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Concurrent Technical Session VI

Pre- and Post- Harvest Factors of Beef Quality

Industry Perspective on Pork Quality

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Traditional pork quality research has focused on the loin and attributes associated with that cut. Researchers have made recommendations to improve loin quality by focusing on genetic selection, animal handling, stunning techniques, and chilling rate. With these recommendations in mind, the industry has worked to decrease variation and improve overall loin quality.

Consumers' first impression of products is largely based on appearance. To be considered for purchase, pork products must therefore appeal to the eye of the consumer or purchaser. Once the product has been purchased an enjoyable eating experience is also required for consideration of future purchase. As the pork industry moves toward selling products at retail based on quality ("Guaranteed Tender Programs" and other high quality programs), being able to differentiate carcasses of better quality becomes more important. This is true not only for the domestic market, but for the export market as well. Unfortunately, late antemortem and early postmortem factors can turn high quality pork into low quality product. To appraise these differences, fresh meat is subjectively evaluated for attributes such as color, firmness, and marbling. Instrumental measurements of both color and tenderness are also influential in determining pork quality. In general, a darker, firmer product with good water holding capacity is preferable to a light, soft product with poor water holding capacity.

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Pork firmness is an important attribute that is commonly measured in the pork industry. It is usually a selection criterion for export product, but has received little attention from the research community into the exact mechanisms behind firm pork. Firmness can range on a 1 to 5 scale (NPPC, 1991), where 1 is very soft and 5 is very firm. Firm pork is usually associated with other quality measurements that indicate better quality; such as dark color, hard fat, and greater water holding capacity (Birmingham et al., 1966; Davis et al., 1975).

Similarly, tenderness is a very complex phenomenon and has received a great deal of attention throughout meat science research. Factors such as genetics, nutrition, and postmortem events all contribute to the ultimate tenderness level. Koohmaraie et al. (2002) evaluated the components of tenderness during aging and described sarcomere length, connective tissue content, and proteolysis of myofibrils and associated proteins as major contributors to postmortem aging. While these factors have been extensively studied in beef (Davis et al., 1979; Sazili et al., 2004; Camou et al., 2007) and sheep (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996; Veiseth et al., 2004), less work has been done to examine tenderness in pork (Wheeler et al., 2000). This lack of research most likely stems from the preconceived notion that pork is always tender. However, this is not always the case. Research indicates shear force for pork can be over 5 kg (DeVol et al., 1988; Wright et al., 2005), a level which would be considered tough.

When discussing pork quality, the subject of pH must also be noted. Due to ease of measurement and the inherent ability to differentiate product of varying quality, pH is one of the most measured pork quality attributes. Both the rate of pH decline and the ultimate pH are extremely important because these 2 factors can have the greatest impact on the final product. The rate of pH decline is important because a high temperature and low pH combination can result in poor quality due to protein denaturation. In pork, a slow rate of pH decline, perhaps hours, is preferable to a rapid decline. If within 45 min (Bendall and Swatland, 1988) postmortem carcass pH is less than 6.0, the potential for pork quality issues is drastically increased. Under ideal circumstances, ultimate pH would

be in the range of 5.6 to 5.8 to maintain color and water holding capacity (Price and Schweigert, 1987) while preventing problems associated with shelf-life (Knox et al., 2008; Holmer et al., 2009).

One notable condition that arises from accelerated pH decline and/or low ultimate pH is pale, soft, and exudative (PSE) pork. Most often PSE pork results from high temperatures (observed early postmortem) and a low muscle pH (Offer, 1991; Cannon et al., 1995). This high temperature/low pH condition damages the meat proteins, which ultimately affects meat quality (Honikel and Kim, 1986). The protein denaturation prevents proteins from binding with water as efficiently, so moisture "pools" on the meat's surface. The ultimate pH of PSE meat is also closer to the isoelectric point of meat proteins compared with dark, firm, and dry (DFD) pork. This results in less water binding capacity (Price and Schweigert, 1987). The decrease in water binding capacity increases the amount of light reflectance (Offer, 1991) and gives PSE meat its characteristic pale and exudative properties.

Although the discovery of a mutation (Fujii et al., 1991) which frequently resulted in PSE meat (Ohene-Adjei et al., 2003) has largely been eradicated from the pig population, problems with PSE still persist. As selection for faster growing, leaner pigs continues, there have been some trade-offs in pork quality. In fact, as of 2003, Stetzer and McKeith (2003) reported approximately 15.5% of pork carcasses still exhibit the PSE condition. As a result of stress before slaughter, metabolic rate increases and lactic acid accumulation drives down the pH at an accelerated rate. Ultimately, such detrimental effects of PSE meat extend to the point where they are discriminated against by consumers (Brewer et al., 1998) and will have a decrease in tenderness compared with DFD meat (van der Wal et al., 1988; Brewer et al., 1999).

With this in mind, researchers have looked at ways to minimize the effects of PSE through the use of accelerated chilling. In general, the chilling of pork carcasses can have a dramatic effect on ultimate meat quality. The common practice within the pork industry is to quickly chill a carcass so that the high temperature/low pH conditions do not result in poor quality. As the rate of chilling increases, the rate of pH decline decreases, which improves pork quality. Multiple methods have been developed to accelerate the chilling process, including blast chills (Milligan et al., 1998; Ohene-Adjei et al., 2002) or cold showering (Maribo et al., 1998), or more aggressive approaches such as immersion in propylene glycol (Weakley et al., 1986) or liquid nitrogen (Jones et al., 1991). Due to the initial quality of the product used (i.e., predisposed to the rapid pH decline) and time of chilling, there are some inconsistent results throughout the literature. However, the accelerated chilling methods generally will have a positive effect on pork quality. Loin color and texture were improved when carcasses were chilled at –32°C for varying times postmortem (Springer et al., 2003). Additionally, removing pork loins at 20 min postmortem and subjecting them to varying degrees of chilling resulted in improved quality (Weakley et al., 1986). However, problems with water loss and tenderness can arise with some accelerated chilling methods (Weakley et al., 1986).

While decreasing temperature slows the rate of pH decline (Marsh, 1954) and has been shown to improve pork quality, current literature may not support this assumption when metabolic rate is increased antemortem. For instance, studies using Halothane positive pigs and the addition of ether liquid nitrogen immersion (Jones et al., 1991) or blast chilling (Ohene-Adjei et al., 2002) have not reported any benefit over conventional chill. Similar results have been reported in Halothane negative pigs which were subjected to aggressive antemortem handling (Hambrecht et al., 2004). In this instance accelerated chilling could not overcome the detrimental effects of aggressive antemortem handling. One caveat to these experiments may be the time postmortem in which samples were handled. If methods of aggressive accelerated chilling (immersion in a chilled solution) are employed early enough postmortem (30 min or less), the normal problems associated with PSE pork may be reduced.

With all of these factors in mind, it is imperative for the industry to send signals to researchers about their specific problems for progress in pork quality to continue. With this, researchers need to design experiments that specifically address economically important problems faced by the industry. Now research needs to be expanded to include recommendations for improvement of additional cuts and wider definition of quality. Common industry issues include color life of case ready pork, food service bacon performance, ham color variation, loin quality consistency, and other traits that have been minimally researched. The industry has responded to the majority of the research based recommendations with positive results for the loin but a new focus is required by researchers on the next generation of pork quality issues.

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Effects of Pork Loin Quality and Enhancement on Consumer Acceptability and Cooking Characteristics of Pork Loin Chops

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INTRODUCTION

The impact of pork quality on consumer preferences has been extensively studied. It is generally thought that as pH decreases, color becomes lighter, marbling decreases and pork becomes tougher, consumer preference is negatively impacted. In addition, consumers tend to cook pork to high degrees of doneness and it is well documented that cooking impacts meat palatability. The pork industry has implemented the use of enhancement, or the addition of nonmeat ingredients in a water-based solution, to improve the juiciness, tenderness and flavor of pork and as degree of doneness increases, enhancement has been shown to have a protective effect on pork palatability. While the effect of quality attributes, degree of doneness and enhancement on consumer perception have been individually studied, the interaction of these effects on consumer perception has not been addressed. The present study was conducted as a cooperative effort between the National Pork Board, The Ohio State University and Texas A&M University, with assistance from colleagues at Iowa State University. Objectives of the study were to evaluate the independent and potential interactive influences of industry standard measures of pork quality (Minolta color (L*), ultimate pH (pH), and intramuscular fat (IMF)), cooked pork Warner-Bratzler shear force (WBSF), endpoint cooked temperature (62.8°C (145°F); 68.3°C (155°F); 73.9°C (165°F); and 79.4°C (175°F)), and enhancement (10% pump rate, 2.5% potassium lactate, 0.35% sodium phosphate, and 0.35% salt) on consumer's perceptions of *longissimus* (LM) palatability. As only one cooking method could be used in the consumer study, the effect of cooking method (clam-style cooker, open-hearth grill, and conventional grill) across the pork quality, temperature and enhancement effects on WBS, color, myoglobin denaturation and pH of LM chops was evaluated.

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MATERIALS AND METHODS

Pork Quality Benchmark Consumer Study

Loins (n = 1340 nonenhanced and n = 455 enhanced) were collected within 3 US packing plants (J.H. Routh Packing, Sandusky, OH; Sioux Preme Packing Company, Sioux City, IA; Hormel Foods, Austin, MN). For selection purposes, a 3 × 3 × 3 categorical classification arrangement of pH, marbling score (NPPC, 2000) and Minolta L* color was used to create a near uniform representation LM quality combinations. Within one packing plant, loins were paired based on LM quality and alternately assigned within a pair to enhancement or nonenhancement treatments. Following estimation of chemical IMF, loins were re-categorized into subclasses defined by fresh 0.10 unit pH, 1% IMF, and 3.9 unit L* increments and selected for testing as a near uniform distribution of LM quality combinations within each plant (n = 228, 228, and 223 nonenhanced loins per packing plant; n = 228 enhanced loins). Whole, boneless loins were collected at approximately 24 h postmortem and cut at the 7th rib for LM quality assessment. Loin pH (HI98240, Hanna Instruments, Italy) was measured using a glass-tipped pH probe (FC201D, Hanna Instruments, Italy) inserted approximately 1 cm under the cut surface in the center of the exposed LM surface. After a 10 min bloom, visual (NPPC, 2000) and instrumental loin color (Minolta L*; Minolta Colormimeter, Model CR-310, 50 mm diameter orifice, 10° standard observer, D^{65} light source; Minolta Company, Ramsey, New Jersey) and

visual marbling scores (NPPC, 2000) were collected by trained personnel. A 1.25 cm-thick section of loin was cut immediately posterior to the 7th rib, subcutaneous fat and connective tissue removed, and the remaining LM sample used for at the Research and Development laboratory of the cooperating packer using needle injection. Weights were collected before individual vacuum sealing for the nonenhanced treatment and before and following enhancement for the enhanced treatment. Loins were transported to The Ohio State University Meat Science Laboratory, Columbus, OH, stored and aged at 2°C for 7 to 10 d.

Beginning at the anterior end, the LM was sliced into 12, 2.54 cm-thick chops that were randomly assigned to 3 destinations (consumer sensory evaluation, trained sensory evaluation, or Warner- Bratzler Shear Force [WBSF] assessment) and within destination to 4 endpoint cooked temperatures (62.8, 68.3, 73.9 or 79.4°C). Allocated chops were individually vacuum-packaged and frozen at –28.8°C.

For WBSF, chops thawed for 48 h at 2°C and cooked using a clam-style cooker (George Foreman grill) to the defined internal temperature. Internal temperature was monitored with copper constant thermocouples inserted into the geometric center of each chop (Digi-sense, Model # 277653 or equivalent). Chops were cooled for 4 h and six 1.27 cm diameter cores were removed. Cores were sheared with a Warner-Bratzler shearing device (Model TA.XT2^{plus} Texture Technologies, Scarsdale, New York) with a probe travel distance of 40 mm from the base at a test speed of 3.33 mm/s.

Consumer recruitment was conducted via telephone interview with parameter targets to include: 1) primary household grocery shoppers, 2) females aged 25 to 49, 3) annual household income of $30,000+, and 4) presence of children under 16 in the household. Male pork consumers were included to represent 35% to 40% of the total respondents. Consumer taste panels were conducted in Chicago IL, Philadelphia, PA, and Sacramento, CA, with 760 consumers secured per site (n = 2280 total consumers). Consumer taste panel sessions were conducted over 2 consecutive weeks within each city (n = 20 consumers per session, 38 sessions per city).

Each consumer was provided samples from 8 different chops, and 5 different consumers assessed samples from each chop. Consumers evaluated 2 enhanced chops and 2 chops from each plant. Chops were randomized to vary in quality parameters within a consumer. Consumer data analyses were conducted in 2 steps: 1) data representing nonenhanced chops were assessed as a set, and 2) data comparing the enhanced chops with the nonenhanced chops collected within the same packing plant were analyzed as a set.

Chops were cooked using a clam-style cooker (George Foreman grill) to the designated internal temperature as described previously for WBSF assessment. Immediately after cooking, chops were cut into 1.27 cm width × 1.27 cm length × 2.54 cm height cubes and consumers were provided 2 cubes per chop. Samples were served under red incandescent lighting to minimize potential degree of doneness color appearance bias. Consumers cleansed their pallets prior evaluating samples with an unsalted saltine cracker and distilled water. The consumer ballot consisted of 7 questions using 8-point, end anchored Hedonic scales. Following the ballot order, the questions were: Overall Like/Dislike, Juiciness Like/Dislike Juiciness, Level of Juiciness, Tenderness Like/Dislike, Level of Tenderness, Flavor Like/Dislike, and Level of Flavor where 1 = dislike extremely, dislike extremely, extremely dry, dislike extremely, extremely tough, dislike extremely, and extremely bland or No Flavor and 8 = like extremely, like extremely, extremely juicy, like extremely, extremely tender, like extremely and extremely flavorful, respectively. Data for Like/Dislike questions will be presented. The final ballot question asked 'How likely would you be to purchase this sample if it were available at a reasonable price in your area?' Likelihood of Purchase response options were: Definitely Would Not Buy, Probably Would Not Buy, May or May Not Buy, Probably Would Buy, and Definitely Would Buy. For analyses, responses were labeled 1 through 5, with a score of 1 representing Definitely Would Not Buy and a score of 5 representing Definitely Would Buy.

Cooking Method Study

Enhanced (n = 657) and nonenhanced (n = 1539) pork loin chops varying in pH, lipid content and Minolta L* color values as described previously were randomly assigned to one of 4 cooking methods (gas grill, clam-shell grill, Hamilton Beach electric grill or oven broiling) and 4 cooked temperature endpoint temperatures (62.4, 68.3, 73.9, and 79.4). Internal cook temperature was monitored as previously defined and cook time and cook yield was determined. Chops were cooked as described previously for clam-shell grill. The Hamilton Beach indoor/outdoor grill (Model 31605A**)** at 325°F surface temperature was used for the Hamilton Beach cooking method. Oven broiling was conducted in an electric oven; chops were placed on a serrated metal pan placed 10.2 cm from the electrical heating source. Chops assigned to gas grill were grilled on a covered propane commercial grill at a temperature of 325°F and chops were cooked with the lid on. Subjective internal cook temperature was assessed by 2 trained evaluator where 0 = extremely rare, majority of the surface area is dark purplish red and 5 = well done, no visible pink and the surface area is gray. Percent myoglobin denaturation, pH, Minoltal L*, a* and b* color space values for the internal cooked surface were determined but results will not be discussed.

Statistical Analyses

Consumer data were analyzed using ordered logistical regression through STATA software (StataCorp, LP, College

Station, TX) and the output parameters summarized using CLARIFY V 2.1 (King et al., 2000). Dependent variables included all consumer response variables. Preliminary statistical models for nonenhanced chops tested the continuous independent variables: cooked temperature, pH, IMF, L*, a*, b*, WBS, NPPC color, and NPPC marbling as linear and quadratic effects, and potential 2-way interactions among independent variables were tested. Plant of origin and city of testing were included as independent effects and, where significant, the effects were accounted for in reporting of the results. A linear covariate for the deviation of cooked temperature from the designated target endpoint temperature was tested in all analyses, found to not be significant, but was maintained in all final models to correctly assess temperature treatment effects. For the analysis comparing enhanced and nonenhanced consumer responses, enhancement treatment and enhancement × cooked temperature interactions were added to the preliminary models while plant of origin was removed from the model. The regression model solutions were used in CLARIFY to estimate predicted mean response levels and predicted consumer response proportions for, and encompassing the range of, each significant ($P < 0.05$) independent effect. Predicted mean consumer responses for a given palatability attribute represent the effect of an incremental change (determined by the authors for each trait) in a given quality trait, while maintaining the remaining significant independent model effects at their respective mean levels.

The cooking method study data were analyzed using Proc Mixed of SAS (v. 9.2, Cary, NC) with cook day (n = 70) included as random effect and cooking method, endpoint temperature, pH category, and their subsequent interactions included in the model. Least squares means were calculated and if differences were found in the Analysis of Variance table ($P < 0.05$), then least squares mean differences were determined using the pdiff function. Interactions were removed from the model if $P > 0.25$.

PORK QUALITY BENCHMARK CONSUMER NONENHANCED RESULTS AND DISCUSSION

Descriptive statistics for fresh pork loin quality attributes, WBSF at each end-point cooked temperature, and consumer responses are provided for nonenhanced (Table 1) loins. Independent effects included in the final models and their significance for the prediction of consumer response variables is presented in Table 2. No interactions or quadratic effects were observed among independent measures tested within the nonenhanced consumer analysis. With a lack of interaction and quadratic effects and given the overall large sample size of the present study, small differences among independent variables were found to be significant. Consumer responses were a linear function of a combination of LM quality traits; IMF, pH, WBSF, and cooked temperature endpoint influenced all consumer responses except flavor. Minolta L*, a* and b* and visual color measurements were not a significant contributors to variation in consumer responses. As moderate correlations between pH and L* (r = –0.58) and visual color (r = 0.62) were found, the effects of L* color space values may have been partially accounted with the inclusion of pH. The lack of interactions and quadratic effects for LM quality traits suggests that there is no evidence of dependencies among LM quality traits used and that there were not threshold levels for a given LM quality trait. Our results suggest that the optimal eating experience is a combination of greater pH and IMF; and lower cooked temperature and WBSF. However, our results are presented as a reflection of each individual LM quality trait with all other LM quality traits at their respective sample mean values. All consumer means are predicted consumer responses from the model.

Correlations among consumer responses to like and level questions within a consumer palatability attribute were, as expected, very large and positive (tenderness, r = 0.92; juiciness, r = 0.87; and flavor = 0.88), contributing to similar regression solutions and predicted responses for a given LM quality trait. Relationships between con-

Table 1. Characterization of loin quality attributes and consumer response variables for nonenhanced loins

Trait	N	Mean	SD	Range
Ultimate pH	679	5.76	0.23	5.34–6.50
Minolta L*	679	52.82	4.28	40.91–65.4
Minolta a*	679	17.42	1.38	11.70–21.02
Minolta b*	679	5.14	1.34	1.93–10.6
NPPC color,[1] 1 to 6	679	3.13	1.01	1.00–5.00
Intramuscular fat, %	678	3.06	1.37	0.43–6.93
NPPC marbling,[1] 1 to 6	679	2.52	1.27	1.00–6.00
Loin purge loss, %	679	1.97	1.92	–4.05 to 10.62
Warner-Bratzler Shear, kg				
Cooked temperature				
62.8°C	678	2.51	0.60	1.26–4.97
68.3°C	676	2.64	0.76	1.23–6.84
73.9°C	677	2.75	0.78	1.24–7.01
79.4°C	675	2.88	0.85	1.46–6.43
Consumer response variables[2]				
Overall dislike/like	13,190	4.84	1.87	1–8
Juiciness dislike/like	13,232	5.15	1.89	1–8
Tenderness dislike/like	13,237	4.92	1.97	1–8
Flavor dislike/like	13,234	4.47	1.92	1–8
Likelihood of purchase[3]	13,183	2.90	1.21	1–5

[1]National Pork Producers Council (NPPC) color and marbling standards (2000).
[2]Consumer responses measured on an 8-point, end-anchored scale.
[3]Consumer responses measured on a 5-point scale.

Table 2. Ordered logistical regression model effects and significance levels for consumer loin eating quality response variables measured on nonenhanced pork loins[1]

	Consumer response[3]				
Model effect[2]	Overall like	Juiciness like	Tenderness like	Favor like	Likelihood of purchase[4]
Cooked temperature, ° C	$P = 0.025$	$P < 0.000$	$P < 0.000$	$P < 0.556$	$P < 0.000$
62.8	4.97	5.43	5.10	4.56	3.01
68.3	4.93	5.28	5.00	4.55	2.97
79.9	4.90	5.13	4.91	4.54	2.93
79.4	4.87	4.97	4.82	4.54	2.89
Intramuscular fat, %	$P < 0.000$	$P < 0.001$	$P = 0.018$	$P = 0.049$	$P < 0.000$
1	4.79	5.14	4.90	4.40	2.86
2	4.85	5.16	4.93	4.47	2.90
3	4.91	5.20	4.96	4.54	2.95
4	4.97	5.24	4.99	4.62	2.99
5	5.03	5.28	5.02	4.69	3.03
6	5.09	5.32	5.05	4.76	3.07
pH	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$
5.40	4.69	4.84	4.59	4.37	2.82
5.60	4.81	5.04	4.79	4.47	2.89
5.80	4.94	5.24	4.99	4.57	2.96
6.00	5.07	5.43	5.19	4.66	3.03
6.20	5.19	5.62	5.39	4.76	3.10
6.40	5.31	5.81	5.58	4.86	3.17
Warner-Bratzler Shear force, kg	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$
1.50	5.36	5.69	5.64	4.86	3.23
2.00	5.10	5.49	5.36	4.73	3.11
2.50	4.99	5.28	5.07	4.60	2.99
3.00	4.80	5.07	4.78	4.47	2.88
3.50	4.61	4.86	4.48	4.33	2.76
4.00	4.41	4.64	4.18	4.20	2.64
4.50	4.22	4.42	3.89	4.07	2.52
5.00	4.03	4.21	3.60	3.94	2.41
5.50	3.83	3.99	3.32	3.81	2.30
6.00	3.64	3.77	3.06	3.68	2.19

[1]Packing plant of loin origin and consumer test cities are accounted for in the ordered logistic regression.
[2]Modeled effect of incremental changes, remaining model effects at their respective mean.
[3]Consumer-Like responses measured on an 8-point, end-anchored scale: 1 = dislike extremely, 8 = like extremely.
[4]Responses measured on a 5-point scale: 1 = definitely would not buy, 3 = may or may not buy, 5 = definitely would buy.

sumers rating of Overall-Like were strongest in relation to Tenderness-Like (r = 0.73), Flavor-Like (r = 0.79) and Likelihood of Purchase (r = 0.78) and somewhat weaker with respect to Juiciness-Like (r = 0.65). Only consumer predicted responses for Like attributes will be presented and discussed to reduce redundancy.

