enter)" until you reach the screen that determines the load cell weight (Cell).

8. Press " +/-" to acquire appropriate load cell weight. For example: 50 kg load cell will be indicated by "50" on screen.

9. Turn TA key back to "run" position and then back to machine configuration. This saves settings in TA.

10. Press the "calibrate" key, then "enter".

11. When TA screen reads the appropriate weight put the actual weight on the TA weight hanger. For example: 50 kg load cell will utilize a 10 kg weight, 5 kg load cell utilizes a 2 kg weight.

12. Press "calibrate" and when screen reads "done", switch TA key back the to "run" position.

13. Remove the actual weight from hanger but do not remove the weight hanger. Do not put the weight away because you will be using it in the computer calibration.

Computer Calibration

1. Go to heading that reads "TA".

2. Click on "Calibrate Force".
3. Press "ok". The computer will then ask you to place the actual weight on the hanger.

4. Once the weight is placed onto the hanger press "ok".

5. The computer will then say "calibration successful". If this is not indicated, or if the calibration unsuccessful, re-calibrate the machine.

6. Remove the weight and the hanger from the TA.

7. Attach the platform to the TA and the appropriate attachment. For Example: For the Kramer shear test attach the 5-blade attachment to TA and from Gel hardness attach TA-10 attachment.

8. When using the WBS attachment an extra set up step is required, if you are not using the WB attachment you may skip this step. Using the up and down arrows on the TA control board lower the WB blade into the slit on the platform until you can feel the blade poke through the platform with the tips of your fingers. Run quick tests and move the platform to make the force in kg as close to 0.000 as possible. This reduces the friction during the analyses.

9. The TA is now ready to analyze samples.

Analysis Procedure and Setting up the Computer Files:

1. Create a personal file for data collection – go to computer desktop.

2. Click "My Computer"

3. Go to Drive "C:\"

4. Click on "My Documents"

5. Open the folder in which you wish to save you results. To create a new folder, right click and scroll to "new" chose the "folder" option.

6. Name your folder (Example: Set 1). If you choose a file name that is too long the computer will not read it (Example: Shear Set 1).

7. In the Texture Expert Program go to your selected project (the minimized window in the bottom left hand corner of the screen, this is the project window.

8. Under settings, push dotted button (ellipses) and make sure your correct folder is selected. Do the same for macro and results. If your desired settings are not entered you must go to a folder with previous tests in it. Copy the macro file, setting files, as well as a result file to your folder. This is done so the computer knows which format to follow.
9. Push "Restart" on the Texture Expert Analyzer Program. You may receive an error message because you have not created any results yet.

10. Under TA go to "Settings" and include the appropriate settings for the test if different than the settings listed there.

11. Click on the "TA" heading on the computer screen. Select "run test". Check to where the results are being sent. The path in black on the middle of the screen indicates this (Example: C:\mydocu\wbs\johnson\set1 will put the results on the C:\ drive under My Documents in Johnson's folder under set 1). If the path is incorrect it can be changed by pressing on the ellipses dots in the large white box. Continually clicking on the top of this screen will take you back to the C:/ drive. Click on the folder into which you wish your results to be placed.

12. Enter the ID of the sample you will analyze under "File ID". Set the "file number" to one so it can count the samples. A low number is necessary because too many characters in the file name will cause an error message to appear. Set the file number to one each time you open a new spreadsheet for results.

13. Place the sample on the machine to be analyzed.

14. Enter the ID of the sample. Dates can be entered under "Batch ID".

15. Press "ok" on the Run Test screen to begin analyzing.

16. You will be prompted to save the results from the previous test. Click "ok".

17. Repeat steps 13-16 as necessary.

18. The analyzer may begin to run slowly after several analyses. If this occurs you `should start a new spreadsheet. To start a new spreadsheet click on the results window to make it active. Select "Save" from the File menu. Then hit the "X" button on the top right corner of the screen. Do not select "exit", as this will close the Texture Analyzer Program. You do not need to open a new spreadsheet. One will start as soon as you run the next test. To get back to the analyzing screen click on the graph page to make that window active.

19. If at anytime the "run test" option is not available go back to the Project screen (the first screen) and click "restart". This should cause the "run test" option to be available again.

Appendix 25: Protocol for use of Taylor clamshell grill

Grill settings:
Gap 2.16 cm (setting on grill between 15 and 16)
Lower grill temp. 102.8 °C
Upper grill temp. 104.4 °C
Cook time – variable (timer set at 900 s, record endpoint time and subtract for final cook time)

Grill preparation:
1. Turn on power box control on wall (it should be locked out) and both grill controls.