The influence of incremental changes in cooked temperature, IMF, ph, and WBSF for dependent consumer response variables are presented in Table 2. Increasing cooked temperature reduced Juiciness-Like, Tenderness-Like and Overall-Like by 0.15, 0.10 and 0.03 units, respectively, for each 5.5°C unit increase in cooked tem-

perature. In contrast, increasing cooked temperature had no influence on flavor score. Predicted mean responses for consumer variables on the 8-point response scale were very close to a score of 5 for each temperature evaluated, a consumer rating of like slightly. This finding indicates that, regardless of end-point cooked temperature, consumer responses were marginal with respect to their perception of how they liked the nonenhanced pork. These results indicate that as degree of doneness decreased for nonenhanced cooked pork chops consumer perceptions of juiciness and tenderness improved, but consumer perception of flavor was not affected. This was similar to results reported by Prestat et al. (2002). Likelihood of purchase results indicated consumer responses on average were neutral or noncommittal when cooked temperature was incrementally increased

As IMF increased, consumer responses incrementally improved in a linear pattern, but improvement was minimal particularly for juiciness and tenderness responses. However, when comparing the highest (6% IMF) with the lowest (1% IMF) means responses, consumers Overall-Like and Flavor-Like increased. These findings support research by Rincker et al. (2008) who reported that IMF had no practical impact on eating quality of pork chops. While the greatest levels of IMF were preferred when compared with the lowest levels, the cost associated with moving populations of pigs from lower to the upper end of the IMF range may not be economically feasible for commercial production. Targeted markets based on customers who are willing to pay a premium for improved quality may see an economic advantage in the production of pork with high levels of intramuscular fat. The impact of increasing IMF on consumer probability of purchase, while significant, was small with responses centered on neutrality, again offering insight that the consumer attitudes toward pork were indifferent with changing levels of intramuscular fat.

As ultimate pH increased from 5.40 to 6.40, predicted mean consumer responses increased and pH category influenced consumer responses to a greater extent than either cooked temperature or IMF levels. Predicted responses for Juiciness-Like and Tenderness-Like increased by 0.20 units for a 0.20 unit increase in pH (1.0 unit increase across the pH range) when pH ranged from 5.4 to 6.4. Ultimate pH is related to water holding capacity (Aberle et al., 2001) and cook loss (Lonergan et al., 2007) which supports the observation that the consumers rated pork with greater ultimate pH more favorably for Juiciness-Like. Predicted mean responses for Overall-Like and Flavor-Like increased as pH increased and there was an increase of about 0.5 units in consumer like as pH ranged from 5.40 to 6.40. When viewed across all consumer response variables, ultimate pH played a large role in impacting consumer preferences than IMF and cooked temperature endpoint. Consumer satisfaction would expectantly be lower for pork that is near an ultimate pH of 5.40. Consumer ratings for likelihood of purchase followed similar trend as discussed for other consumer attributes. Research has addressed how and what determines loin ultimate pH, but no definitive strategy has been identified to consistently increase loin pH to the mean level, much less the upper level of pH described, indicating that methods to increasing pH are somewhat tenuous

As WBSF increased, consumer ratings decreased from a very neutral rating for chops with the lowest WBSF (1.50 kg) to a very unfavorable level at the highest WBS level (6.00 kg). Consumer ratings of Tenderness-Like were predicted to be greater than 5.00 when WBSF was ≤2.50 kg, a value near the average of the pork measured in this study. Pork chops with a 4.00 kg WBSF had consumer Tenderness-Like ratings near 4.10 and as WBSF level increased to 6.00 kg, consumer ratings continued to declined to near 3.0. Correlations between WBSF and the consumer rating for Tenderness-Like ($r = -0.27$) were moderate, but indicative that consumer ratings decreased as WBSF increased. A WBSF level of ≤2.50 kg was needed before consumer responses for Tenderness-Like, Overall-Like and Juiciness-Like to be 5 or higher or slightly favorable. The large incremental changes in consumer responses that reflect the sizable changes in WBS tenderness provide strong evidence that tenderness is one of the primary contributors to a consumer's perception of pork eating quality.

PORK QUALITY BENCHMARK CONSUMER NONENHANCED CONCLUSIONS

Consumer perceptions of pork eating quality were greatly influenced by and reflective of differences in fresh pork ultimate pH and cooked pork Warner-Bratzler Shear force. Cooked temperature endpoint and IMF influenced consumer ratings, but there influences were smaller. The absence of significant interactions among and between quality indicators and end-point temperature suggests that, for nonenhanced pork loin, consumer's perceptions of eating quality (flavor, tenderness, juiciness, overall desirability) would be optimized in a fresh pork loin with greater pH and IMF, lower cooked WBS, and a chop that is cooked to a lower degree of doneness.

PORK QUALITY BENCHMARK CONSUMER ENHANCED AND NONENHANCED RESULTS AND DISCUSSION

Descriptive statistics for sampled pork loin quality attributes for enhanced and nonenhanced loins (Table 3) show that loins across the enhancement treatment had similar LM quality traits. Model effects and significance levels (Table 4) indicate that there as a cooked temperature and enhancement treatment interaction for consumer preference attributes that resulted in a slight change in the magnitude of the difference between enhanced and nonenhanced chops across pork quality traits, but predicted means did not change in rank. Quadratic pH was the only nonlinear effects observed, and there was a slight plateau for consumer responses as loin pH increased.

Table 3. Summary statistics for nonenhanced (n = 228) and enhanced (n = 227) loins served in consumer taste panels

	Nonenhanced				Enhanced			
Item	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
NPPC color,[1] 1 to 6	3.08	1.07	1.00	6.00	3.22	1.03	1.00	6.00
NPPC marbling,[1] 1 to 6	3.08	1.35	0.49	6.86	3.15	1.40	0.22	6.84
Ultimate pH	5.78	0.23	5.34	6.48	5.78	0.24	5.34	6.65
Minolta L*	53.77	4.45	43.80	65.40	53.13	4.51	41.00	67.50
Minolta a*	17.26	1.32	13.60	21.02	16.79	1.33	13.00	20.40
Minolta b*	4.44	1.03	2.00	6.80	4.34	1.08	2.00	7.30
Intramuscular fat, %	3.06	1.34	0.49	6.86	3.15	1.40	0.22	6.84
Loin purge, %	2.62	2.08	0.00	10.62	3.77	1.41	1.08	7.56
Post-pump pH	—	—	—	—	5.91	0.22	5.24	6.47
Warner-Bratzler Shear, kg								
62.8°C	2.37	0.51	1.29	3.99	1.67	0.41	0.97	3.41
68.3°C	2.44	0.54	1.23	4.32	1.65	0.43	1.00	3.45
73.9°C	2.62	0.67	1.34	5.50	1.62	0.37	0.88	3.32
79.4°C	2.78	0.75	1.53	5.94	1.72	0.42	1.04	3.55

[1]National Pork Producers Council (NPPC) color and marbling standards (2000).

Enhancement of LM improved all consumer responses across the primary pork quality indicators (IMF, pH, WBSF) as well as cooked temperature (Table 5). The enhancement × cooked temperature interaction resulted in a slight reduction in nonenhanced chop ratings as cooked temperature increased and either a slight improvement or no change in consumer ratings for enhanced chops as cooked temperature increased. At the lowest cooked temperature, the difference between enhanced and nonenhanced chops for Overall-Like was 0.88 units and increased to 1.13 units at the greatest cooked temperature.

Enhancement improved consumer ratings for Flavor-Like by about 1.20 unit across the range of each LM quality trait (data not presented). Improved flavor most likely was the result of the addition of salt from the enhancement brine. Vote et al. (2000), Prestat et al. (2002), and Keeton (1983) found that salt addition improved flavor in enhanced beef and pork meat. Nonenhanced chops were clearly less flavorful and the effect of enhancement im-

Table 4. Model effects and significance levels of ordered logistic regression analyses of consumer response variables for pork loin eating quality of nonenhanced and enhanced loins

	Consumer response variable, *P*-value				
Model Effect	Overall like	Juiciness like	Tenderness like	Flavor like	Likelihood of purchase
Cooked temperature	0.364	0.000	0.011	0.929	0.005
Enhancement	0.000	0.000	0.000	0.013	0.000
Enhancement × temperature interaction	0.008	0.000	0.007	0.000	0.000
Intramuscular fat	0.000	0.002	0.287	0.041	0.004
Ultimate pH	0.000	0.000	0.004	0.000	0.008
Ultimate pH–quadratic	0.001	0.000	0.007	0.001	0.010
Minolta L*	0.423	0.892	0.167	0.617	0.094
Warner-Bratzler Shear	0.000	0.000	0.000	0.000	0.000
City of testing	0.000	0.000	0.181	0.000	0.005

proved flavor perception substantially with a larger impact as cooked temperature increased (Table 5). The difference between enhanced and nonenhanced consumer scores for Tenderness-Like increased from 0.80 units at 62.8°C to 1.08 units at 79.4°C indicating that enhancement protected against heat-induced toughening. Overall, as internal cook temperature increased, consumer rating generally decreased across all consumer attributes in nonenhanced pork chops as previously reported, but enhancement mitigated the negative effects of cooking to higher internal temperatures on consumer preferences.

Loin ultimate pH effects were quadratic for all consumer attribute responses. Enhanced and nonenhanced pork chops with a 5.40 pH had less favorable consumer ratings than pork chops with higher pH. Optimal loin pH levels varied across attributes, reflecting slightly different points of inflection across the pH range (Table 5). Predicted mean responses for Juiciness-Like were lowest for pork chops in the 5.40 and 5.60 pH classes, indicating that low ultimate pH reduced consumer juiciness perception, likely due to the lower inherent water holding capacity capabilities of the loin, regardless of enhancement. AS pH increased, consumer Tenderness-Like mean responses increased and were most favorable at a pH of ~6.20 (5.47 units and 6.38 units for nonenhanced and enhanced loins, respectively). Predicted means for Flavor-Like were the highest for enhanced (5.81) and nonenhanced (4.61) chops at a loin pH of 5.80 and 6.00. Predicted mean consumer responses for

Table 5. Predicted[1] means for consumer assessment of pork eating quality measured on enhanced (E) and nonenhanced (N) pork loins measured across loin cooked temperature, pH, and intramuscular fat levels

	Cooked temperature, °C							
	62.8		68.3		73.9		79.4	
Consumer response[2]	N	E	N	E	N	E	N	E
Overall like	5.12	6.00	5.10	6.06	5.08	6.12	5.05	6.18
Juiciness like	5.61	6.34	5.48	6.35	5.36	6.36	5.23	6.38
Tenderness like	5.39	6.20	5.33	6.22	5.27	6.26	5.20	6.28
Flavor like	4.61	5.71	4.61	5.77	4.61	5.84	4.60	5.91
Likelihood of purchase[3]	3.13	3.71	3.08	3.75	3.03	3.78	2.99	3.82

	pH											
	5.40		5.60		5.80		6.00		6.20		6.40	
	N	E	N	E	N	E	N	E	N	E	N	E
Overall like[xy]	4.77	5.81	4.97	5.99	5.09	6.10	5.13	6.13	5.08	6.08	4.94	5.97
Juiciness like[xy]	5.06	6.26	5.28	6.24	5.43	6.36	5.49	6.41	5.46	6.39	5.36	6.30
Tenderness like[xy]	4.97	5.96	5.20	6.16	5.36	6.29	5.44	6.36	5.46	6.38	5.42	6.34
Flavor like[xy]	4.37	5.59	4.53	5.74	4.61	5.81	4.60	5.81	4.50	5.72	4.32	5.56
Likelihood of purchase[3,xy]	2.88	3.59	2.99	3.70	3.06	3.76	3.09	3.79	3.08	3.78	3.02	3.73

	Intramuscular fat, %											
	1		2		3		4		5		6	
	N	E	N	E	N	E	N	E	N	E	N	E
Overall like[xy]	4.96	5.98	5.02	6.03	5.08	6.08	5.14	6.14	5.20	6.19	5.26	6.24
Juiciness like[xy]	5.32	6.27	5.37	6.31	5.41	6.35	5.46	6.39	5.51	6.43	5.55	6.47
Tenderness like[x]	5.26	6.21	5.28	6.23	2.30	6.24	5.33	6.25	5.34	6.27	5.37	6.28
Flavor like[x,y]	4.46	5.68	4.53	5.74	4.59	5.80	4.67	5.86	4.73	5.92	4.80	5.98
Likelihood of purchase[3,xy]	2.99	3.70	3.03	3.73	3.06	3.76	3.09	3.79	3.12	3.81	3.15	3.84

[x]Main effect of enhancement significant ($P < 0.01$)

[y]Main effect of pH or intramuscular fat percentage significant within designated rows ($P < 0.05$).

[1]Independent effect of a main effect with remaining LM quality effects adjusted to their respective mean value.

[2]Consumer responses assessed using an end-anchored, 8-point Hedonic scale; like variables: 1 = dislike extremely, 8 = like extremely.

[3]Likelihood of purchase: 1 = definitely would not buy, 3 = may or may not buy, 5 = definitely would buy.

Overall-Like were optimized at a loin pH between 5.80 and 6.20.

Intramuscular fat effects were significant for Overall- and Juiciness-Like ratings within enhanced and nonenhanced loins; however, the effect of increasing IMF were very small and likely of practical value only when comparing a chops with 1% and 6% IMF. Tenderness-Like was not affected by IMF. These results do not support a threshold level for IMF or that IMF is a major contributor to consumer overall acceptability when consumers evaluate enhanced and nonenhanced pork loins. Most likely, enhancement mitigates the slight effect reported for nonenhanced pork chops previously discussed.

Increasing WBSF in both enhanced and nonenhanced chops reduced predicted mean responses for each consumer descriptive attribute and the effect of WBSF represented the largest overall impact on consumer responses. Enhanced chops were more tender than nonenhanced chops and as cooked temperature increased, enhanced and nonenhanced chops increased in WBSF. Enhanced and nonenhanced chops cooked to 62.8°C differed in WBSF by 0.7 kg and when cooked to 79.4°C, WBSF differed by 1.05 kg. There was a smaller range and a reduction in the maximum level of WBSF evaluated within the enhanced chop data set. Results of logistical regression analyses were projected to reflect the range of WBSF observed in the nonenhanced subset of data, and therefore reflect an extension of the regression line for enhanced product to reflect nonenhanced WBSF variation.

Predicted mean consumer responses for Juiciness Like (Figure 1) indicated a reduction in consumer ratings of approximately 0.20 units for each 0.50 kg incremental increase in WBSF for both enhanced and nonenhanced chops. These results indicated that Juiciness-Like is influenced by the level of tenderness/toughness. The most tender WBSF category (1.5 kg) had Juiciness-Like ratings 1.78 and 1.61 units higher than the toughest WBS category (6.0 kg) for nonenhanced and enhanced chops, respectively. In addition, enhancement improved Juiciness-Like ratings by approximately 1 consumer unit. The correlation between WBSF and Juiciness Like rating in the present study was r = −0.19 and r = −0.09 for nonenhanced and enhanced chops, respectively, which were generally low.

The relationships between WBSF and the direct assessment of Tenderness-Like by the consumer (Figure 2) were negative (r = −0.22, nonenhanced chops; r = −0.11, enhanced chops). For nonenhanced chops, a 2.53 unit (31.6%) reduction in consumer ratings on the 8-point scale was observed when comparing tender (1.5 kg) to very tough (6.0 kg) pork. For enhanced chops, predicted mean responses declined by 2.41 units (30.1%) when comparing 1.5 and 6.0 kg WBS levels indicating a similar rate of decline for the nonenhanced chops; however, the chops from enhanced loins had 1.0 unit greater predicted Tenderness-Like mean rating at each point across the range of WBSF categories studied. With a response level of 5 representing the first full unit on the favorable side of the response scale, the enhancement treatment allowed chops to reach a WBSF level of up to 4.0 kg before the predicted mean responses declined to less than 5 on the scale. In contrast, chops from nonenhanced loins needed a WBSF level of ≤2.5 kg to maintain a predicted mean consumer response that was greater than 5.

Increasing WBS from 1.5 to 6.0 kg reduced the predicted mean consumer responses for Overall-Like (Figure 3) for nonenhanced chops 1.51 units (18.9%) and 1.4 units

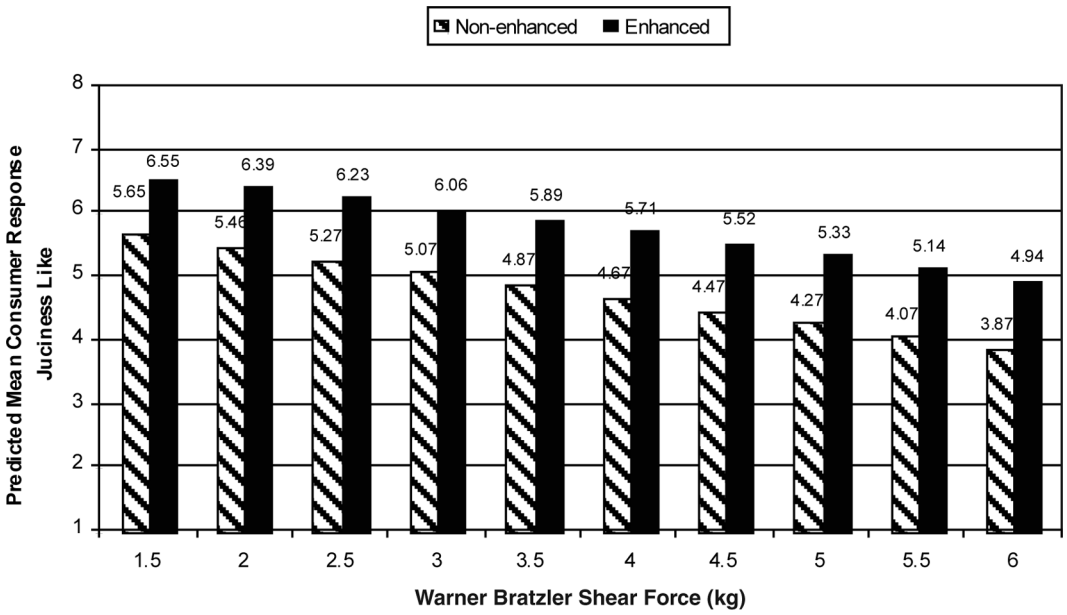

Figure 1. Predicted mean consumer responses for Juiciness-Like of nonenhanced and enhanced chops across Warner-Bratzler Shear Force values. (*Please note that these are preliminary results and do not necessarily represent the final results.*)

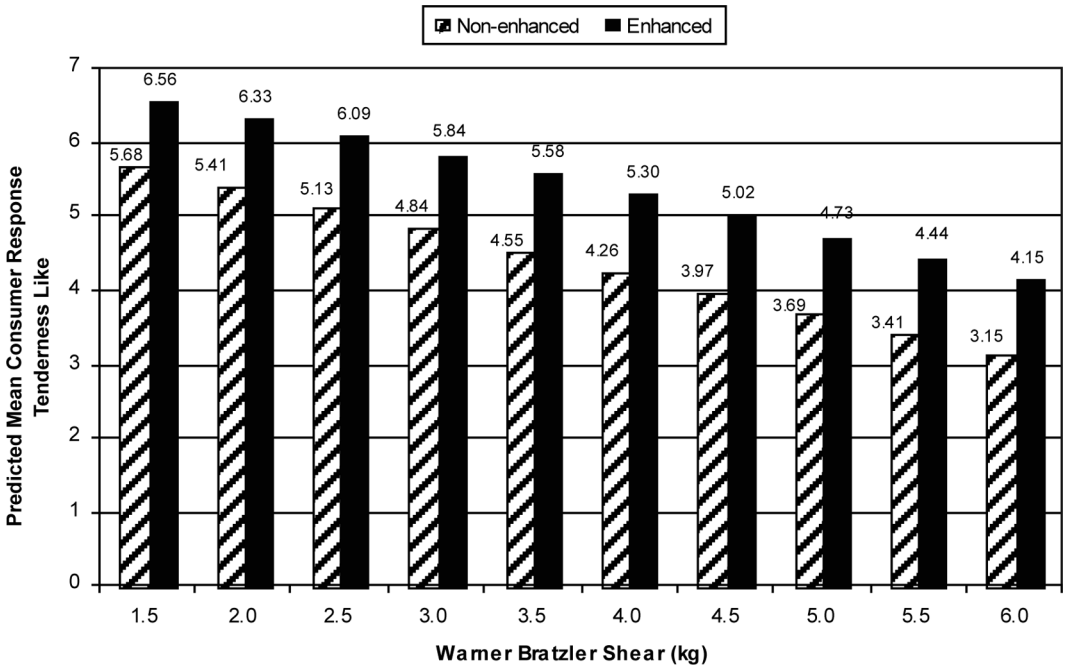

Figure 2. Predicted mean consumer responses for Tenderness-Like of nonenhanced and enhanced chops across Warner-Bratzler Shear Force values. (*Please note that these are preliminary results and do not necessarily represent the final results.*)

(17.5%) for enhanced chops, indicating a culmination of results described previously for juiciness and tenderness, and flavor related descriptive attributes. When assessing Overall-Like at the average WBSF of nonenhanced chops (~2.5 kg) the predicted mean response for Overall-Like was near 4.96 while at the mean WBSF of enhanced chops (~1.6 kg) the enhanced chops were rated near 6.20. Similar to previous discussions regarding WBSF, enhancement created a shift in the acceptable level of WBSF as it relates to the consumer's rating for Overall-Like. To achieve a predicted mean response of 5 on the 8-point scale, nonenhanced chops needed to have a WBSF value of ≤2.5 kg while enhanced chops would receive a predicted mean rating of 5 or greater with a WBSF value of 5.5 kg or less.

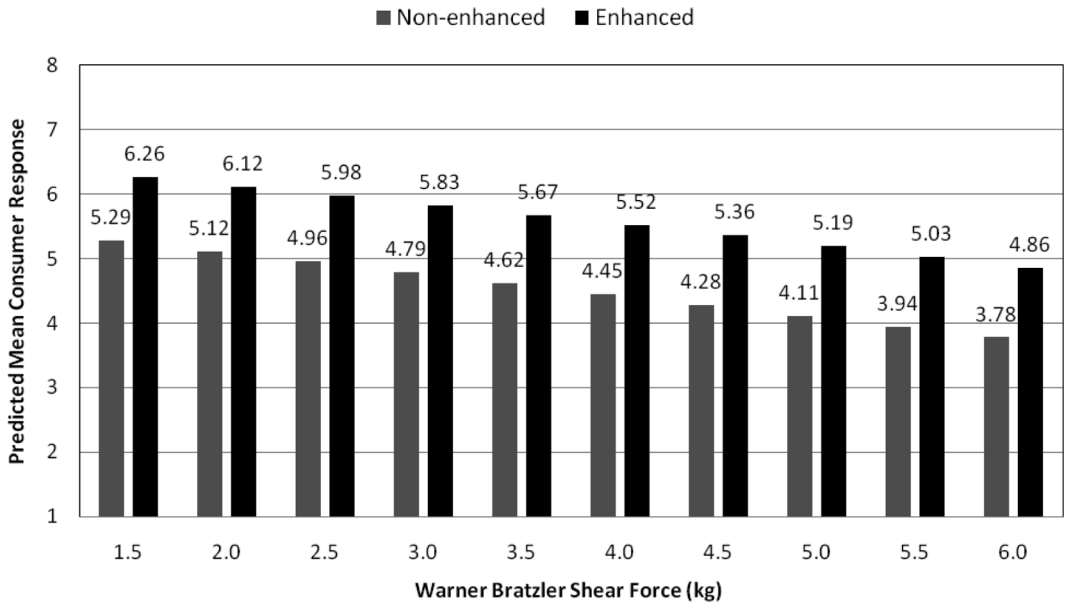

Figure 3. Predicted mean consumer responses for Overall-Like of nonenhanced and enhanced chops across Warner-Bratzler Shear Force values. Consumer Response, end-anchored, 8-point Hedonic Scale: 1 = extremely dislike, 8 = extremely like. (*Please note that these are preliminary results and do not necessarily represent the final results.*)

PORK QUALITY BENCHMARK CONSUMER ENHANCED AND NONENHANCED CONCLUSIONS

Consumer perceptions of pork loin eating quality and their likelihood of purchase were clearly improved as the result of enhancement across the range of loin intramuscular fat, pH, and WBSF. In direct comparisons, increasing cooked temperature of nonenhanced chops resulted in small, but consistent, reductions in consumer satisfaction. For enhanced loins, as cooked temperature increased, consumer ratings were either not changed or improved slightly indicating a need for different target end-point cooking recommendations for enhanced and nonenhanced product to maximize consumer satisfaction. Intramuscular fat effects were linear in relation to consumer perceptions of eating quality traits, but the incremental impact was very small and only of value when comparing the ends (1% and 6%) of the range. Optimal loin pH values to maximize consumer preferences were near pH levels of 5.80 to 6.20; and at lower pH, consumer ratings declined, with declines outside the range and most severe decline occurring when evaluated at a pH of 5.40. Tenderness, measured as WBS, had a large impact on consumer preference. As tenderness decreased, consumer ratings decreased incrementally and effects were not as great for enhanced pork chops. Development of an instrument to estimate tenderness on fresh pork products assist the pork industry in meeting consumer satisfaction for improved tenderness of nonenhanced pork chops, as well as providing a tool to assess environmental and or genetic influences that contribute to variation on pork tenderness.

COOKING METHOD RESULTS AND DISCUSSION

In the Pork Quality Benchmark Consumer Study one cooking method was used. Due to the size of the study and the logistical inability to evaluate different cooking methods, a companion study was conducted to understand the effects of cooking method on cook yield, internal color as an indicator of cooked pork chop degree of doneness, and WBSF. Pork not utilized in the consumer study, but selected, processed and evaluated as a component of the consumer study, were used in this study.

Table 6. Least squares means for cook yield, subjective color and Warner-Bratzler shear force for nonenhanced and enhanced pork chops cooked as affected by cook methods, cook temperature endpoint, pH category, and fat category

	Nonenhanced			Enhanced		
Item	Cook yield, %	Internal color[1]	Shear, kg	Cook yield, %	Internal color[1]	Shear, kg
Cook method	<0.0001	<0.0001	<0.0002	<0.0001	<0.0001	0.02
Clam-shell grill	87.95[d]	3.91[b]	2.27[a]	91.77[c]	3.50[b]	1.61[a]
Gas grill	80.18[a]	3.86[b]	2.48[b]	84.52[a]	3.82[c]	1.72[a]
Hamilton Beach grill	87.10[c]	3.29[a]	2.21[a]	91.49[c]	2.57[a]	1.63[a]
Oven broil	85.21[b]	3.82[b]	2.26[a]	89.78[b]	3.49[b]	1.77[b]
Cook temperature endpoint, °C	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	0.09
62.8	87.71[d]	3.21[a]	2.08[a]	91.21[d]	2.63[a]	1.63
68.3	86.17[c]	3.49[b]	2.21[a]	90.24[c]	3.20[b]	1.62
73.9	84.16[b]	3.91[c]	2.42[b]	88.68[b]	3.61[c]	1.74
79.4	82.41[a]	4.27[d]	2.52[b]	87.45[a]	3.95[d]	1.74
pH category	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	0.0003
Low	82.82[a]	4.07[c]	2.42[b]	87.82[a]	3.83[c]	1.82[b]
Med	84.86[b]	3.75[b]	2.45[b]	89.49[b]	3.40[b]	1.69[b]
High	87.66[c]	3.24[a]	2.04[a]	90.87[c]	2.82[a]	1.53[a]
Fat category	0.66	0.003	<0.0001	0.09	0.86	0.02
Low	85.26	3.62[a]	2.52[b]	89.79	3.39	1.67[a]
Medium	85.02	3.80[c]	2.20[a]	88.83	3.34	1.78[b]
High	85.05	3.75[b]	2.20[a]	89.55	3.32	1.60[a]
Residual	4.470	0.847	0.517	3.926	0.948	0.413

[a-d]Least Squares Means within a column and sub-item classification without a common superscript differ ($P < 0.05$).

[1]Subjective internal cooked color: 0 = extremely rare, majority of the surface area is dark purplish red, and 5 = well done, no visible pink and the surface area is gray.

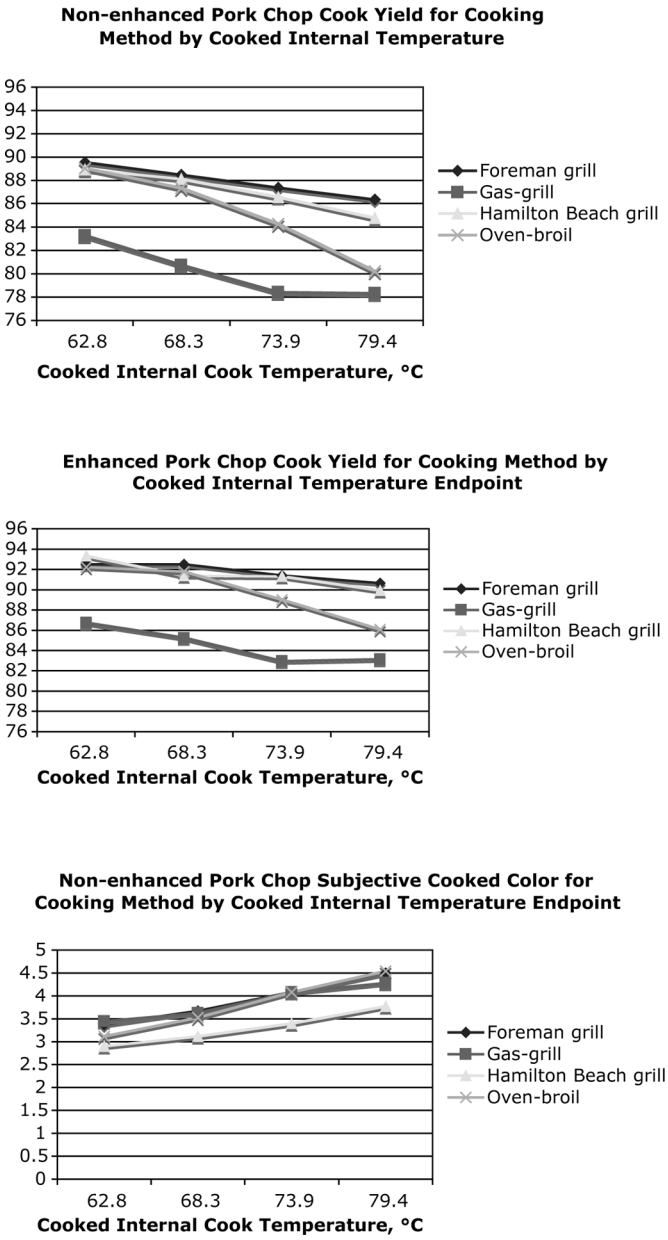

Figure 4. Interactions for cooked Internal cook temperature endpoint and cooking methods for (a) cook yield for nonenhanced, (b) cook yield for enhanced, and (c) internal cooked color for nonenhanced pork chops where 0 = extremely rare, majority of the surface area is dark purplish red, and 5 = well done, no visible pink and the surface area is gray. (*Please note that these are preliminary results and do not necessarily represent the final results.*)

Cook yield, internal color and WBSF for enhanced and nonenhanced pork chops for main effects of pork quality are presented in Table 6. Cook method and cook temperature endpoint affected cook yield, internal cooked color and WBSF for nonenhanced and enhanced pork chops. In general, enhanced pork chops had higher cook yields, lower degree of doneness and WBSF than nonenhanced chops. Pork chops cooked using the clam-shell and Hamilton Beach grills had similar cook yields and WBSF that were higher than chops cooked using other methods, regardless of enhancement treatment. Chops cooked on the gas-grill had the lowest cook yields, highest degree of doneness and WBSF. As cook temperature endpoint increased, nonenhanced and enhanced pork chops decreased in cook yield, had higher visual degree of doneness and were tougher; however, enhanced pork chops were not affected at the same magnitude as nonenhanced pork chops. The interaction for internal cook temperature and cooking method on cook yield are presented in Figure 4a and 4b for nonenhanced and enhanced pork chops, respectively. For chops cooked using the George Foreman or Hamilton Beach grills, cook yields were similar when chops were cooked to either 62.8, 68.3 or 73.9°C; but for pork chops cooked to 79.4°C, chops cooked on the Hamilton Beach grill had slightly lower cook yields than chops cooked to 79.4°F on the clam-shell grills. Oven broiled pork chops cooked to 62.8 or 68.3°C had similar cook yield as pork chops cooked to 62.8 or 68.3°C using either the clam-shell or Hamilton Beach grills. As cook temperature increased to 73.9 and 79.4°C, cook yield decreased concomitantly for oven-grilled pork chops indicating that as pork chops are cooked to higher degrees of doneness, cook yield is affected to a greater extend for oven-broiled pork chops than for clam-shell or Hamilton Beach grilled pork chops. Nonenhanced pork chops cooked using the Hamilton Beach grill had slightly lower degrees of doneness than pork chops cooked using the other cooking methods when cooked to 68.3, 73.9 and 79.4°C.

As pH increased cook yields increased and visual degree of doneness and WBSF decreased for nonenhanced and enhanced pork chops. Fat level or IMF and minimal effects on cook yields and WBSF for enhanced and nonenhanced pork chops, but low fat, nonenhanced pork chops had lower degree of doneness scores than pork chops containing medium and high levels of IMF.

COOKING METHOD RESULTS AND DISCUSSION

Cooking method impacted cook yield, degree of doneness and WBSF; however, enhanced and nonenhanced pork chops had similar attributes to pork chops cooked on a Hamilton Beach electric grill. Consumers more commonly cook pork chops using either oven-broiling or gas-grilling and differences in cook yield, degree of doneness and WBSF were reported in enhanced and nonenhanced pork chops cooked using these methods compared with the clam-shell method. (*Please note that these are preliminary results and do not necessarily represent the final results.*) The question is if cooking method effects reported are substantial enough to impact interpretation of consumer results of the Pork Quality Benchmark Consumer study or if consumers cook using gas-grill or oven-broil where cook yields were lower and the resultant chops are tougher (higher WBSF), do the relationships of enhancement, cook temperature, pH and IMF with con-

sumer preference change. As cooking method impacted WBSF and consumers responded negatively to increases in WBSF, most likely cooking method effects most likely would strengthen or magnify effects reported in the Pork Quality Benchmark Consumer Study. By using the clamshell cooking method, the cooking method with the least effect on cook yield and WBSF, we may have reduced the variability in pork chops across quality traits. If this is true, relationships may have been under estimated, not over estimated. As results were highly significant and data analyses indicated that results were very robust in the Pork Quality Benchmark Consumer Study, utilizing these results across cooking methods to pork consumers will most likely accurately reflect consumer reactions to quality traits of pH, internal cook temperature, IMF and tenderness, and enhancement.

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HACCP Reassessment: Implementation of a Synergistic Intervention System to Reduce or Eliminate Escherichia coli O157:H7 in Frozen Ground Beef Patties

Kurt Sorensen

INTRODUCTION

Maid-Rite Steak Company, Inc. produces several hundred raw and cooked meat and poultry products among 4 separate production facilities. Early in 2007 and in the midst of multiple *E. coli* O157:H7 public health events and recalls, we embarked on reassessing our food safety system with the goal of making our ground beef products significantly safer.

ASSESSMENT

The first step in the reassessment was to assign risk to our product lines. We concluded the focus should be on frozen burgers that are sold to the general public via retail distribution (supermarket chains). Even though stringent Purchase Specification and Approved Supplier Programs were in place and all incoming materials were N-60 sampled and tested negative for E. coli O157: H7, we believed that technologies could be implemented that could further reduce the risk associated with ground beef production. Next, we evaluated production practices and source materials. In all practicality, we needed to maintain flexibility with vendors and the types of raw beef materials purchased. Our grinding practices rely on the ability to utilized multiple types of raw beef components, including fresh beef trim, frozen boneless beef, intact vacuum packaged boneless subprimals, etc. In addition, it is unavoidable for a small beef grinding operation such as ours to limit the scope of point source material purchasing. Traceability exercises show point source lots spreading across multiple days and possibly week's worth of production. Considering our purchasing and grinding practices we evaluated the scope of how a potentially contaminated lot of raw material would impact our operations. To do this we evaluated the well documented "comet" effect theory and considered how applicable the concept is to our grinding operation. The 'comet" effect is supported with studies and data showing that E. coli O157:H7 is not an environmental contaminant; rather, it is introduced by raw material and subsequent raw material will "clean the system out" in time. (See, ICMF book, Microorganisms in Food 7, the contaminated raw materials cause a "comet-like" effect so that the contamination decreases over time to zero as the system cleans itself out.) While applicable and considered in our definition of a lot, we determined that we could not solely rely on pathogenic testing to impart safety into the process because potential cross contamination from food contact surfaces may occur even with the application of the "comet" principle (ICMF 2002). This conclusion resulted in devising a system that redefined and minimized our definition of a "lot". At multiple points during the production day the application of an antimicrobial intervention onto a food contact surface was needed to justify that potential contamination from one point source lot to the next would be eliminated or reduced to a non-detectable level. Enhanced operational sanitation would further support the separation of a new point source lot by minimizing potential cross contamination from food contact surfaces from one lot to the next lot.

Ultimately we determined that systems would need to be established to verify the effectiveness of the enhanced sanitation system and support the "comet" effect in the event of a presumptive positive. One way to accomplish

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this is to establish a finished product statistically based sampling program for E. coli O157:H7.

REASSESSMENT

The outcome of our reassessment defined the elements needed to minimize the risk on multiple fronts. All HACCP, SSOP and prerequisite controls needed to be enhanced to obtain a new level of food safety.

Having flexibility with 3 raw production facilities, we shifted all ground beef production to one facility and initiated a separate purchasing strategy for this facility; thereby, minimizing the potential impact of a supplier initiated event or a consumer initiated food safety event.

The next step was the implementation of enhanced sanitation or flood sanitation in between "sampled lots". The purpose of this enhanced sanitation is to provide multiple points of separation in the production process and reduce or eliminate the possibility of cross contamination from one sampled lot to the next. This enhanced operational sanitation includes the purging of all interim products from the system, rinsing the food contact surfaces with water, and finally the application of a processing aid to all food contact surfaces. We evaluated several processing aids and determined that Acidified Sodium chlorite (ASC) from Biocide International, Inc. provided the functionality we needed for this procedure. After each enhanced sanitation and prior to resuming production, the sanitary conditions are verified through a combination of documented visual inspection and by sponge sampling multiple food contact surfaces and testing for Enterobacteriaceae. This environmental testing helps to verify that food contact surfaces return to a "pre-operational" state between each lot. Additionally a validation study was conducted to evaluate the effectiveness the Acidified Sodium Chlorite (Biocide tm) as applied to food contact surfaces for reducing E. coli O157:H7 and Salmonella. The final report provided by Kansas State University Food Safety & Security demonstrated the effectiveness of an Acidified Sodium Chlorite spray treatment vs. a water rinse in reducing contamination with E. coli O157: H7 and Salmonella on stainless steel food contact surfaces (Figures 1 & 2).

SYNERGISTIC INTERVENTION

After the implementation and verification of the sanitation element to support lot definition we shifted our focus to developing an innovative approach to control potential pathogenic contamination by way of an antimicrobial intervention. The use of "hurdle" technology has advantageous benefits should the most effective antimicrobials be utilized in a multiple intervention approach (Pohlman and McElyea, 2003). However, much of the research conducted to date does not lend itself to a grinding operation, such as ours. The antimicrobial intervention needed to account for the multiple types of raw components, including frozen block of boneless beef.

The first antimicrobial treatment we evaluated and implemented involves using an anti-microbial spray application to beef trim before grinding. Within our ground beef operation we have positioned multiple systems that apply ASC to treatable surfaces of boneless beef. However, the solution needed to account for all components entering the system, including frozen beef.

The main obstacle when trying to decontaminate frozen beef trim is the lack of untreatable surface area. We have addressed this problem by positioning multiple pieces of equipment including chambers that are flooded with high concentrations of ASC that provide decontamination of the entire exterior of the frozen block itself then mechanically rip the block apart to expose more surface area for further decontamination with ASC. To further address the frozen beef components, we began evaluating a second antimicrobial treatment: The UV/PHI Food Sanitation Tunnel (RGF Environmental). This tunnel provides exposure of beef trim or course ground beef to Photohydroionization cells (PHI/cells) technology that include germicidal (UV) Lamps. The UV/PHI cells are designed to catalyze oxidative reactions including the conversion of Oxygen

0 seconds ASC Treatment - Average reductions - 0.2 Log CFU/cm^2
15 seconds ASC Treatment - Average reductions - 1.92 Log CFU/cm^2
30 seconds ASC Treatment - Average reductions - 2.37 Log CFU/cm^2
60 seconds ASC Treatment - Average reductions – 3.42 Log CFU/cm^2

Figure 1 : E. coli O157:H7.

0 seconds ASC Treatment – Average reductions – 0.1 Log CFU/cm^2
15 seconds ASC Treatment – Average reductions – 2.02 Log CFU/cm^2
30 seconds ASC Treatment – Average reductions – 2.60 Log CFU/cm^2
60 seconds ASC Treatment – Average reductions – 3.91 Log CFU/cm^2

Figure 2: Salmonella.

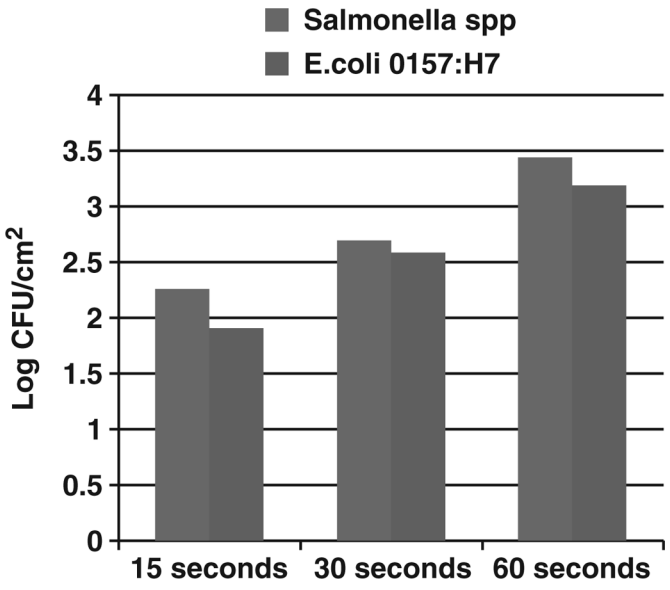

Figure 3. Average reductions (Log CFU/cm2) of *Salmonella* spp. and *E. coli* O157:H7 in boneless beef trimmings treated using a solution Acidified Sodium Chlorite and in the Food Sanitation Tunnel for periods of 15, 30 and 60 s.