2. Choose STK 4 (setting for item #4) on each grill and allow to warm up until "TOO COOL" no longer is displayed on LED displays.

3. While the grill is warming up complete the following:
   a. Apply the stick-resistant Teflon cloths to both upper grills.
   10. Start with left side of grill (top of grill should be in "down position").
   11. Insert left bar through top spring, cloth, and bottom spring in that order.
   12. Secure left side.
   13. Lift top.
   14. Insert right bar through cloth.
   15. Pivot bottom of right bar on right back edge of grill and pull the top of the bar over the top edge.
   16. Secure right side.
   17. Repeat for right side of grill

4. ake sure display on grill read "STK 4 900"

Cleaning Grill
1. Turn off power to grills and lock-out power box on wall.

2. Let grill cool a minimum of 10 min.

3. Use rubber squeegee to wipe sown stick-resistant Teflon cloths on top burners.

4. Remover cloths, clean in sink, dry and lay flat.

5. Pour a small amount of water on lower grill surface and use metal scraper to release cooked-on material (repeat as necessary).

6. Use scrub pad dipped in water to rinse both top and bottom grill burners.

7. Apply soap from spray bottle and scrub with pad.

8. Rinse out pad and use in clean water to rinse both top and bottom burners.
9. Clean back trough with trough scraper.
10. Remove and clean out fat trap.
11. Let grill dry.

Appendix 26: Protocol for cooking, coring, and shearing

Cutting 2.54 cm steaks

1. Take fresh, boneless ribeyes and cut 2.54 cm steaks using cutting box and knife.

2. Make sure each steak is 2.54 cm thick and not more than 2.54 cm thick.

Record steak weight and pre cook (initial) temperature of each steak.

Thermocouples

1. Insert thin thermocouples into the geometric center of the steak. First, insert probe (needle) through a small section of meat on the end of the cut, before insertion into the center of the cut. Insert the needle into the side of the meat (in the center of the depth of the steak) and push completely through the cut. Remove the probe from the end of the thermocouple and then pull the thermocouple back into the center of the meat. The end of the thermocouple should not be touching bone or fat. (See 2f and figure 2 from *The Guidelines for Cooking Procedures* – AMSA).

Cooking

1. Open top of grill.

2. Apply thin layer of Crisco® on lower grill surface.

3. Stack grill with steaks from front to back rapidly.

4. Close grill top.

5. Press time button on grill.

6. Cook steaks to 71 °C (remove from grill around 68 °C, temp will rise to 71 °C).

7. Remove steaks from front to back rapidly.

8. Scrape lower grill surface with metal scraper to release buildup.

9. Scrape buildup off upper grill surface (Teflon surface) with rubber squeegee as needed.

10. Place next rep of steaks on grill and repeat cooking process.

11. Record final cook temperature (highest temperature steak rises to) for each steak.

12. Allow steaks to drain on cooking rack for 5 min.

13. Record weight of cooked steak.

14. Cover steaks with saran wrap and store at 4 °C for 24 h.

Coring steaks with Craftsman 8 in. drill press and ½ in. corer drill bit.

1. Remove one steak at a time from refrigerated storage to core and shear.
2. Cut cross section off end of steak to determine muscle fiber direction.
3. Position base of drill press to obtain angle parallel to muscle fibers.
4. Slowly and firmly lower the corer through the entire depth of the steak.
5. Remove the corer from the steak.

Warner-Bratzler shear of ½ inch core with Texture Analyzer – HD*i*

1. Place ½ inch core in the center of the WBS shear plate.
2. Run a test on the TA-HD*i*.
3. Allow TA-HD*i* to return to start position.
4. Clean plate and base stand on TA-HD*i*.
5. Repeat steps 1-4.

Beef: core and shear 6 samples on each steak.

Standardized Warner-Bratzler Shear Force Procedures for Genetic Evaluation

Committee Members:

Jeff Savell, Texas A&M University, Chair; Rhonda Miller, Texas A&M University; Tommy Wheeler, MARC; Mohammad Koohmaraie, MARC; Steven Shackelford, MARC; Brad Morgan, Oklahoma State University; Chris Calkins, University of Nebraska; Mark Miller, Texas Tech University; Michael Dikeman, Kansas State University; Floyd McKeith, University of Illinois; Glen Dolezal, Oklahoma State University; Bill Henning, Pennsylvania State University; Jan Busboom, Washington State University; Roger West, University of Florida; Fred Parrish, Iowa State University; Scott Williams, University of Georgia.