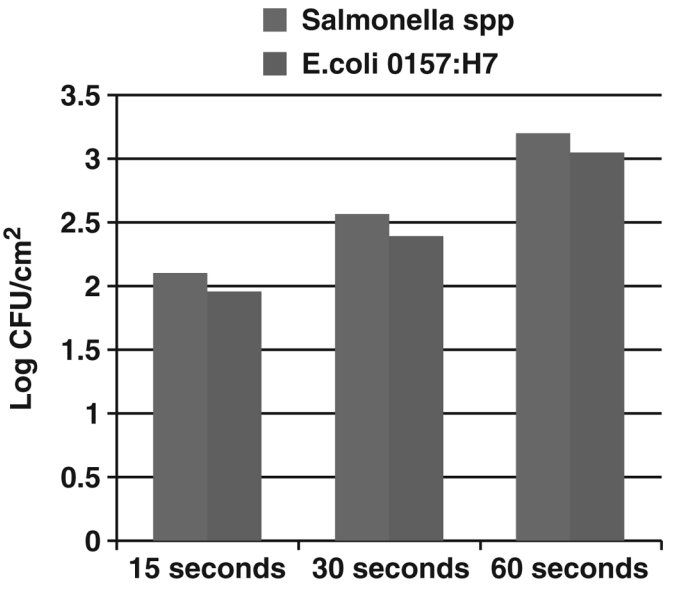

Figure 4. Average reductions (Log CFU/cm2) of *Salmonella* spp. and *E. coli* O157:H7 in boneless beef trimmings treated using a solution Acidified Sodium Chlorite and then ground and treated in the Food Sanitation Tunnel for periods of 15, 30 and 60 s.

to Ozone and water vapor to vaporized Hydrogen peroxide.

Engineers at Cozzini, Inc. helped us integrate the UV/PHI Food Sanitation Tunnel into our operation by positioning it onto a conveyor structure that would expose beef trim as it is transitioned from the course grinder to the final blender.

At this point, we believed the combining the 2 antimicrobial treatments (ASC combined with UV/PHI) they would provide systematic and potentially synergistic means of product decontamination. The intervention technologies were positioned simultaneously in our grinding system so that all beef trim fresh or frozen would be adequately treated.

INTERVENTION VALIDATION STUDY

In order to validate our intervention strategy we had the mutli-hurdle intervention approach replicated in a laboratory environment. In September 2008 Kansas State University Food Safety & Security released the final report Treatment of Beef Trimmings for Control of Escherichia coli and Salmonella spp. using a Combined Treatment of Acidified Sodium Chlorite and a UV Based Photohydrionization Cell technology. A combination treatment of beef trimmings using Acidified Sodium Chlorite (BioCide) and treatment using a UV based Advanced Oxidation Cell (RGF Environmental) was evaluated as a means of increasing the reduction of surface contamination on beef trimmings. The combination of treatments was specifically evaluated for reducing levels of *Escherichia coli* O157:H7 and *Salmonella* spp. on the surface of inoculated beef trimmings. Trimmings were first treated using a solution of Acidified Sodium Chlorite and then subjected to treatment by the UV and oxidative gases produced by a UV based Photohydroionization Cell (PHI).

Boneless beef trimmings were surface inoculated with a 5-strain cocktail of *E. coli O157:H7* and *Salmonella* spp. The trimmings were then treated in a spray cabinet using an Acidified Sodium Chlorite solution. The reductions associated with this treatment were measured by removing half of treated, inoculated trimmings and conducting microbiological analyses. The remaining trimmings were treated in the UV/PHI Food Sanitation Tunnel for periods of 0, 15, 30 and 60 s to determine the effect of the combined treatment. In addition, inoculated beef trimmings were treated using only the UV/PHI Food Sanitation Tunnel. This was done to measure the effect of the UV/PHI treatment independent of the Acidified Sodium Chlorite treatment. The target surface inoculation for all tests was 6.0 Log CFU/cm^2. After each treatment and combination of treatments, the beef trimmings were tested to determine reductions of each pathogen tested (Figures 3 & 4). Inoculated beef trimmings were also treated with a solution of Acidified Sodium Chlorite and then ground through a coarse plate (3/4″) and treated with the UV/PHI panel. This was done to simulate our process that involves the sequential treatment of trimmings and coarse ground beef.

The results of this study demonstrate that both the treatment with a solution of Acidified Sodium Chlorite and the treatment using the UV based Photohydroionization cell are effective interventions for controlling *E. coli* O157:H7 and *Salmonella* on the surface of beef trimmings and coarse ground beef. The effectiveness of both interven-

tions is enhanced when they are combined and applied in sequence. The combined reduction for this combination of treatments was 3.45 Log CFU/cm^2 for *Salmonella* and 3.20 Log CFU/cm^2 for *E. coli* O157:H7. The study also demonstrated that the combined reductions when the Acidified Sodium Chlorite was applied to inoculated beef trimmings and the UV/PHI treatment was applied to coarse ground beef for a period of 60 s was 3.20 Log CFU/cm^2 for *Salmonella* and 3.05 Log CFU/cm ^{2} for *E. coli* O157:H7. The results of this study suggest that this combination of treatments is a highly effective means of controlling microbiological contamination on beef trimmings and in ground beef.

SYSTEM VERIFICATION AND VALIDATION

To further strengthen our total food safety system and verify the system is functioning as intended, a robust finished product sampling protocol has been implemented. During patty forming, 13 - 25 gram units (grabs) of ground beef is collected at a frequency of approximately every 10-15 minutes. This 25 gram sample is composited into one 325 gram sample representing the entire "sampled" lot. The entire 325 gram sample is enriched and analyzed with Biocontrol GDS Assurance methodology.

ACKNOWLEDGMENTS

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Environment Management System: Surface and Air Sanitation for Food Quality and Safety: Review of Efficacy for the Meat Industry

Sherri Clark

INTRODUCTION

The primary factors influencing food safety risk and quality management are: environmental conditions including temperature, humidity, chemicals, and atmospheric gases; sanitation and cleanliness; packaging; product handling; process control; and people management. Research shows that temperature control is still the primary factor influencing the maintenance of food quality (Kader, 2002), and the lack of proper temperature maintenance is one of the primary contributing factors in foodborne disease outbreaks (Olsen et al., 2000; Lynch et al., 2006). However, temperature control and a wide range of current food safety practices are not fully meeting the needs of the food industry. This is illustrated by what is known about the incidence of microbial contamination early in the meat supply chain and by recent events that demonstrate the continued presence of some of this contamination in finished products.

As example of early contamination, one study on beef carcasses in 8 plants determined that total plate counts for hide-on carcasses ranged from 8.2 to 12.5 log CFU/cm^2 and after hide removal ranged from 6.1 to 9.1 log CFU/cm^2. *E. coli* counts averaged more than half of these numbers (Bacon et al., 2000). These same carcasses still retained 0.9 log CFU/cm^2 of *E. coli* after multiple sequential interventions for decontamination and chilling. Government statistics on recalls and foodborne illness illustrate the continued presence of some contamination in finished products. USDA FSIS reports an average of 22 recalls per year in 2005 - 2008 for bacterial contamination related meat recalls from different companies. There have been 2 so far in 2009 (USDA, 2009a). Also, early 2009, the CDC reported that the incidence of infections caused by *E. coli* 0157, *Salmonella*, *Listeria* and 6 other foodborne pathogens "did not change significantly when compared with the preceding 3 years" (CDC, 2009). This information and the continued regulatory attention to food safety and increased government inspections (USDA, 2009b: Notice 18–09; USDA, 2009c: Directive 6410.1) emphasize the need for filling gaps in full supply chain food safety strategies and the opportunity to use value-added technologies to supplement them and further reduce the risks.

INGERSOLL RAND'S ENVIRONMENT MANAGEMENT SYSTEM (EMS)

Multiple hurdle strategies for microbial decontamination have been used with good results in meat slaughter and processing facilities (Pohlman and McElyea, 2003; Sofos et al., 2003). One such hurdle technology that has been used is the application of a reactive oxygen species (ROS) such as hydrogen peroxide, or ozone (gas and liquid phase) (Reagan et al., 1996; Kim et al., 1998; Pohlman and McElyea, 2003; Baird et al., 2006). However, these applications have had somewhat limited efficacy or other limitations such as high ROS gas concentrations above human safety levels or surface only application that could not effect subsequent environmental cross contamination.

Ingersoll Rand has acquired an exclusive global license from AirOcare, Inc. to market and further develop a microbial intervention system for perishable supply chain applications. This system creates multiple ROS to significantly reduce the microbial load, including food pathogens and food spoilage organisms, in the air and on surfaces of a treated space. The Environment Management System (EMS) technology was developed in Chile in the mid 1990s and is used extensively in the Chilean per-

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ishable cold chain from farm to table. It employs a cold plasma generator to create anti-microbial ROS that are released into the air. This application is different from the use of direct product contact with cold plasma plumes as a sanitizing agent which has more recently come under investigation (Niemira and Sites, 2008; Perni et al., 2008).

The EMS system pulls air through the plasma generator where highly reactive oxidative properties of short-lived ROS such as hydroxyl radical, atomic oxygen, singlet oxygen, and peroxynitrite break down bacteria, molds, viruses, odor causing compounds and other volatile organic compounds (VOCs) inside the EMS reaction chamber. The more stable ROS, hydrogen peroxide and ozone, move out into the treated space where the reduction of microbes and odors continues in the air and on surfaces that the air, as a carrier of the ROS, can reach. The hydrogen peroxide and ozone are produced and maintained in the treated space at levels below human safety limits using a dynamic control system with ozone as a marker.

The efficacy of this system as a microbial intervention has been shown in medium and ambient temperature applications across the perishable supply chain for multiple products including fruits and vegetables, flowers, meats, and poultry. The following section will detail efficacy studies of the system in meat slaughter, processing and storage applications.

EMS EFFICACY FOR THE MEAT INDUSTRY

Multiple studies (published and unpublished) have been conducted to demonstrate the ability of the EMS system to reduce the microbial load (general and specific microbes) on surfaces and in the air of a treated space. The following summaries of these studies are presented in review of the EMS system efficacy for application in the meat industry. Note that some of the studies were performed before Ingersoll Rand licensed the technology so they refer to an AirOcare system. Ingersoll Rand has not changed the basic technology or function of the equipment. Only improvements in ease of installation, ease of service and those required for applicable certifications have been made.

Air Cleaning Study Results

Effect of a Reactive Oxygen Species-Generating System for Control of Airborne Microorganisms in a Meat-Processing Environment (Patel and Nou, 2008)

- Researcher and Location – Jitu R. Patel and Xiangwu Nou; USDA ARS Food Safety Laboratory, Beltsville, MD, USA
- Environment – Meat processing room within ARS laboratories
- Microorganisms – *Serratia marcescens* and lactic acid bacteria (*Lactococcus lactis* and *Lactobacillus plantarum*)
- Summary of Study and Results –
  - Cold plasma ROS equipment (produced by AirOcare for this study; exactly comparable to EMS equipment) was installed in the processing room and tuned so that the ozone marker was maintained at an average level of 0.0389 ppm in the room.
  - Natural ambient bacterial load in the room of < 1.5 log CFU/m^3 were reduced to undetectable levels within 2 h.
  - The potential environment of a meat processing facility with higher microbial load was simulated by artificially contaminating the air with a nebulized solution of the *S. marcescens* and lactic acid bacteria.
  - Initial populations of airborne *S. marcescens* in the ROS treated room ranged from 3.99 to 4.88 log CFU/m^3. These populations were reduced by >3 log over controls in 2 h with further reduction to undetectable levels within 24 h.
  - Initial populations of airborne lactic acid bacteria in the ROS treated room ranged from 4.23 to 4.97 log CFU/m^3. These populations were reduced by >2 log over controls in 2 h with some further reduction within 24 h.
  - The ROS treatment significantly reduced airborne bacterial load in 2 h and has application for controlling airborne contamination in meat-processing facilities.

Field Study: Air sampling and total plate count (TPC) analysis for facility bioload reduction by EMS in a beef processing facility (Falkenberg, 2008a)

- Researcher and Location – Rick Falkenberg (Scientific Air Solutions, Turlock, CA, USA); a large beef slaughter and processing facility in the north central US
- Environment – Cattle kill floor (~274,000 ft^3) and ground beef processing room (~360,000 ft^3)
- Microorganisms – Total bacterial and fungal plate count as representation of microbiological load
- Summary of Study and Results –
  - EMS equipment was installed in the 2 areas as follows
    + Kill floor– equipment installed to treat makeup air (~50K cfm). System was tuned so that the ozone marker was maintained at an average level of 0.030 ppm in the room.
    + Ground beef room– equipment installed to recirculate room air. System was tuned so that the ozone marker was maintained at an average level of 0.030 ppm in the room.
  - Pre-treatment TPC from air samples: (control samples were taken from outside of the rooms for this field test due to the high influx of outside makeup air being brought into the building)

+ Kill floor – Average 2,282 CFU/m³ from 19 25L air samples (single outside control sample - 2,200 CFU/m³)
+ Ground beef room - Average 2,779 CFU/m³ from 19 25L air samples (single outside control sample - 3,360 CFU/m³)

– 63-d Post-treatment TPC from air samples:
+ Kill floor – Average 231 CFU/m³ from 19 25L air samples (single outside control sample – 1,840 CFU/m³)
+ Ground beef room - Average 161 CFU/m³ from 19 25L air samples (single outside control sample – 1,640 CFU/m³)

– Final reduction being maintained in the facility due to EMS treatment (adjusted due to difference in outside temperature and bioload between pre and post-treatment sampling)
+ Kill Floor - 87.9% bioload reduction (adjusted based on 16% reduction in outside control bioload). This is equal to 0.98 log CFU/m³ reduction.
+ Ground beef room – 88.2% bioload reduction (adjusted based on 52% reduction in outside control bioload). This is also equal to 0.98 log CFU/m³ reduction.

Field Study: Air sampling and total plate count (TPC) analysis for facility bioload reduction by EMS in a ready-to-eat (RTE) sandwich commissary (Falkenberg, 2008b)

- Researcher and Location – Rick Falkenberg (Scientific Air Solutions, Turlock, CA, USA); a large sandwich commissary
- Environment – meat slicing room (~27,000 ft³) and sandwich/wrap assembly room (~220,000 ft³)
- Microorganisms – Total bacterial and fungal plate count as representation of microbiological load
- Summary of Study and Results –
  – EMS equipment was installed to recirculate room air in both rooms with the systems tuned so that the ozone marker was maintained at an average level of 0.030 ppm.
  – Pre-treatment TPC from air samples:
    + Meat slicing room – Average 550 CFU/m³ from 4 25L air samples
    + Sandwich assembly room - Average 370 CFU/m³ from 21 25L air samples
  – 16-d Post-treatment TPC from air samples and reductions being maintained:
    + Meat slicing room – Average 78 CFU/m³ from 4 25L air samples, which results in a 78.9% (0.88 log CFU/m³) reduction.
    + Sandwich assembly room - Average 20 CFU/m³ from 21 25L air samples, which results in a 96.3% (1.07 log CFU/m³) reduction.
  – The EMS equipment was then turned off for 13 d and then back on for 48 d to validate that the bioload reduction was due to the EMS treatment and that continuous treatment was necessary to maintain the lower bioload rather than one single "cleaning" treatment. The timing of the treatments varied due to the schedule of the facility. Data not shown here indicates that the treatment normally equilibrates to the lower bioload in 2–14 depending on bioload influx and then maintains "intervention" levels.
    + While the EMS equipment was off, the bioload in the 2 rooms increased by 91.7% for the meat slicing room and 66.4% for the assembly room.
    + After the EMS equipment was turned back on for 48 d, the bioload was reduced again to a final reduction from the original untreated levels of 85.5% (0.95 log CFU/m³) for the meat slicing room and 87.6% (0.97 log CFU/m³).

Field Study: Air sampling and total plate count (TPC) analysis for facility bioload reduction by ROS in a retail chicken cooler (Falkenberg, 2007b)

- Researcher and Location – Rick Falkenberg (Scientific Air Solutions, Turlock, CA, USA); a retail grocery store
- Environment – Retail chicken cooler (630 ft³)
- Microorganisms – Total bacterial and fungal plate count as representation of microbiological load
- Summary of Study and Results –
  – ROS equipment (produced by AirOcare for this study; exactly comparable to EMS equipment) was installed to recirculate room air with the systems tuned so that the ozone marker was maintained at an average level of 0.030 ppm.
  – Pre-treatment TPC from air samples showed an average of 3500 CFU/m³ from 2 25L air samples
  – 6 mo post-treatment TPC from air samples showed an average of 1490 CFU/m³ which is still at a very high and unacceptable level. The EMS equipment was serviced and properly adjusted for proper performance.
  – Subsequent 17-d post-treatment TPC from air samples showed an average of 40 CFU/m³ which indicated that the EMS treatment had brought the cooler to acceptable levels with a total reduction of 98.9% or 1.99 log CFU/m³.

Product and Equipment Surface Study Results

Treatment of Beef Carcass Tissue in Simulated Cold Storage for Control of *Escherichia coli* 0157:H7 and *Salmonella* using EMS Surface and Air Sanitation Technology (Marsden, 2009a)

- Researcher and Location – James L. Marsden, Food Safety Systems, LLC, Manhattan, KS, USA

- Environment – 4′ X 4′ X 4′ test chamber; tests run with and without EMS treatment to simulate post-slaughter cooling / storage times
- Microorganisms – *Escherichia coli* 0157:H7 and a 5 species cocktail of *Salmonella*
- Summary of Study and Results –
  - Cold plasma EMS equipment was installed in the test chamber. During EMS treatment of bacterial samples the system maintained an average level of 0.085 ppm ozone and 0.103 ppm vaporized H_2O_2 in the chamber.
  - A set of beef carcass tissue samples was inoculated with 6.3 log CFU/cm^2 of the *Salmonella spp.* and a second set was inoculated with 6.3 log CFU/cm^2 *E. coli 0157:H7*.
  - In separate tests for the *Salmonella* and *E. coli*, the inoculated beef carcass tissue samples were treated with ROS in the test chamber for 0, 10, 20 and 60 min. Separate control runs were performed without EMS treatment.
  - The resulting log reductions are shown in Table 1.
  - The ROS treatment significantly reduced contamination of both bacteria within 10 min of treatment (>than 1.5 log CFU/cm^2) with additional reductions of > 2.5 log CFU/cm^2 in 60 min.
  - In addition, testing showed no detectible ozone or hydrogen peroxide residue on the beef carcass tissue samples and TBA (thiobarbituric acid) values were similar between the treated and untreated samples, 0.32 and 0.30, respectively.

Treatment of Inoculated Sliced Turkey Breast for Control of *Listeria monocytogenes* using EMS Surface and Air Sanitation Technology (Marsden, 2009b)

- Researcher and Location – James L. Marsden, Food Safety Systems, LLC, Manhattan, KS, USA
- Environment – 4′ X 4′ X 4′ test chamber; tests run with and without EMS treatment to simulate in-line processing times
- Microorganisms – Five strain cocktail of *Listeria monocytogenes*
- Summary of Study and Results –
  - Cold plasma EMS equipment was installed in the test chamber. During EMS treatment of bacterial samples the system maintained an average level of 0.080 ppm ozone and 0.125 ppm vaporized H_2O_2 in the chamber.
  - A set of turkey breast slices (newly removed from retail packaging) was inoculated with 6.2 log CFU/cm^2 of the *Listeria monocytogenes.*
  - The inoculated turkey breast slices were treated with ROS in the test chamber for 0, 15, 30 and 60 s. A separate control run was performed without EMS treatment.
  - The resulting log reductions are shown in Table 2.
  - The ROS treatment reduced contamination of *L. monocytogenes* by 1.10 log CFU/cm^2 within 15 s with additional reduction 2.1 log CFU/cm^2 in 60 s.
  - In addition, testing showed no detectible ozone or hydrogen peroxide residue on the beef carcass tissue samples and TBA (thiobarbituric acid) values were similar between the treated and untreated samples, 0.16 and 0.14, respectively.

Evaluation of AirOcare Reaction Chamber and Reactive Oxygen Species for Reducing Microbial Populations on Stainless Steel, Plastic and Polyethylene Surfaces (Marsden, 2007)

- Researcher and Location – James L. Marsden, Department of Animal Sciences & Industry, K-State Food Science Institute, Kansas State University, Manhattan, KS, USA
- Environment – 4′ X 4′ X 4′ test chamber; tests run with and without ROS treatment (ROS equipment produced by AirOcare for this study; exactly comparable to EMS equipment) to simulate environmental treatment of processing and storage facilities
- Microorganisms – A 5 species cocktail of *Salmonella, Pseudomonas aeruginosa, Eschericia coli,* and *Listeria monocytogenes*
- Summary of Study and Results –
  - Cold plasma ROS equipment was installed in the test chamber. During EMS treatment of bacterial samples the system maintained

Table 1.

	Salmonella (log cfu/cm^2)		*E.coli* 0157:H7 (log cfu/cm^2)	
Treatment Time	EMS Treated	Control	EMS Treated	Control
0 min	6.30	6.30	6.50	6.50
10 min	4.60	6.25	4.90	6.40
20 min	4.10	6.25	4.40	6.40
60 min	3.50	6.10	3.90	6.30

Table 2.

	Listeria monocytogenes (log cfu/cm^2)	
Treatment Time	EMS Treated	Control
0 s	6.20	6.20
15 s	5.10	6.20
30 s	4.50	6.10
60 s	4.10	6.15

an average level of 0.043 ppm ozone in the chamber.
  - A set of food contact surface coupons (one each of stainless steel, polyethylene, and plastic) was inoculated with 5 to 6 log CFU/cm^2 of each bacteria listed above.
  - In separate tests for each bacterium, the set of food contact surface coupons was treated with ROS in the test chamber for 12, 24 and 48 h. Separate control runs were performed without EMS treatment.
  - The resulting log reductions are shown in Table 3 (all reductions in control samples were minimal (>1 log CFU/cm^2); ND = non-detectible).
  - The ROS treatment significantly reduced contamination on all surfaces in 12 h with further reductions to non-detectible levels within 24 h.

AirOcare Technology, Creating a Safe Food Environment by Eliminating Influenza A, mRSA, Norovirus, and Rhinovirus on Various Inoculated Surfaces (Falkenberg, 2007a)

- Researcher and Location – Rick Falkenberg, Food Safety and Process Technology Laboratory, Scientific Air Solutions, Turlock, CA, USA
- Environment – 4′ X 4′ X 4′ test chamber; tests run with and without ROS treatment (ROS equipment produced by AirOcare for this study; exactly comparable to EMS equipment) to simulate environmental treatment of processing and storage facilities
- Microorganisms – mRSA (methicillin-resistant *Staphylococcus aureus*), *Influenza A* (bird flu strain), *Norovirus*, and *Rhinovirus*
- Summary of Study and Results –
  - Cold plasma ROS equipment was installed in the test chamber. During EMS treatment of bacterial samples the system maintained an average level of 0.040 ppm ozone in the chamber, with a temperature of 75.0°F and 40%RH.
  - A set of food contact surface coupons (one each of stainless steel, plastic, and flooring tile) was inoculated with ~7 log CFU/ 5 g of each bacteria/virus listed above.
  - In separate tests for each microorganisms (performed in triplicate), the set of food contact surface coupons was treated with ROS in the test chamber for 30 min, 1, 2, 4, 8, 12, and 24 h. Separate control runs were performed without EMS treatment. Negative controls using sterile water as the inoculums were also run.

Table 3.

	Log Reductions (log cfu/cm^2)											
	Salmonella			E.coli			Listeria			Pseudomonas		
EMS Treatment Time	Steel	Plastic	PE	Steel	Plastic	PE	Steel	Plastic	PE	Steel	Plastic	PE
12 h	ND	3.8	3.1	ND	4.1	3.3	ND	2.7	2.1	ND	3.9	3.6
24 h	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
48 h	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 4.

	Log Reductions (log cfu/5 g)											
	mRSA			Influenza A			Norovirus			Rhinovirus		
EMS Treatment Time	Steel	Plastic	Tile	Steel	Plastic	Tile	Steel	Plastic	Tile	Steel	Plastic	Tile
30 min	3.51	3.71	3.42	3.32	3.07	2.99	3.19	3.28	3.06	2.31	2.11	3.21
1 h	5.35	5.01	5.13	4.72	4.41	4.46	4.85	4.73	4.51	4.81	4.79	4.98
2 h	5.63	5.39	5.28	5.87	5.65	5.37	6.01	5.97	5.72	6.05	5.99	6.12
4 h	6.25	5.91	6.01	6.32	6.11	6.17	6.99	6.90	6.89	6.99	6.89	6.93
6 h	6.63	6.41	6.39	6.86	6.81	6.43	6.96	7.01	6.87	7.01	6.95	7.03
8 h	6.97	6.99	6.87	6.98	7.00	6.99	7.00	7.01	7.00	7.00	7.05	7.00
12 h	6.93	6.95	6.86	6.91	6.89	6.94	6.88	6.95	6.96	6.93	6.97	6.94
24 h	6.92	6.97	6.83	6.89	6.88	6.94	6.93	6.94	6.94	6.94	6.95	6.91

- The resulting log reductions are shown in Table 4 (all reductions in control samples were minimal (>0.1 log CFU/cm^2)).
- The ROS treatment resulted in a 99.5% log destruction in 8 h for all microorganisms and surfaces. The largest log reduction (3.3 log CFU/ 5 g average) was seen after the first 30 min.
- The ROS treatment showed a slightly greater log reduction on the bacterium tested than on the viruses.
- The stainless steel surface showed the greatest log reduction followed by the tile and the plastic, respectively.

CONCLUSION

The results of all of the laboratory and field studies reviewed above shows efficacy of the Ingersoll Rand Environment Management System (EMS) Surface and Air Sanitation technology as an intervention step to enhance food quality and reduce food safety risk by reducing facility bioload including specific food pathogens.

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Bovine Satellite Cells as Precursors of Intramuscular Adipocytes

B. J. Johnson and K. Y. Chung

INTRODUCTION

Currently, feedlot producers tend to overfeed pens of cattle to ensure adequate marbling. This practice results in poorer feed efficiencies and higher cost of gains. Several investigators have tried to develop efficient feeding programs to increase marbling in bovine muscle tissue. Cattle can accumulate adipose tissue almost indefinitely, and strong evidence exists to indicate that some portion of the increase in adiposity in the mature animal is derived from proliferation and differentiation of pre-existing adipogenic precursor cells (Harper and Pethick, 2004). Several studies have investigated key factors that regulate resident myogenic cells to be converted to marbling adipocytes. Adipogenic transcriptional factors such as C/EBPs, PPARs, and SREBP have been expressed as major markers during transdifferentiation. However, myogenic transcriptional factors such as myogenin, Myo D, and Myf5 were down-regulated during adipogenic differentiation. A recent report further showed that G-protein coupled receptors (GPRs) were highly involved in adipogenic development in a variety of tissues (Briscoe et al., 2003; Brown et al., 2003). Interestingly, GPR 43, 41, 40 and 120 activated by free fatty acids were a major initiation mechanism of adipogenic development in the adipocytes (Hong et al., 2005; Gotoh et al., 2007). Another growth promotant study demonstrated that anabolic steroids caused progenitor cells to go down the myogenic pathway and block entry to the adipogenic pathway. However, synthetic progestin not only decreased proliferation of muscle-delivered cells (Sissom et al., 2006) but also enhanced adipogenic development in the beef muscle (Hutcheson et al., 1993; Chung and Johnson., unpublished).

Transdifferentiation of Myoblasts into Adipoblasts

Several in vitro studies have demonstrated not only a role for adipogenic transcriptional factors, PPARγ and C/EBPα (Hu et al.,1995; Torii et al., 1998; Poulos and Hausman, 2006) but also a role of extrinsic regulation factors, fatty acids and thiazolidinediones (TZD; Grimaldi et al., 1997). These 2 adipogenic transcriptional factors have been focused as a adipogenic markers (Figure 1), which gradually expressed under the transdifferentiation process of myoblasts. Fatty acids and TZD was used as regulator of adipogenesis in the myoblasts. Yu et al. (2006) suggested that transdifferentiation of myoblasts to adipocytes was greater in cells containing PPARγ overexpression vectors. This data demonstrated that PPARγ overexpressed mouse myoblasts were greater than expressed adipogenic genes such as lipoprotein lipase (LPL), adipocyte fatty acid binding protein (aP2), and glycerol-3 phosphate dehydrogenase (GPDH) compared with nontreated cells. Singh et al. (2007) investigated the effect of a potent TZD (ciglitizone) on transdifferentiation of porcine muscle satellite cells to adipocytes. Porcine muscle satellite cells became multinucleated myotubes, therefore, indicating normal myogenic differentiation. Exposure of these muscle satellite cells to ciglitizone completely ameliorated fusion (formation of multi-nucleated myotubes) and caused formation of cells containing lipid droplets suggesting conversion of muscle cells to adipocytes. Further investigations revealed that in ciglitizone-treated groups, expression of C/EBPα and PPARγ were upregulated. High expression of C/EBP restricted mitotic growth of preadipocytes and promoted differentiation of preadipocytes (Umek and McKnight, 1991). C/EBPα specific antibody and nucleic acid hybridization analysis was conducted on liver, lung, adipose, intestine, and placenta tissues (Birkenmeier et al., 1989), but not in the muscle tissue. Therefore, PPARγ and C/EBPα can be used as specific markers for indicating myoblast transdifferentiation. Consequently, the expression of PPARγ was sufficient to block muscle differentiation and result in transdifferentiation of muscle satellite cells to adipocytes (Figure 2).

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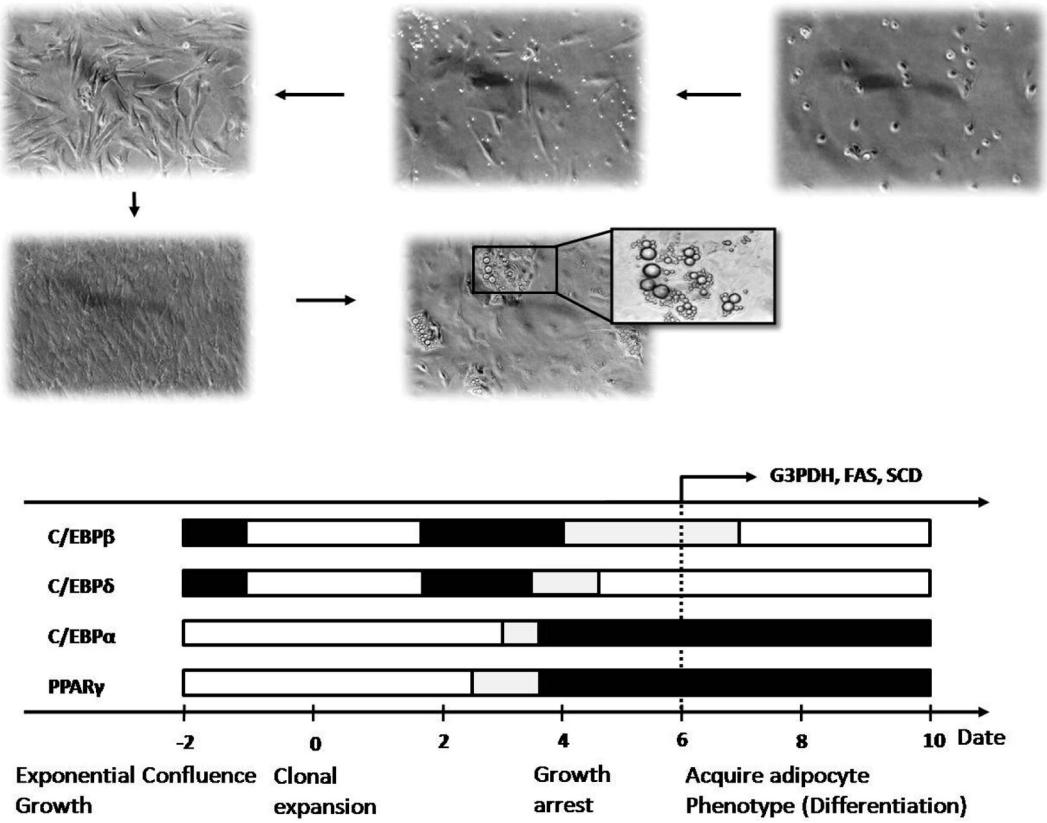

Figure 1. The time-dependent roles of transcription factors related to preadipocyte differentiation. Transcription factors regulated by specific ligands are involved in triggering adipocyte differentiation and expressing functional genes.

A transcriptional regulator had recently been discovered, PRDM16 (PRD1-RF1-RIZ1 homologous domain containing 16), which controls transcriptional linage from skeletal myoblasts to brown adipocytes (Seale et al., 2008). This data reported that both brown adipocyte and skeletal muscle cells contained a high thermogenic capacity regulated by adipogenic transcriptional factors such as PGC-1s and PPARs. PRDM16 co-activated with PPARγ as a transcriptional regulator and highly induced brown adipogenesis in C2C12 and primary myogenic cell. This study also suggested that high UCP-1containing brown adipocytes in the skeletal muscle can be activated as a progenitor cell of the marbling development. Because brown adipocytes transformed white adipocytes under non-adrenergic condition. Large portion of brown adipocytes lose their characteristics and assume white adipocyte characteristics during growth (Cinti, 2006).

Singh et al. (2003) used the pluripotent, immortalized cell line, C3H 10T1/2 to investigate the direct effect of androgens on myogenic and adipogenic differentiation. Interestingly, the number myogenic cells and myosin protein levels increased in a dose-dependent fashion in response to both testosterone and dihydrotestosterone (DHT) addition. At the same time these 2 steroids decreased the number of adipocytes formed by the 10T1/2 cells and downregulated both adipogenic protein expression. These profound effects were blocked by a specific androgen receptor antagonist, bicalutamide, indicating the steroids were mediating these cell fates through the androgen receptor on the pluripotent cells. Although conducted with rodent pluripotent cells in a cell culture model, these data increase our understanding of the potential effects of anabolic steroids used in implants on the push of primitive muscle-derived cells to stay muscle cells and not become adipocytes. Thus offering us a cellular explanation of how growth promotion could positively impact skeletal muscle growth and simultaneously inhibit marbling. Future research will need to investigate the effects of these products on in vivo changes.

TZD and long chain fatty acids can induce transdifferentiation from myoblast cell to adipocytes by acting as specific ligands for PPARγ (Hu et al., 1995; Teboul et al., 1995; Grimaldi et al., 1997; and De Coppi et al., 2006). TZD has been used as an antidiabetic agent to improve insulin sensitivity and glucose uptake by activating GLUT4 (Mukherjee et al., 2000). Grimaldi et al. (1997) reported that TZD and LCFA treated myoblasts reduced the formation of multinucleated myotubes and the expression of myogenic genes in the mouse cell model. Treatment with unsaturated LCFA such as linoleic, linolenic, or archidonic acid have been reported to promote the lipid accumulate process in mouse myoblast. However, there are not many studies reported on the functional activation of monounsaturated fatty acid (MUFA), even if MUFA is largest percentage of fatty acid composition in bovine adipose tissue (Chung et al., 2006). This data also shows

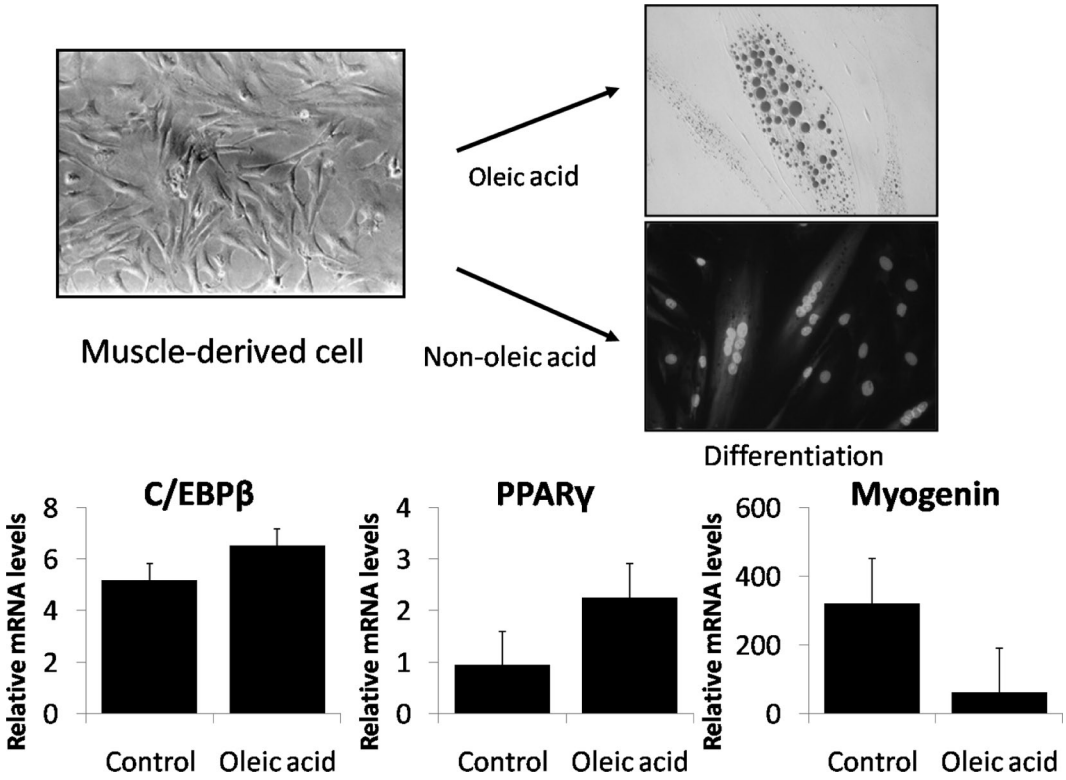

Figure 2. The role of morphological and genetic markers change related to in vitro fate of bovine muscle-derived cells, which is regulated by oleic acid. Relative C/EBPβ PPARβ and myogenin mRNA levels in total RNA isolated from transdifferentiation inducing bovine MDC cultures.

that the proportion of monounsaturated fatty acid had a significant positive correlation with not only intramuscular fat content but also melting point in the intramuscular fat of cattle. Therefore, we hypothesized that MUFA may be a critical factor to enhance the adipogenic pathway in bovine muscle derived cells. When we searched for suitable transdifferentiation mixtures in bovine muscle derived cells, the oleic acid contained mixture were more effective in accumulate lipid droplet of bovine myoblasts. Figure 2 demonstrated the role of mRNA level of transcriptional factors related to in vitro fate of bovine mesenchymal cells, which is regulated by transdifferentiation factors (Insulin, Oleic acid, and Ciglitizone). It is illustrated that relative C/EBPβ, PPARγ ($P < 0.05$), and Myogenin ($P > 0.05$) mRNA levels from bovine satellite cell cultures after 4 d of treatment with adipogenic differentiation factors (IOC) and non treatment(Cont).

Our preliminary data supports the hypothesis that within postnatal skeletal muscle there is a population of progenitor cells that have unique characteristics to become a variety of cell types. Furthermore, under appropriate stimuli, bovine muscle derived cells, mouse myoblasts (C2C12), and mouse mesenchymal cells (C3H 10T 1/2) can be induced down the adipose tissue pathway to form marbling, rather than the muscle pathway, which normally would aid in supporting additional muscle growth (Figure 3).

G Protein Coupled Receptors (GPRs) Regulated by Free Fatty Acid

Although fatty acids are an essential energy source and structural material in the cellular membrane, high concentrations of circular fatty acids cause a risk of atherosclerosis and obesity (Kliewer et al., 1997). Long-chain fatty acids (LCFAs) are byproducts of dietary intake, adipogenesis, and hepatic elongation and desaturation. Short-chain fatty acids (SCFAs) are byproducts of the fermentation of starches and fibers by anaerobic rumen microbes in the bovine species. Circulating fatty acid is not only used to affect vascular epithelial cells but also delivered to peripheral tissue and modulates physiological response. Within the last decade, some studies have suggested that fatty acids can modulate the nucleic transcription of genes regulating the adipogenic differentiation of adipose tissue (Kliewer et al., 1997). Fatty acid and hyperlipidemic drugs, such as thiazolidinedione, regulated PPARs as a ligand, while PPARs regulate the expression of target genes by binding to PPAR response elements (PPAEs). Recently research has shown that LCFA may specifically affect to cell surface protein, G-protein-coupled receptor 40 (GPR40) (Briscoe et al., 2003). GPR40 is highly expressed in pancreatic cells under treatment of medium and LCFA and these treatment stimulate secretion of significant levels of insulin from pancreatic cells (Briscoe et al., 2003). GPR120 activated by a ligand, LCFA, is highly expressed in the several depots of adipose tissue (Gotoh

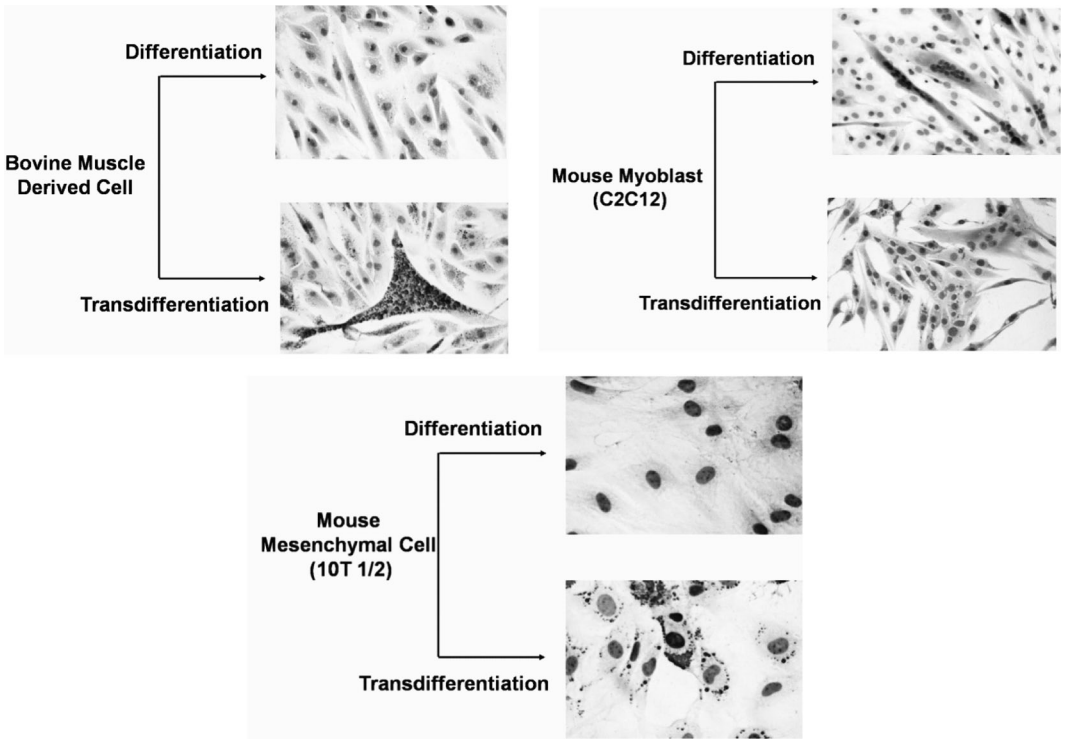

Figure 3. The role of morphological change related to in vitro fate of bovine muscle-derived cells, mouse myoblast (C2C12), and mouse mesenchymal precursor cell (10T 1/2), which were regulated by transdifferentiation factors.

et al., 2007). GPR 120 expression in mouse adipocytes is highly correlated with differentiation of preadipocytes. This data suggests that levels of GPR 120 mRNA increase during preadipocyte differentiation and decrease differentiation when GPR 120 gene expression is knocked down using small interference RNA (siRNA).