An initiative to standardize the protocol for Warner-Bratzler shear force determinations was identified at the National Beef Tenderness Plan Conference in April 1994. The purpose of this protocol is to allow for consistent collection of Warner-Bratzler shear force determinations across institutions for comparative evaluation. These data then can be used in progeny testing and in the development of carcass EPDs for meat tenderness. Any institution abiding by these guidelines can then be certified to collect Warner-Bratzler shear force determinations for the beef industry. The objective is to assist the beef industry ion culling live animals that produce tough meat.

Conversion of live animals to carcasses

The process of conversion of the live animal to the carcass can have a significant effect on meat tenderness. Therefore, the slaughter process and the environmental conditions during slaughter should be controlled as closely as possible. Conditions that should be monitored and could affect Warner-Bratzler shear force values include electrical stimulation and postmortem chilling. Although these factors can affect the ultimate tenderness of beef, these variables are probably uncontrollable by the researcher.

Appendix 27: Sensory ballot

Meat Descriptive Attribute Ballot

Name_____ Rep_____ Date_____

Sample	Juiciness	Muscle Fiber Tenderness	Overall Tenderness	Connective Tissue	Off-Flavor Intensity	Beef Flavor Intensity	Fat Flavor Intensity	Mouth Coating

Juiciness

8 Extremely Juicy
7 Very Juicy
6 Moderately Juicy
5 Slightly Juicy
4 Slightly Dry
3 Moderately Dry
2 Very Dry
1 Extremely Dry

Muscle Fiber and Overall Tenderness

8 Extremely Tender
7 Very Tender
6 Moderately Tender
5 Slightly Tender
4 Slightly Tough
3 Moderately Tough
2 Very Tough
1 Extremely Tough

Connective Tissue

8 None
7 Practically None
6 Traces
5 Slight 5 Moderate
4 Moderate
3 Slightly Abundant
2 Moderately Abundant
1 Abundant

Off-flavor Intensity and mouth coating

8 Abundant
7 Moderately Abundant
6 Slightly Abundant
5 Slightly Intense
4 Slight
3 Traces
2 Practically None
1 None

Flavor Intensity

8 Extremely Intense
7 Very Intense
6 Moderately Intense
5 Slightly Intense
4 Slightly Bland
3 Moderately Bland
2 Very Bland
1 Extremely Bland

Appendix 28: Sensory random order

Storage Day 0

Replicate	Treatment #	Treatment	Random Code
2	4	Average Choice Control	778
3	4	Average Choice Control	699
2	2	Injected Select	775
3	2	Injected Select	794
1	1	Control Select	475
1	3	Low Choice Control	856
2	3	Low Choice Control	176
1	4	Injected Select	797
4	2	Average Choice Control	456
3	1	Low Choice Control	280
4	4	Control Select	861
2	1	Control Select	473
4	1	Low Choice Control	166
1	2	Average Choice Control	883
3	3	Control Select	582
4	3	Injected Select	128

Storage Day 14

Replicate	Treatment #	Treatment	Random Code
1	4	Injected Select	285
4	1	Low Choice Control	625
4	2	Average Choice Control	426
3	1	Low Choice Control	622
1	1	Control Select	777
1	2	Average Choice Control	180
2	1	Control Select	556
1	3	Low Choice Control	717
2	2	Injected Select	109
2	3	Low Choice Control	144
4	3	Injected Select	162
3	4	Average Choice Control	809
3	3	Control Select	395
3	2	Injected Select	548
2	4	Average Choice Control	707
4	4	Control Select	477

Storage Day 28

Replicate	Treatment #	Treatment	Random Code
4	1	Low Choice Control	566
4	2	Average Choice Control	752
1	2	Average Choice Control	986
1	4	Injected Select	706
3	1	Low Choice Control	174
1	1	Control Select	180
2	2	Injected Select	627
2	1	Control Select	345
3	4	Average Choice Control	697
3	3	Control Select	555
1	3	Low Choice Control	612
4	4	Control Select	797
2	3	Low Choice Control	385
4	3	Injected Select	860
3	2	Injected Select	824
2	4	Average Choice Control	110

Storage Day 42

Replicate	Treatment #	Treatment	Random Code
4	1	Low Choice Control	511
2	3	Low Choice Control	414
3	1	Low Choice Control	175
1	1	Control Select	434
2	2	Injected Select	515
3	2	Injected Select	990
1	3	Low Choice Control	735
1	2	Average Choice Control	158
3	3	Control Select	609
4	4	Control Select	755
4	3	Injected Select	464
1	4	Injected Select	632
2	1	Control Select	598
2	4	Average Choice Control	268
4	2	Average Choice Control	746
3	4	Average Choice Control	226