Both GPR41 and GPR43 are not only activated by SCFA, such as acetate or propionate, but also regulated adipogenesis in adipose tissue (Brown et al., 2003; Hong et al., 2005). These reports suggest that physiological effects of SCFA or LCFAs in peripheral tissue may be regulated through a cell-membrane anchored receptor. The mechanism of action of receptor mediated fatty acid binding is not well defined. Recent reports have identified LCFA and SCFA as a ligand for GPR40 (Itoh et al., 2003) and GPR 120 or GPR41 and GPR43 (Brown et al., 2003), respectively. Our preliminary data suggests that GPR43 specifically affected by short chain fatty acids were greater in non-implant muscle tissue than anabolic steroid implant muscle tissue (Data not shown). This result suggests that anabolic steroids inhibit GPR43 protein expression.

CONCLUSIONS

Bovine satellite cells have been considered to be multipotential characteristic cell and regulated by several free fatty acids and thiazolidinedione isoforms. Although there are still many questions for developing in vitro and in vivo transdifferentiation systems, novel chemicals and hypothesis induces understanding transdifferentiation of bovine satellite cell. GPR 41, 43, and 120 highly existed at adipose tissue was activated by short chain or long chain free fatty acids. An increased understanding of how these fatty acids are affecting the cellular aspects of the transdifferentiation of myoblasts to adipoblasts will allow us to enhance marbling fat during physiological growth of beef cattle. Understanding the GPR regulation mechanism induced by agonist such as fatty acid or TZD will beneficial in to control developing adipogenesis or in vitro transdifferentiation from myoblast to adipoblast.

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Comparison of Intramuscular and Subcutaneous Preadipocytes

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The positive relationship between marbling, or intramuscular fat, and overall palatability of meat has long been recognized (Savell and Cross, 1988). The importance of marbling to palatability is punctuated by the fact that purveyors, retailers, and restaurateurs collectively ranked insufficient marbling as the greatest quality challenge facing the beef industry in 2005 (Smith et al., 2006). Likewise, the 2005 National Beef Quality Audit investigators identified "recognizing the importance of marbling as a value-determining trait" and "minimizing production of excess fat" among the industry goals key to improving quality and reducing nonconformity (Smith et al., 2006). In fact, undesirable quality grade and yield grade distributions were estimated to cost the beef industry $26.81 and $20.92 per head, respectively (Smith et al., 2006). Consequently, inappropriate fat deposition typically costs the beef industry over a billion dollars annually.

Despite the importance of intramuscular fat deposition in determining product quality, the goal of maintaining or increasing intramuscular fat, while reducing subcutaneous, intermuscular, and internal fat depots, has proven to be challenging. Adipose tissue deposition involves both hyperplasia, which is an increase in the number of fat cells (adipocytes), and hypertrophy, which is the enlargement of adipocytes due to lipid accumulation. Although the relative contribution and timing of these processes varies somewhat among anatomical fat depots, fat deposition does occur simultaneously in multiple depots. The ability to selectively increase or decrease fat accumulation in specific depots of growing and finishing livestock would require that biological differences exist in the regulation of fat deposition among depots. Interestingly, intramuscular fat deposition appears to be dependent on hyperplasia until at least 15 mo of age in cattle (Cianzio et al., 1985), whereas subcutaneous fat accumulation in cattle over 8 mo of age results primarily from adipocyte hypertrophy (Hood and Allen, 1973; Cianzio et al., 1985). Subcutaneous adipocytes become substantially larger than intramuscular adipocytes. Smith and Crouse (1984) reported that acetate provides 70 to 80% of the acetyl units to lipogenesis in subcutaneous bovine adipose tissue but only 10 to 25% in intramuscular adipose tissue. In contrast, glucose supplies 50 to 75% of the acetyl units for lipogenesis in intramuscular fat. These differences between depots warrant optimism regarding the opportunity to develop management strategies to simultaneously improve beef quality and yield.

Mature adipocytes result from the differentiation of preadipocytes, which are sometimes referred to as stromal-vascular cells. Preadipocyte differentiation is a transformation from a fibroblast-like cell to a lipid-filled cell. These changes are controlled by multiple signaling pathways and result from altered expression of many genes, enzymes, and other proteins that allow adipocytes to effectively store and release lipid (Ailhaud et al., 1992; Grégoire et al., 1998; Fernyhough et al., 2007; Hausman et al., 2009). Because intramuscular fat accumulation is dependent on an increase in the number of adipocytes, differentiation of preadipocytes is an important regulatory step in the deposition of marbling.

To better understand the regulation of fat deposition in different anatomical locations of cattle, we isolated stromal-vascular cells from bovine intramuscular, subcutaneous, and perirenal fat. Subsequently, we established clonally derived (pure) preadipocyte populations from these depots and used these cells to develop culture conditions suitable for differentiation of bovine preadipocytes (Grant et al., 2008a). A clonally derived cell culture has the advantage of being free of contaminating nonadipogenic cell types, and it has the limitation that all cells are derived from one progenitor cell. Conversely, stromal-vascular cell cultures reflect the preadipocyte diversity of the initial isolate from adipose tissue, but may also contain some nonadipogenic cell types. Our experiments with bovine stromal-vascular cells and clonally derived preadipocytes have thus far produced similar results.

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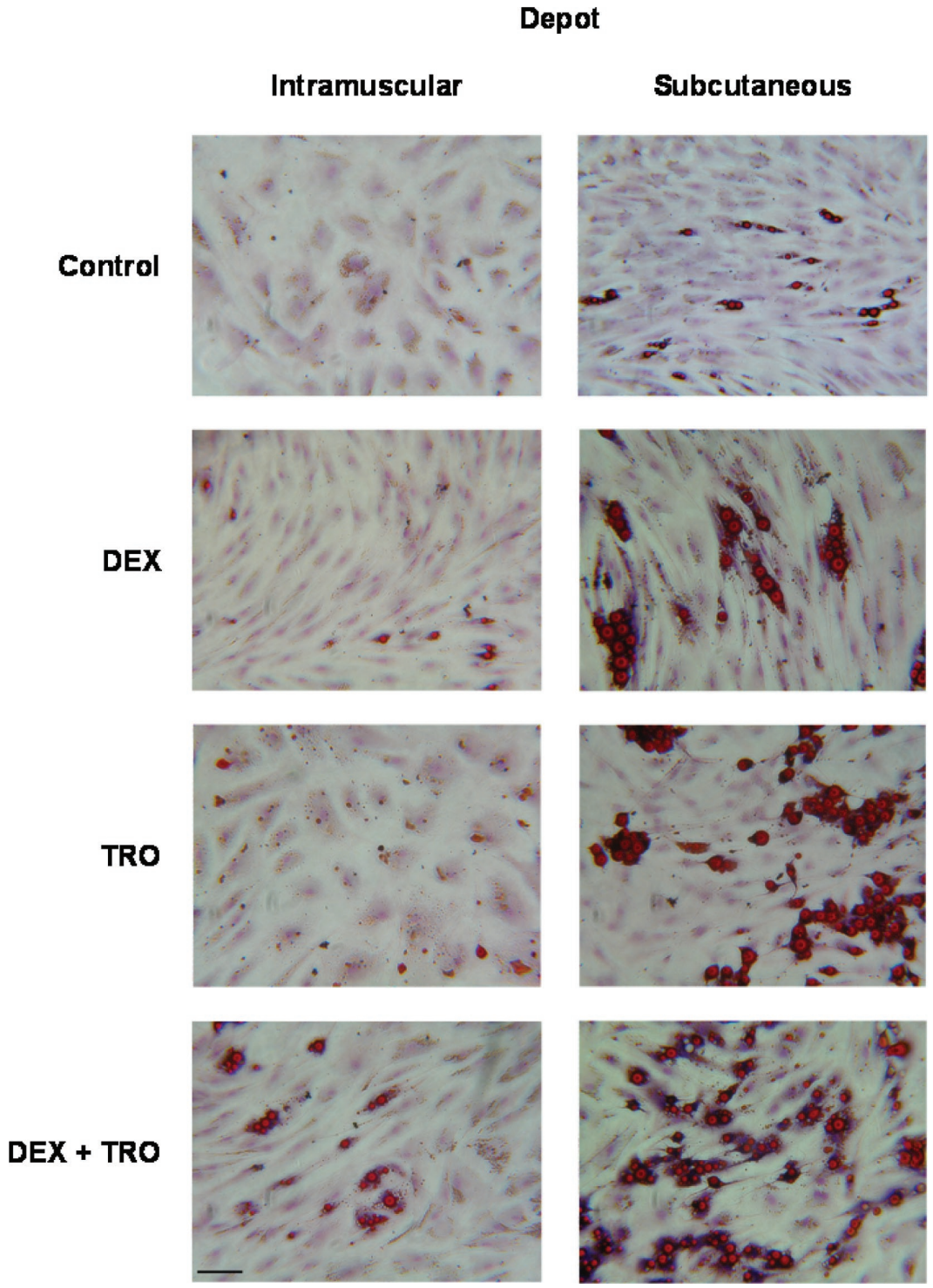

Figure 1. Photomicrographs of intramuscular and subcutaneous adipogenic stromal-vascular cell colonies exposed for 10 d to differentiation media. Treatments included no additions (control), addition of 0.25 µ*M* dexamethasone (DEX) for the first 48 h, 40 µ*M* troglitazone (TRO) for 10 d, or their combination. Cells are stained with oil red O and lightly counterstained with Giemsa stain. Bar = 100 µm. Figure reprinted with permission from Grant et al. (2008b).

Grant et al. (2008a) demonstrated that biochemical and morphological differentiation of bovine preadipocytes was stimulated by addition of a serum lipid supplement (ExCyte, Serologicals Corp., Norcross, GA), insulin, dexamethasone, and troglitazone, which belongs to the class of insulin-sensitizing drugs called thiazolidinediones (**TZD**). These drugs act as ligands for peroxisome proliferator-activated receptor γ (**PPARγ**), which in turn dimerizes with a retinoid X receptor and subsequently binds to PPAR response elements in the promoters of genes that promote adipogenic differentiation (Kliewer et al., 1992; Spiegelman, 1998; Fernyhough et al., 2007). Poulos and Hausman

(2006) demonstrated that the TZD ciglitazone increased the number of preadipocytes from subcutaneous adipose tissue but not muscle-derived stromal-vascular cultures. Although dexamethasone and troglitazone induced similar increases in differentiation of bovine intramuscular and subcutaneous preadipocytes, the percentage of differentiated cells within adipogenic colonies isolated from subcutaneous fat was 6.4-fold greater than that from intramuscular fat (Grant et al., 2008b). Figure 1 illustrates the adipogenic effects of dexamethasone, troglitazone, and their combination on bovine preadipocytes and clearly shows that intramuscular preadipocytes have a lesser propensity to differentiate than subcutaneous preadipocytes.

The reasons bovine intramuscular preadipocytes have a lesser propensity to differentiate in culture than subcutaneous preadipocytes are currently unclear. Ortiz-Colón et al. (2009) demonstrated that isolated bovine intramuscular, subcutaneous, and perirenal preadipocytes express glucocorticoid receptor-immunoreactive protein bands of similar size and abundance. In addition, glucocorticoid receptors in cells from all depots were downregulated by exposure to dexamethasone. Abundance of PPARγ2 was also shown to be similar in intramuscular and subcutaneous preadipocytes (Ortiz-Colón, 2006). Interestingly, we have shown that ibuprofen preferentially enhances differentiation of cultured bovine intramuscular compared with subcutaneous preadipocytes (Buskirk et al., 2007). Ibuprofen is a PPARγ ligand (Lehmann et al., 1997) that induces expression of adipose differentiation-related protein, also called adipophilin (Ye and Serrero, 1998). Collectively, our observations indicate that development of distinct anatomical fat depots may be differentially regulated by specific PPARγ ligands. A more complete understanding of the biological mechanisms controlling the accumulation of fat in distinct adipose tissue depots will provide opportunities to more effectively increase marbling and decrease unwanted fat in livestock.

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Biological Markers of Intramuscular Fat (IMF) Content

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INTRODUCTION

The amount of intramuscular fat (IMF) and its fatty acid composition play major roles in the quality attributes of meats, including sensory properties and healthy considerations. It is generally assumed that IMF content positively influences sensory quality traits, including flavor, juiciness, and tenderness of meat or firmness of fish, whereas a low amount of fat induces a less tasty meat. The amount of visible fat is also regarded as a quality criterion of beef in many developed countries as it is judged positively in Asia and North America whereas an excess of visible fat is mainly unpopular in European countries. Therefore, a better understanding of the biological mechanisms determining the amount and composition of IMF remains a hotspot of research conducted in most countries, to satisfy consumers' expectations and ensure the competitiveness of meat production all over the World. This review paper will deal with biological markers of IMF in farm animals. A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of biologic processes." Biomarkers may be phenotypic traits or gene characteristics.

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IMF AND MARBLING: DEFINITION AND ROLE IN PALATABILITY OF MEAT, VARIABILITY BETWEEN MUSCLE TYPES AND CUTS

IMF content covers the sum of phospholipids mainly found in cell membranes, triglycerides (which are the main forms of energy reserves) and cholesterol within muscle tissue. In muscles of mammals and avian species, triglycerides are mainly stored within intramuscular adipocytes (about 80% at least, Gondret et al., 1998) but also within myofibers cytoplasm in droplets in close-vicinity to mitochondria (5 to 20% of total triglycerides). Marbling is the term used in the beef industry to refer to the appearance of white flecks or streaks of IMF between the bundles of muscle fibers. Marbling is an integral part of beef grading in the United States of America, especially because a segment of consumers is willing to pay a premium for guaranteed quality beef. Marbling in beef is not regarded in the SEUROP or European grading system. Marbling is less visible in pork than in beef, because extractable lipids in pork loins range only from 0.76% to 8% (Rincker et al., 2008).

It is generally accepted that IMF positively influences flavor, juiciness, tenderness and/or firmness and the overall acceptability of meat in different species, although research results are in fact quite controversial. There is a general agreement that very low levels of IMF lead to dry and less-tasty meat. The minimum amount of IMF to achieve acceptable consumer satisfaction is about 3–4% for beef, and 5% for sheep meat. In fresh pork meat, flavor and juiciness were also significantly enhanced when IMF levels increased above approximately 2.5% (reviewed by Pethick et al., 2007). Similarly, it has been shown that the contribution of marbling to the variation in palatability may explain only 10 to 15% of the variance in palatability in beef (reviewed by Pethick et al., 2007). However, when variations in tenderness are controlled, the contribution of marbling to palatability is more important due to its specific contribution to juiciness (about 38.4% of the variation; Jeremiah et al., 2003) and flavor (Thompson, 2004).

In France, some producers specialized in the production of beef from young bulls of late-maturing breeds (Belgian Blue, "Blonde d'Aquitaine," etc) would consider that IMF content is too low (<3%) to ensure meat taste.

The chemical composition of muscles is relatively constant (about 75% of water, 19 to 25% of proteins, and 1–2% of minerals and glycogen). However, their lipidic part is highly variable, both between species, between individuals in a given species, and between muscles and cuts. Meat from pork, rabbit and poultry is generally lower in fat than beef and lamb. Among poultry, chicken and turkey have especially low IMF level (<1% in breast meat). As in mammals and poultry, huge differences in IMF are observed among fish species (from less than 3% to more than 18%) (Medale et al., 2003). An important point, which is often not clear to consumers, is that whole meat products trimmed of salvage fat (e.g., trimmed steaks, chops, roasts, casserole cubes) make only a small contribution to the daily fat intake of human diets re-inforcing the idea that IMF does not play a negative role in human nutrition.

DEVELOPMENT OF IMF AND ITS RELATION WITH MUSCLE GROWTH

From a developmental point of view, marbling is the last adipose tissue to be deposited in finishing animals, although adipose tissue starts to accumulate in the early weaning periods (Harper and Pethick, 2004). At the metabolic level, IMF content in species such as cattle and pigs which can synthesize fat within muscle tissue results from the balance between uptake, synthesis and degradation of triacylglycerols. In avian and most fish species, lipid synthesis is negligible if any in the muscle (Rollin et al., 2003). Therefore, endogenous lipids are mainly synthesized in liver then exported to extrahepatic tissues including to the muscles by the blood-stream. Taken together, the accretion rate of IMF content would depend not only on the variation during development of number and intrinsic metabolic activity of adipocytes inside the muscle tissue, but also on muscle growth rate and metabolic activity of other organs.

Intramuscular adipocytes originate from multi-potent stem cells (reviewed by Harper and Pethick, 2004). Stem cells derived either from mesenchymal stem cells or from satellite cells have received specific attention. However, the mechanisms involved in deriving different lineages to obtain adipose cells rather than muscle or fibroblastic cells remain largely elusive. Interaction between adipocytes and myoblasts in the very early stages of growth is likely to occur and influence the respective differentiation of both cellular types. Another complexity is added by the possible existence of inter-conversion of muscle satellite cells into adipocytes as shown in vitro (for review, see Chung and Johnson, 2008). Much research is now needed to identify molecular markers related to mesenchymal stem cell differentiation or satellite cells inter-conversion, to better understand the origin of intramuscular adipocytes and subsequent development of IMF content.

Triglycerides are generally initially stored within muscle fibers and then in intramuscular adipocytes in mammals. A noticeable specificity of birds is that muscle lipid is high at hatching and mainly stored in intramuscular adipocytes (Chartrin et al., 2007). During growth, intramuscular adipocytes will increase both in size and number as shown for instance in rabbits (Gondret et al., 1998). These results suggest that hyperplasia of adipocytes not initially visible (and hence their early differentiation) plays an important role in marbling during growth (Albrecht et al., 2006).

Because fat is deposited at a lower rate than muscle growth during the first periods of postnatal life and at a greater rate than lean tissues when animals get older, the concentration of fat in muscle (i.e., IMF content) will inevitably increase later in an animal's life. This does not mean that the rate of fat accretion in intramuscular adipocytes is late maturing relative to other depots. In this context, the level of IMF at the start of the growth period (even if it is low) is likely a key determinant of the final level of IMF after finishing at least in cattle (reviews from Pethick et al., 2006, 2007). Because assessing IMF content at this period of time is difficult, this emphasizes the usefulness of early predicting markers to predict the ability of an animal to deposit IMF.

It is also generally accepted that a high muscle mass is associated with a low fat mass in the carcass, and with also less IMF. This suggests that muscle tissue and non-muscular adipose tissue interplay during the process of IMF accumulation, through changes in hormones regulating metabolism and fluxes of energy-yielding nutrients. Thus, attempts have been made to predict IMF level from blood metabolic parameters (Adachi et al., 1999). However, indicators of carcass fatness and muscle types (red oxidative vs. white glycolytic) are generally poor markers of the ability of animals to deposit IMF. Indicators of adipocyte number are probably more relevant. To date, the best marker of adipocyte number which can be assessed is probably the content in adipocyte-type fatty acid binding protein (A-FABP, encoded by FABP4 gene) as shown in pigs (Damon et al., 2006) and cattle (Jurie et al., 2007). Because leptin is secreted by enlarged adipocytes, leptin expression at the mRNA level in muscles may be also a good indicator of IMF mass and hence marbling (Bonnet et al., 2007). The activity levels of various lipogenic enzymes in skeletal muscles have been also related to IMF content (Mourot and Kouba, 1999 in pigs; Bonnet et al., 2007, in cattle) but not in all studies (Damon et al., 2006). It is now clear that IMF accumulation during growth is the result of a balance between fatty acids synthesis and their oxidation, rather than upregulation of a single pathway in mammals (Gondret et al., 2004, in rabbits, Kolditz, Borthaire, et al., 2008,in fish). Thus, the turn-over of fatty acids within the muscle must be considered. In addition, the capacity for lipid uptake by muscle tissue is also a major determinant of the control of IMF level in ducks

(Chartrin et al., 2006) and fish (Kolditz, Borthaire, et al., 2008). Taken together, the relationships between IMF content and metabolic markers could depend on the source of variation in IMF. This is the reason why some authors have preferred to combine both blood indicators and metabolic enzyme activities to predict IMF variability, but the equations of prediction differ between muscles (Gondret et al., 2004).

GENETIC AND GENOMIC MARKERS OF IMF CONTENT

Genetic Markers

Various genetic markers associated to IMF deposition or marbling have been reported: they include the thyroglobulin (TG) gene in cattle (Barendse, 2002), the diacylglycerol Acyltransferase 1 or 2 (DGAT1; DGAT2) gene in cattle (Thaller et al., 2003), pigs (Nonneman and Rohrer, 2002) and chicken (Bourneuf et al., 2006). However, the results are often inconsistent. For instance, the thyroglobulin marker (commercialized as the GeneSTAR marbling marker) has been reported to have no effect in Simmental steers (Rincker et al., 2006).

From scientific knowledge on how IMF starts to accumulate in the muscle, it is then obvious that genes belonging to adipogenic process within muscles could be also good candidates for predicting IMF content. Genes involved in intracellular fatty acid transport within skeletal muscles have been notably proposed (Gerbens et al., 1999, in pigs). This list of genetic markers associated with IMF or marbling is not exhaustive (for a recent review, see Gao et al., 2007). However, none of these markers are omnipotent between species, between breeds and also between traits under study (IMF content, marbling or other ones). This may be not a problem anymore due to the advent of genomic selection (Meuwissen et al., 2001) as an approach based on markers which cover the whole genome. But this type of approach emphasizes the need for large phenotypic databases for discovery and validation, and therefore an ontology system to better define and standardize the measurements of phenotypes. An international undertaking entitled "Animal Trait Ontology" has been initiated with the goal to make easier the comparison of phenotypic information between species (Hughes et al., 2008).

Genomic Principles and Main Findings

Nowadays, scientists have access to gene networks and interactions thanks to the development of transcriptomic and proteomic tools which allow the high-throughput detection of genes and proteins differentially expressed between different conditions without any knowledge a priori. Several studies dealing with functional genomics in livestock animals have been published so far (reviewed by Hocquette et al., 2007, and Cassar-Malek et al., 2008, for cattle). This allowed a great number of differentially expressed genes associated with IMF level to be identified such as NAT1 (i.e., a translational suppressor), and some genes associated with the thyroid hormone pathway, with the unsaturated fatty acid synthesis and fat deposition including FABP4 or with the muscle structure (Liu et al., 2009, in pigs and for review in cattle, see Lehnert et al., 2006, and Hocquette et al., 2007). Especially, a recent combination of transcriptome and proteome analyses (Kolditz, Paboeuf, et al., 2008) in the liver of 2 rainbow trout lines divergently selected for IMF revealed that major changes induced by the selection procedure were not only related to lipid metabolism (synthesis and transport), but also to other pathways such as protein and amino-acid metabolism pathways.

An important challenge for researchers is to turn the current knowledge about gene expression into practical biological assays useful for the meat industry. Unlike geneticists (who have developed commercial DNA tests), biochemists missed this challenge. Some scientists do not believe in RNA-based methods to develop robust quantitative assays (Lehnert et al., 2006) although those methods have been standardized before proteomics tools. Alternatives are to develop protein-based assays or to detect the biomarkers in peripheral blood instead of within muscle samples. In addition, it is probable that expression profiling will become integrated with genotyping outputs in the next future. The combination of linkage genetics and expression profiling (genomics) is called "genetical genomics" and is forecasted to become important (Kadarmideen et al., 2006).

NUTRITIONAL REGULATION OF IMF LEVEL AND POTENTIAL OUTPUTS

Metabolic Specificities of Intramuscular Adipose Tissue and Practical Outputs

Much evidence is accumulating to indicate intramuscular adipocytes have specific features compared with adipose cells in other fat depots. First, they displayed low activity levels of enzymes of lipogenesis in pigs (Gardan et al., 2006) and in cattle (Bonnet et al., 2007). Recent proteomic investigation reveals that not only lipogenesis, but also indicators of lipolysis, fatty acid oxidation and basal energy metabolism are lower in abundance in those adipocytes (Gondret et al., 2008). Differences in gene expression between IMF depot and subcutaneous adipose tissue have been also reported (Ross et al., 2005; Gardan et al., 2006). Additional evidence for unique intramuscular fat metabolism is seen in cattle where marbling adipocytes preferentially use glucose/lactate carbon for fat synthesis while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis (Rhoades et al., 2007; Smith et al., 2009 for a review). Furthermore, a higher level of GLUT4 expression and higher activities of metabolic enzymes involved in the conversion of glucose into long-chain fatty acids (namely phosphofructokinase and ATP-citrate lyase) were detected in intramuscular adipose tissue compared with subcutaneous fat in this spe-

cies (Hocquette et al., 2005). Although these studies did not provide any biomarkers, they could be used to propose new feeding strategies. For instance, greater dietary absorption of the gluconeogenic precursor propionate earlier in the growth period might result in increased IMF deposition and greater marbling scores. Generally speaking, diets which promote glucose supply to the muscle invariably increase IMF deposition in ruminants.

Manipulating Dietary Protein, Energy and Micronutrients

In various species, overfeeding induces a greater IMF content (e.g., Chartrin et al., 2007 in ducks), but also more fat in organs other than muscles (i.e., liver in fatty-goose or duck, subcutaneous fat in pigs…). The most successful nutritional strategy in non-ruminants to render fat accumulation in muscle independent to that in other parts of the body has been obtained with subtle protein deficient diets (e.g., D'Souza et al. 2008, in pigs). The picture is however somewhat different in fish where fat deposition is increased by feeding diets with high protein levels (Reinitz, 1983), due to the particular ability of this species to synthesize fat from dietary proteins when protein intake exceeds the level needed for body protein synthesis.

In cattle, it is also known that vitamin A or β-carotene deficiency is associated with an elevated IMF content, both in young Wagyu steers and Australian Angus cattle (for review, see Pethick et al., 2006). Other micronutrients potentially involved in adipogenesis have also been studied in cattle (for review, see Kawachi, 2006; Smith et al., 2009).

Taken together, nutritional manipulation of IMF independently from body fat depots has proved to be more difficult to achieve than genetic strategies. In addition, the biological mechanisms which explain the variability of IMF content differ between genetic and nutritional factors. The nutritional regulation of IMF also differs between ruminants, monogastrics and fish due to their digestive and nutritional particularities.

CONCLUSION

Early events that determine the number of intramuscular adipocytes are likely crucial in the final determination of muscle lipid content in animals at commercial slaughter. IMF depot also develops largely in parallel with other fat depots, but is also dependent on muscle growth. Critical developmental time periods have to be re-examined by expression experiments using genomic tools. There is also additional evidence that intramuscular adipocytes later in the growth period are metabolically different to other depots (subcutaneous fat) and an understanding of this would appear important to underpin strategies for genetic and non genetic manipulation of IMF. Several adipogenic factors and metabolic enzymes that explain variations in IMF content could be likely used mainly for genetic purposes if DNA markers are identified in those genes. But, recent studies have put light on the importance of metabolic balance between various pathways, rather than the control of one single pathway for the control of IMF. New targets and markers will be identified in the future using combined genomic, transcriptomic, and proteomic approaches. From the increasing biological knowledge, it is however clear than only a few of them will go to application as tests because of lack of effective commercial utility. So, before a biomarker brings any benefit over other criteria, it needs to address the following 4 concepts: "easier, better, faster and cheaper." It must also be validated at farm level or food-production chain and this should be a priority of research in the case for the control of IMF content.

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Functional Properties of Muscle Proteins: Implications for Processed Meat Product Characteristics

Joseph G. Sebranek

WHAT IS PROTEIN FUNCTIONALITY?

Definitions

Protein functionality is a term that can be used to describe many different properties of proteins for practical applications in food products. Merriam-Webster's dictionary defines functionality as "… the quality or state of performing or being able to perform a function… concerned with actual use rather than theoretical possibilities…" Thus, protein functionality in processed meats may be considered to be a combination of properties that affects expected practical performance of the protein in a finished product. Protein functionality in meat systems is also sometimes called technological quality, a term that aptly describes the interactive role that muscle proteins play in conjunction with processing technology to achieve desired quality in processed meats. Protein functionality should not be confused with the term "functional foods." Functional foods is a term used to describe nutritional properties of foods or ingredients that provide an additional physiological benefit beyond their basic nutrition.

In meat processing, the protein properties of interest for functionality typically include the ability to bind and retain water, the ability to encapsulate and bind fat and the ability to form a cohesive, heat-set gel. Color is also a function of muscle proteins that is extremely important to processed meats but color is normally considered as a separate issue in discussions of processed meat properties.

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Basis for Differences in Protein Functionality

Proteins are biological products designed for a wide range of specific biological functions in living organisms. As a result, proteins have a broad range of hydrophilic-hydrophobic properties, interphasic properties, intermolecular interactions and thermal response behavior (Damodaran, 1994). The hydrophilic-hydrophobic properties dictate how the protein will interact with water which translates into solubility and water binding. Interphasic properties determine how well proteins can form films between immiscible media, a critical property for emulsion behavior in meat batters. Intermolecular interactions provide proteins with the ability to form junctions between protein molecules and other components to affect viscosity, cohesion, stickiness and elasticity. The response of proteins to thermal treatments is a protein-protein interaction that determines gelation, water and fat retention and finished product texture.

Factors which determine the specific functional behavior of individual proteins include both chemical and physical properties of the protein. Amino acid composition is obviously important but the sequence of amino acids as well as the proportions of different amino acids will greatly affect the hydrophilic-hydrophobic properties of the protein and its behavior in forming films and gels. The molecular size, shape and conformation of the protein chain will affect solubility, intermolecular interactions and molecular rearrangement during thermal treatments. Conformation of protein molecules will also affect surface polarity, electrical charges and hydrophobic-hydrophilic areas of the protein exposed to the medium. These properties are intrinsic to the protein source and, as a result, functionality changes are limited by the range of protein sources available. On the other hand, protein functionality can be drastically altered by the environment. This is where processing treatments play a critical role. Physical stresses such as muscle fragmentation (grinding or chopping) or tumbling of meat pieces provides greater exposure of proteins to subsequent chemical modifications achieved by salts, pH changes and shifts in redox potential. Thermal treatments typically provide the final modification of pro-

tein structures and interaction by inducing the unfolding and crosslinking between protein chains that occurs during heat-set gelation.

MUSCLE PROTEINS

General Categories and Functional Properties

Muscle proteins are generally categorized into 3 main groups: sarcoplasmic, myofibrillar and stromal. Sarcoplasmic proteins are the soluble proteins in the muscle cell sarcoplasm and consist of enzymes, pigments and many relatively small peptides. These proteins are water soluble or low-salt soluble and make up about 30 to 35% of muscle protein (Asghar et al., 1985; Au, 2004). The properties of these proteins that are significant to protein functionality are a relatively high surface charge/polarity, relatively low molecular weights of 15 to 60 kDa and a globular structure. The relatively high surface charge/polarity facilitates solubility in water and provides for some functionality in meat systems but the low molecular weight and globular structure tend to limit the functional role of these proteins. The role of sarcoplasmic proteins may become relatively more important for functionality in reduced salt products of less than one percent because of the reduced role of salt-soluble proteins in a low-salt environment (Miyaguchi et al., 2004). Calpain is a sarcoplasmic protein that is not significant to functionality by itself, but may impact some functional properties such as water holding capacity because of selective proteolysis of structural myofibrillar proteins by calpain that permits greater protein swelling and better retention of water by muscle myofibrils (Huff-Lonergan and Lonergan, 2005; Bee et al., 2007). Post mortem proteolysis during aging also results in greater myofibrillar protein solubility (Xiong, 2000).

Myofibrillar proteins are the structural and contractile apparatus of living muscle which can be extracted from meat with about 2 percent or more salt (2.5–3% brine). These proteins make up about 55% of total muscle protein (Asghar et al., 1985) and provide for the majority of the functional properties sought in processed meats (Xiong, 2000). Myosin comprises 43% of this group and actin 22% (Asghar et al., 1985; Xiong, 2000), thus these 2 proteins make up about 2/3 of the total myofibrillar proteins in muscle and over 1/3 of the total protein content. Consequently, these proteins have an overwhelming role in meat processing and most research efforts have focused on these 2 myofibrillar proteins.

Myosin is considered by far the most important, not only because it makes up almost half of the myofibrillar protein group, but because it is a long filamentous molecule with some 4,500 amino acids and a molecular weight of close to 500,000 (500 kDa) (Clark et al., 2002). The molecule is very long and slender, about 160 nm long and 10–13 nm in diameter, with a mixture of highly hydrophobic and hydrophilic sections that make it nicely designed to interact with both polar and non polar media. These are key properties that give myosin a very large capacity to interact with water, fat and other protein molecules.

Actin, while making up over 20% of the myofibrillar protein group is considered much less important than myosin for functionality because it is a globular protein made up of 375 amino acids (Clark et al., 2002). This means that actin has considerably less capacity to interact with other molecules in terms of both size and shape. In post rigor meat, actin is complexed with myosin to form actomyosin as a result of rigor development, and the complex is largely retained in most meat processing applications. The actomyosin complex functions quite similarly to myosin though functional capacity is slightly reduced. It is thought that actin provides some structural hindrance to myosin functionality when present or may provide a simple dilution effect to result in slightly less functionality than that of myosin alone (Xiong, 2007).

The remainder of the myofibrillar proteins have not been studied for functional properties to the same extent as myosin and actin and consequently, their roles are not as clear. However, titin, the third most abundant myofibrillar protein and the largest with over 30,000 amino acids, appears to be one of the major targets for post-mortem degradation by calpain and as such could provide an indirect contribution to some functional properties such as water binding ability by release of other myofibrillar proteins (Davis et al., 2004).

Stromal proteins of muscle provide a significant part of the supporting connective tissue between muscle fibers, muscle bundles and individual muscles. The primary protein in this group is collagen. The collagen molecule is composed of a very high proportion of non polar, hydrophobic amino acids making collagen a largely nonfunctional protein in processed meats (Xiong, 2007). Untreated collagen typically results in emulsion breakdown and water losses if present in excess amounts in emulsified products. However, because collagen is converted to gelatin with moist heat, properly treated and dispersed collagen can contribute a firm, elastic cold-set gel that retains water well when incorporated into processed meat products (Eilert et al., 1993, 1994; Osburn et al., 1998).

Protein Isoforms

It has become clear that several isoforms of the myofibrillar proteins exist. At least 10 isoforms of myosin heavy chains and several myosin light chains have been reported (Lefevre et al., 1999; Pette and Staron, 2000; Clark et al., 2002; Au, 2004) and the combinations of isoforms of each means that several distinct isomyosins are possible. The implications of the isoforms are 2 fold; first, the difference in amino acid composition may introduce variation in functionality. However, these changes are typically quite small. In the case of actin, for example, the isoforms found in muscle have been reported to vary in 10 of the 375 amino acids in this protein (Clark et al., 2002). On the other hand, the isoforms are indicative of different muscle fiber types and the resultant meat quality changes

that may occur as a result of fiber type difference. (Bowker et al., 2004; Choi et al., 2006). It has also been reported that myosin isoforms may differ in their response to changing ionic strength such as the addition of salt. White muscles require lower salt concentration to begin swelling and water uptake than red muscles. Further, maximum swelling of white muscle at equal ionic strength and pH was found to be greater than for red muscle (Xiong, 1999). This suggests that the isoforms of myosin may be enough different in amino acid composition or sequence to affect interaction of these proteins with salt and affect subsequent water binding ability.

Critical Factors for Protein Functionality

Meat protein functionality is largely affected by factors that also affect the solubility and extractability of the proteins, and the swelling ability of the remaining myofibrils or myofibrillar fragments. Species and muscle fiber type are well recognized as important determinants of protein solubility. For example, proteins from white muscles (poultry breast) are considerably more soluble than proteins from red muscle (poultry leg) (Xiong, 2000). In general, the functional differences seem to favor white muscle in many comparisons, and this would imply species differences as predicted by white muscle fiber content (Li-Chan et al., 1985; Lan et al., 1995a). However, direct comparison of species for protein solubility is confounded by differences in post mortem muscle biochemistry, and muscle chilling rates. It is clear that nonmeat ingredients and processing technology offer much more potential to increase protein solubility and functionality than inherent muscle differences.

For a given meat source, protein solubility and functionality will increase with pH, ionic strength (added sodium chloride), specific ion effects (polyphosphates) and added water. Consideration of the effects of processing treatments on protein functionality can be important. For example, the role of temperature during preparation of emulsion/batter products is well recognized. The mechanical effects of chopping or emulsifiers result in a temperature increase while particle size is being reduced. The increase in temperature represents increased mechanical energy input which increases protein extraction and mobilization, and increased subdivision and softening of the fat. Use of vacuum is another in-process consideration that is important to protein functionality. Vacuum results in increased capacity of proteins to bind fat and water with proportionally greater effects observed for vacuum with sarcoplasmic than myofibrillar proteins (Tantikarnjathep et al., 1983). These authors suggested that if air bubbles are incorporated into a meat emulsion, this will result in some protein film formation around the air thus reducing the total protein available to stabilize fat and water.

Product handling treatments that may impact protein functionality after the blending and mixing of meat batches include the amount of time before the meat mixture is placed in its' final form and the subsequent thermal treatment. The time factor becomes a consideration because meat proteins will slowly form salt-induced crosslinks following blending or mixing. If allowed to remain undisturbed for an extended period before stuffing into casings or other final form, the result is a firmer, harder mixture that experiences more pressure and stress during pumping or stuffing. The physical change in form during pumping or stuffing may also disrupt some of the protein bonds that have developed. The protein disruption can result in a loss of fat and water binding as a result of stuffing or other forms of physical transfer.

Thermal treatment is the final processing step that can have a large impact on protein functionality. There has been an extensive amount of research on the effects of heating conditions on protein functionality. An orderly transition of the proteins through the physical rearrangements that occur as a result of heating is necessary and the heating rate becomes an important factor for the formation of a meat protein gel.

FUNCTIONAL PROPERTIES

Water-Holding Ability

The water binding ability of processed meats encompasses both inherent water present in muscle and water added as part of product formulation. Because added water can be a large portion of the formulated product weight in some cases, it is critical to provide as much water-binding ability by the proteins as possible. Effective water retention is a critical quality attribute in terms of product appearance, eating quality and yields. While there are many extrinsic and intrinsic factors including animal genetics, nutrition, preslaughter and post slaughter treatments, and chilling that affect water binding of meat (Cheng and Sun, 2008), the fundamental factors that control water retention are pH and salt (NaCl), both of which induce myofibrillar swelling (Bertram et al., 2004).

Water molecules in meat form a monomolecular layer on proteins that is very tightly bound to available charged and polar sites on protein molecules. A somewhat looser hydration shell of 2–3 additional layers of water molecules is held by hydrogen bonding to the monomolecular water layer. The great majority of water in meat (~80%) is held very loosely by capillary and surface tension force. This water is highly sensitive to structural changes within and between the proteins and can be manipulated by pH and salt (Offer and Knight, 1988). Changes in pH and salt concentration that create greater capillary space within the protein structures provide for greater retention of the loosely held water (Offer and Knight, 1988). Generally speaking, water binding ability of proteins is a function of the pH relative to the isoelectric point, which for meat proteins is about pH 5.2. Because the isoelectric point is defined as the pH at which protein net charge is zero, this is the point of minimum electrostatic repulsion between proteins, minimum swelling and minimum water binding ability. The classical pH/water binding relation-

ship for meat shows that water binding increases when pH is above or below the isoelectric point. A pH above or below the isoelectric point increases charges on proteins and increases the electrostatic repulsion between proteins to induce myofibrillar swelling. This provides more intramyofibrillar space for the weak H bonding and capillary forces to increase binding of additional water.

Salt and pH-adjusting ingredients used in processed meats for improving water binding are effective due to swelling effects on meat proteins. Salt (NaCl) for example, increases the electrostatic repulsion between proteins because selective binding of chloride ions to positively charged sites on the protein reduces positive charges and results in greater net negative charge. Because meat proteins normally have a net negative charge at the typical postmortem muscle pH (5.8–6.0), increasing the net negative charge will increase swelling. Maximum swelling of myofibrils has been reported to occur at 0.8–1.0 N NaCl (4.6–5.8%) (Offer and Trinick, 1983), however, there are differences between species and muscle types, probably because of different myosin isoforms (Xiong, 1999). Phosphates increase pH to create increased electrostatic repulsion but also serve to increase actomyosin dissociation and depolymerization of thick and thin filaments to allow greater swelling.

Tumbling of intact meat pieces with salt and phosphate is also used to increase the retention of water by the myofibrillar proteins. While tumbling facilitates the distribution and equilibration of salt and phosphate, the mechanical activity of the process is believed to reduce some of the restraining cellular and myofibrillar structures in muscle to permit greater separation and swelling of the proteins. Moisture retention of processed meats will also be a function of the thermal treatment used for cooking the product. Generally speaking, the cooking method used to deliver heat energy, the temperature of the medium, the heating rate in the product and the internal endpoint temperature of the product will all affect the amount of water retained or lost during the cooking process. The effect of these processes on the denaturation and gelation behavior of the meat proteins will determine how effectively the proteins can trap and retain water during and after the heating process.

Solubility and Extractability

Solubility and extractability are important properties of meat proteins, not so much by themselves but because the amount of protein made available in solution affects many of the other functional properties expected of the proteins (Vojdani, 1996). In the case of meat products, for example, high protein solubility is especially critical to emulsion film formation, adhesion of meat pieces in restructured products and gelation during heating. Solubility of proteins depends on both the hydrophilic-hydrophobic balance of the amino acids that facilitate interaction with the solvent (water) and the conformation of the protein that allows the exposure at the surface of the molecule of more or less charged and polar amino acids.

Protein solubility is strongly affected by pH because, at the isoelectric point, proteins usually have the least solubility. One of the advantages of pre-rigor meat for further processing of comminuted products is that the pH is considerably higher than that of post-rigor meat and the protein solubility is significantly greater as a result. Of course, pre-rigor also means that the actin-myosin crossbridges have not formed to the same extent as in post-rigor meat and this means that proteins, myosin in particular, will be much easier to solubilize. The practice of adding salt to pre-rigor meat to create a pre-rigor pre-blend is an especially powerful tool to maximize protein solubility and functionality in comminuted, processed meats. The addition of salts that increase ionic strength and modify pH (NaCl and phosphates) is an important means of increasing protein solubility. In fact, this is necessary to achieve any reasonable amount of protein solubility and functionality because of the need to solubilize myosin.

While pH, salts and mechanical action achieved by mixing, tumbling or similar treatments are important means of increasing protein solubility, there are significant differences in meat sources that affect the degree of solubility achieved. Postmortem conditions such as the rate of lactic acid production and the muscle chilling rate can greatly impact protein solubility by partially denaturing some of the muscle proteins. The lack of functionality in pale, soft, exudative pork and poultry muscle is due in part to the restricted protein solubility that results from these conditions. However, muscle fiber type plays a part here as well. White, glycolytic muscle fibers are generally more responsive to extraction procedures and salt effects (Xiong, 2004). The different isoforms of myosin that characterize different muscle fiber types may well be different in solubility because of differences in amino acid composition, sequence or conformation.

Emulsion/Batter Stabilization

The blending and stabilization of finely divided fat in a water-based meat mixture is a significant challenge to meat processors who manufacture emulsion/batter products because of the high interfacial tension that develops between fat and water phases. The key to producing a stable mixture in meat emulsions or batters is to first create an interfacial protein film on the surface of the fat droplets to reduce interfacial tension and then suspend the fat droplets more rigidly within a protein gel formed by heat processing.

Formation of an interfacial protein film is a critical function of myofibrillar proteins, particularly myosin or most likely actomyosin in the case of post mortem muscle. The properties of the interfacial film depend on the amount and type of proteins extracted by the chopping or milling action used to prepare the emulsion and by the ionic strength and pH of the mixture. Clearly, the critical func-

tion of the proteins is to display both hydrophobic affinity for fat and hydrophilic affinity for water.

Myosin provides a distribution of polar and non polar amino acids that facilitates orientation between 2 unlike phases. The myosin molecule also has a very high length-to-diameter ratio which facilitates molecular flexibility and rearrangement at the film interface (Xiong, 2000). Studies of the role of specific segments of the myosin molecule have suggested that the heavy meromyosin portion of the myosin molecule seems to be a good candidate due to a specific hydrophobic area on the protein chain (Asghar et al., 1985). Other meat proteins are less important though sarcoplasmic proteins and actin probably play minor roles. Sarcoplasmic proteins are capable of emulsifying fat but are probably limited by their globular nature as demonstrated by Tantikarnjathep et al. (1983) and by reduced solubility in high ionic strength solutions. Flexibility and elasticity of the interfacial protein film are likely to be important to withstand the physical stresses of moving the emulsion from one place to another and stuffing the mixture into casings. The protein film has been reported to consist of at least 3 layers (Gordon and Barbut, 1990), and the thickness of the film is likely to contribute to stabilizing the mixture as well. One of the effects of utilizing the myofibrillar proteins in the interfacial film is that these proteins are not likely to contribute as much water-binding to the system as they would otherwise. Consequently, some water binding ability is lost in an emulsion batter system due to loss of some myofibrillar protein in that function. The concept of pre-emulsified fat using a non meat protein such as casein or soy provides for retention of more of the myofibrillar proteins for water binding and texture formation during subsequent heat treatments. For these applications, fat, non meat protein and water are chopped before product manufacturing to form a stable fat emulsion. The pre-emulsified fat is then added to the meat product as the fat component of the final mixture.

Gelation

Heating of meat proteins results in a series of intra- and intermolecular changes that result in cross linking of the protein chains into a continuous inter-linked 3-dimensional gel structure. If formed correctly, the gel provides for trapped and retained water and fat as well as texture and mouthfeel of the cooked product. Consequently, formation of a strong, uniform gel by proteins in a meat product is a critical process for virtually every cooked product. It is especially critical in emulsion/batter products where failure to stabilize fat can result in dramatic, unsightly large pools of fat and water in the cooked product. However, gelation is also critical to binding together of meat pieces in restructured or tumbled products and for texture formation in almost all cooked meats.

As with the formation of the protein film in emulsions, protein gelation in meat products is dependent primarily on myosin (Samejima et al., 1984; Morita and Ogata, 1991). The long filamentous nature of the myosin molecule, and its abundance in meat have made this protein the key constituent of meat protein gels. Consequently, the factors which affect myosin properties (ionic strength, pH, isoforms, other proteins) will impact the strength and stability of myosin gels (Westphalen et al., 2005; Liu et al., 2008). For example, the negative effects of rapid post mortem pH changes which occur in development of pale, soft exudative (PSE) pork are known to damage protein functionality. Even at equivalent extracted protein concentration, gels from PSE pork muscle have been observed to have less than half the gel strength (rigidity) and 11% greater water loss (Camou and Sebranek, 1991b). Wang et al. (2009) recently reported that actomyosin from PSE pork demonstrated structural change (unfolding) at lower temperatures and showed less hydrophobic interaction and disulphide bonding than actomyosin from normal pork.

While myosin is the primary component of meat protein gels, it is important to remember that a meat product mixture will also include muscle fiber fragments, myofibrils, sarcoplasmic proteins, connective tissue, non-meat ingredients like spices, sugars, curing agents, starches and non-meat proteins. Some of these, particularly non meat proteins, may interact with myosin and participate as a co-factor in gel formation or may dilute the protein gel by remaining within the gel structure (Camou and Sebranek, 1991b). These interactions have potential to strengthen or weaken the resulting gel and offer opportunity to modify the properties of the mixture. An example would be the softening of high protein myosin gels though occurs in low fat products to improve what is often considered to be excessive hardness or rigidity.

The heat-set gelation mechanism of myosin is a stepwise process in response to heat application. Under the typical conditions in processed meats (pH 6.0, 2.5% NaCl) gelation starts with unfolding of myosin molecules at 35°C - 40°C. Above 40°C, the protein molecules begin to form aggregates as evidenced by increases in turbidity when protein solutions are heated (Xiong, 2000). The initial unfolding of the protein at these temperatures probably exposes reactive groups in the protein structure that form the initial cross-protein bridges. It has been suggested that the transition at this temperature is not affected by pH (Asgar et al., 1985), and consists of the transformation from αhelix structure to a random coil exposing reactive groups to permit molecules to combine into aggregates. This is believed to permit head-to-head interaction of myosin molecules which results in the formation of groups or aggregates of myosin molecules. Following aggregation, additional heating at about 55°C and above results in crosslinking between the aggregates to form a full 3-dimensional network. The final increase in temperature above 55°C is likely to involve other proteins as they are denatured and deposited within the myosin structures as the heating is continued.

There are several additional factors that affect meat protein gels as might be expected given the dependence

on myosin availability and functionality. Gel rheology, for example, can be affected by pH even though the protein responses to temperature remain the same (Westphalen et al., 2005). A low pH resulted in stronger gels but less water retention once temperature increased above 60°C. A temperature range of 60°C-75°C resulted in the strongest relationship between pH, gel characteristics and water-holding capacity. The authors concluded that pH may need to be monitored to assure consistent gel properties in cooked products, and that pH could be used to alter or control meat protein gels to achieve desired rheological properties. Heating rate has often been suggested as important to gelation because of the need for an orderly enfolding, aggregation and gel formation sequence by the proteins (Lan et al., 1995b). A slow heating rate (17°C/hr) have been shown to result in less protein loss in water exudate than a fast heating rate (93°C/hr) (Camou and Sebranek, 1991a).

CONCLUSIONS

While there has been a great deal of research on meat protein functionality and a very significant amount of useful information is now available on the fundamental behavior of these proteins in processed meat products, there are still many facets of the relationships between meat proteins and processing treatments that are not well understood. Meat is a very complex biochemical and physical mixture which is affected differently by the many varieties of processing treatments that are imposed on this mixture. In addition, new meat processing technologies continue to develop which introduce new questions about the behavior of meat proteins in the environments created by the process.

Research on high pressure processing, for example, suggests that this process has significant effects on meat protein gelation and other functional properties (Colmenero, 2002). However, the literature is not clear about the effects of high pressure at different temperatures, pressures or duration of treatment. While pressure-assisted gelation has been observed, the understanding of conditions necessary to control and predict the outcomes of the process on gel properties is not complete. Novel ingredients that promote protein crosslinking such as transglutaminase are being introduced as a means of facilitating meat product gelation (Ahhmed et al., 2007; Ionescu et al., 2008), and may offer a means of improving the usefulness of relatively nonfunctional proteins.

Developments of novel, specialized uses for muscle proteins such as that achieved by the surimi industry has demonstrated the advantages of improved understanding of muscle protein functionality. Continued research and more complete understanding of meat proteins in processing environments can be expected to produce more new and novel uses of these valuable and versatile proteins.

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Impact of Chemical Ingredients and Processing on Muscle Protein Functionality

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INTRODUCTION

The functionality of muscle proteins in meat processing is affected by a variety of intrinsic and extrinsic factors. Intrinsic factors are related to the physicochemical properties of proteins in raw materials, for example, the structure and morphology, the presence of free sulfhydryls, molecular size, hydrophobicity, and solubility characteristics. Because these properties are inherently related to specific muscle fiber types and animal species, the choice of raw materials is important but limited. On the other hand, meat processors can exploit the functionality of muscle proteins through various extrinsic means, for example, the use of specific formulations and processing conditions (comminution, marination, and temperature and rate of cooking). For product formulations, both micromolecule and macromolecule (starch, gums, nonmuscle proteins, etc.) compounds are used. For the purpose of discussion in this paper, all these compounds will be referred to as "chemical ingredients" (Table 1).

Nonmuscle proteins and protein hydrolysates (peptides), e.g., soy protein, soy protein hydrolysate, and sodium caseinate, are used in meat processing to improve textural characteristics and water-binding potential in finished products. Many of the peptides directly interact with muscle proteins to confer the overall functionality of the composite muscle food systems. On the other hand, common micromolecule ingredients known to influence protein functional performance in meat processing include monobasic salts (NaCl, KCl, etc.), divalent cationic salts ($CaCl_2$, $MgCl_2$, etc.), various alkali and acid compounds, and different phosphates. Studies in recent years have shown that lipid and nonlipid free radicals, which are commonly generated in meat processing, can also have a profound impact on the functionality of muscle proteins, hence, textural quality of meat products. To control oxidation, antioxidant compounds are used. Moreover, the cross-linking enzyme microbial transglutaminase is extremely beneficial in improving gelling properties of muscle proteins under both low- and high-salt conditions. Overall, the intended roles of many of the large and small molecular chemical ingredients are affected by the specific meat processing conditions.

This paper will briefly discuss the major and most important chemical ingredients used by the meat industry in the manufacture of healthy and convenience food products. Emphasis will be placed on the fundamental mechanisms by which different ingredients affect the gelling, emulsifying, texture-forming, water-binding, and hydration properties of muscle proteins. Polysaccharide-based ingredients (starch, gums) will not be included because their roles in meat products are often independent of muscle proteins.

SALTS

According to the Merriam-Webster dictionary, a salt is "any of various compounds that result from replacement of part or all of the acid hydrogen of an acid by a metal or a group acting like a metal." Thus, salts are ionic compounds composed of cations (positively charged) and anions (negatively charged). Although salts are electrically neutral compounds, in aqueous solutions, such as the aqueous phase of meat products, cations and anions are dissociated, making them chemically active substances that can react with proteins and other muscle constituents.

Salts, usually NaCl, are almost always used in processed meats to not only impart flavors but also enhance protein functionality. Salts affect protein functionality through 2 distinct mechanisms: aiding in myofibrillar protein extraction and modifying protein-protein interactions. When used as a protein extraction agent, salts at an application level of approximately 2% or higher raise the ionic strength of the sarcoplasm to above 0.5, thereby causing the myosin filaments to depolymerize and myofibrils to swell for an improved hydration and water-holding capacity (Hamm, 1986). When salted raw meat is finely

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Table 1. Common and potential chemical ingredients used in meat processing to promote muscle protein functionality

Ingredient type	Example	Main effects on muscle protein functionality
Salts	NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$, $NaNO_3$, Na_2SO_4,	Water-binding, protein solubility, gelation, emulsification, myofibril hydration, protein extraction
Phosphates	Na_3PO_4 (ortho-), $Na_4P_2O_6$ (pyro-), $Na_5P_3O_8$ (tripoly-), $(NaPO_3)_n$ (hexameta-)	Protein extraction, myofibril hydration, gelation
Alkalis	$NaHCO_3$, alkali phosphates	Protein extraction, myofibril hydration
Acids	Citric acids, malic acid, lactic acid, acetic acid, acidic phosphates	Protein extraction, myofibril hydration
Oxidants	$FeCl_2$, H_2O_2, ascorbate, $KBrO_3$, lipoxygenase	Gelation, emulsification
Antioxidants	Tocopherols, BHA, BHT, ascorbate, erythorbate	Gelation, emulsification
Nonmuscle proteins	Preheated soy protein, preheated whey protein, protein hydrolysates, caseinate	Gelation, emulsification, meat binding
Enzymes	Transglutaminase	Gelation, meat binding, emulsification

chopped in the presence of salts, myofibrils disintegrate and dissociate, leading to the extraction and solubilization of actomyosin, myosin, actin, and most other myofibrillar proteins, a process known as 'salting-in'. Solubilization is achieved through ionic interactions of cations and anions from ionized salts with charged side chain groups of proteins to enhance electrostatic repulsions between proteins and promote the protein-water interaction.

The most commonly used salt in meat products is sodium chloride (NaCl). Because excess dietary intake of sodium is linked to hypertension, there have been considerable efforts in partial substitution of non-sodium salts for NaCl in meat products. Early research by Hamm (1960) showed that at an ionic strength of 0.40 and pH of 5.5 and 6.4, sodium salts enhanced the water-holding capacity of porcine muscle homogenates in the order of $I^- > Br > Cl^- > F^-$. For chlorides with different metal ions, the following order of efficacy in water-holding was observed: $Li^+ > Ca^{2+} > Mg^{2+} > Na^+ > K^+$ at pH 5.5, and $Li^+ > Na^+ > K^+ > Mg^{2+} > Ca^{2+}$ at pH 6.4. A similar finding was reported for trout muscle after treatment with different chlorides (Regenstein, 1984). For sodium salts, NaCl and Na_2NO_3 exhibited stronger water-binding potential than Na_2SO_3 and sodium citrate.

Gordon and Barbut (1992) compared 4 chloride salts (KCl, LiCl, $MgCl_2$, and $CaCl_2$) as potential substitutes for NaCl in reduced-sodium meat emulsion products. The 1.5% NaCl, KCl, and LiCl treatments extracted similar proteins and produced similar protein profiles in the membrane of meat emulsions. At an equal ionic strength of 0.43, the 3 monovalent salts had similar protein extraction efficacies, while $CaCl_2$ was least effective in extracting proteins, especially myosin and actomyosin. As a result, $CaCl_2$, and also $MgCl_2$, were less capable of stabilizing fat and binding water, which was evidenced by the porous structure in cooked batters as revealed by electron microscopy and the excessive amount of water released.

Divalent cations also have a profound effect on myofibrillar protein extraction and gel-forming properties. In a previous study, we found that $CaCl_2$ or $MgCl_2$ at concentrations of less than 5 mM promoted the extraction of chicken myofibrillar proteins, including myosin (Xiong and Brekke, 1991). Similar cation effects were reported for turkey muscle protein extraction (Nayak et al., 1996). The efficacy of cations varied depending on muscle fiber types. While the extractability of white muscle (chicken breast) myofibrillar proteins was increased by $CaCl_2$ or $MgCl_2$ at less than 5 mM, it was decreased at greater than 10 mM $CaCl_2$. However, the extractability of red muscle (chicken leg) proteins increased 40–70% upon treatment with 10 mM $CaCl_2$ or $MgCl_2$ and remained unchanged within the 10–100 mM concentrations (Xiong and Brekke, 1991).

The presence of <10 mM of Ca^{2+} promoted protein aggregation and enabled both types of proteins to form a more rigid gel with a stronger water-holding capacity. However, when the Ca^{2+} concentration exceeded 10 mM, gelation was suppressed for white myofribrillar proteins but not for red myofibrillar proteins (Figure 1). It appears that the specific role of $CaCl_2$ in the gelation, water-binding, and emulsification of muscle proteins was concentration-dependent, ostensibly related to the carboxyl-Ca^{2+}-carboxyl bridges. If non-sodium salts are used to partially replace NaCl (0.15 M), then all the IA and IIA groups of cations in the periodic table (K, Li, Mg, Ca) would have an equal or superior water- and fat-binding capacity (Whiting, 1987). Hence, low concentrations of divalent cation salts are desirable, but neither $CaCl_2$ nor $MgCl_2$ is suitable for use as a total NaCl replacement in meat batters.

PHOSPHATES

In the United States, phosphates have become almost indispensable ingredients in meat processing due to their superior water-binding and protein extraction capability. Phosphates can be divided into 2 general groups: open-chain phosphates and ring-structured phosphates.

Open-chain phosphates vary in chain length. Monophosphate, also known as orthophosphate, includes sev-

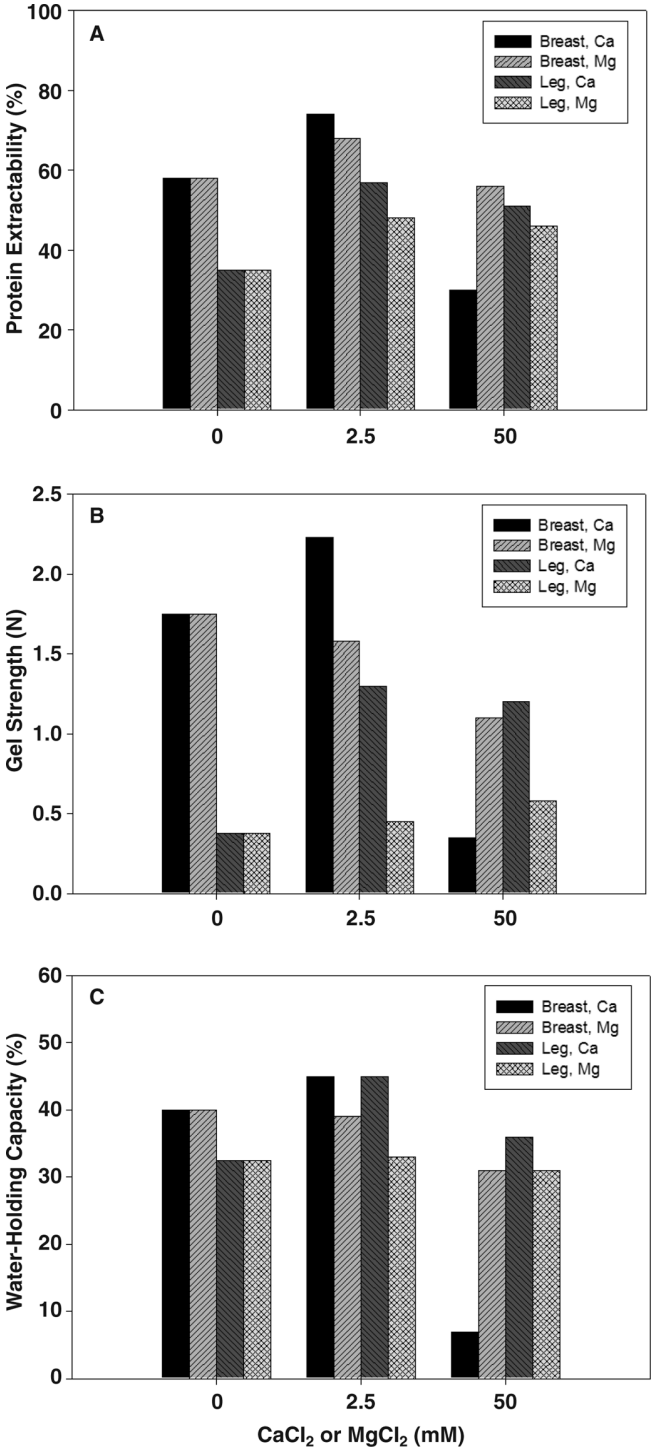

Figure 1. Effects of $CaCl_2$ and $MgCl_2$ on protein extractability (A), gel strength (B), and gel water-holding capacity (C) of chicken breast (white) and leg (red) myofibrillar proteins at pH 6.0. Total ionic strength for all samples was 0.6 as adjusted by NaCl solutions. Adapted from Xiong and Brekke (1991).

eral forms that differ from one another only in the number (mono-, di-, and tribasic) and type (usually Na^+ and less commonly, K^+) of cations. They are comprised of one phosphorous atom tetrahedrally surrounded by 4 oxygen atoms, 3 of which are ionizable and, thus, can bind with cations. Monobasic orthophosphate contains one alkali metal ion and 2 hydrogens, dibasic orthophosphate has 2 metal ions and one hydrogen ion, and tribasic orthophosphate is fully neutralized with 3 metal ions and gives rise to a high pH when dissolved in aqueous solutions. Pyrophosphate, a diphosphate, also exists in acidic, neutral, or basic forms that differ in the number of metal ions (Na^+, K^+) attached to the phosphate molecule. Tripolyphosphate, next in the family of open-chain phosphates, has 3 phosphorous atoms and varying amounts of metal ions to exist as acidic, neutral, or basic compounds.

On the other hand, ring-structured phosphates, generally referred to as metaphosphates, are condensed phosphates. They are composed of up to 15 or more orthophosphate units connected via oxygen atoms to form bulky cyclic compounds. Among them, sodium tetrametaphosphates and hexametaphosphates [$(Na\ PO_3)_n$, n = 10–15] are commonly used in meat products. Commercial hexametaphosphates usually are mixtures of metaphosphates with different numbers of phosphate units.

Open-chain phosphates, especially pyrophosphate, have a propensity to precipitate in aqueous solutions containing substantial amounts of calcium. Even in the absence of calcium, pyrophosphate has a relatively low solubility when compared with polyphosphates. Thus, pyrophosphate is often used in combination with tripolyphosphate or hexametaphosphate which are more tolerant to Ca^{2+}. Hexametaphosphate can form soluble complexes with Ca^{2+} and, therefore, is suitable for use in various meat marinades. Possible mechanisms by which phosphates enhance water-binding ability and meat hydration in marinated muscle foods have been reviewed (Xiong, 1999). These include the ionic effect and pH alteration contributed by phosphates and the ability to remove Ca^{2+}, thereby reducing myosin aggregation. For pyrophosphate and tripolyphosphate, the ability to dissociate the actomyosin complex in the presence of Mg^{2+} also contributes to water-binding in meat. The dissociation of actomyosin enables myofibril lattices to expand, thereby allowing increased water entrapment (Offer and Trinick, 1983).

While significant hydration of salt-marinated meat begins at approximately 0.6 M (i.e, 2.5–3.0%) NaCl, which parallels significant enlargements of the myofibril diameter, the addition of as little as 10 mM pyrophosphate can lower the required level of NaCl to 0.4 M for meat hydration. Furthermore, the extraction of myofibrillar proteins in the presence of pyrophosphate or tripolyphosphate occurs preferably at both ends of the A-band of the sarcomere, ostensibly due to the actomyosin-dissociating effect as myosin is cross-bridged with actin at the A-I band junction (Xiong et al., 2000). Interestingly, marination in the presence of orthophosphate or metaphosphate does not induce the above extraction pattern, which is similar to that of NaCl-only marination. The ability of sodium phosphates to facilitate water-binding in meat follows the general order of: pyrophosphate ≥ tripolyphosphate > hexametaphosphate > orthophosphate.

The dissolution of the actomyosin complex leads to an increased myosin extraction, therefore, an improved functionality such as gelation. The effect of phosphates on the functionality of myofibrillar proteins is influenced by the ionic strength. Both pyrophosphate and tripolyphosphate facilitate the gelation of myofibrillar proteins at 0.3–0.4 M NaCl but decrease the gel strength at above 0.5 M NaCl, while hexametaphosphate either promotes or has little effect on protein gelation (Robe and Xiong, 1993). Similar phosphate effects have been observed in emulsified batters in which pyrophosphate and tripolyphosphate do not necessarily help stabilize meat emulsions. The functionality changes are attributed to the phosphate-mediated alterations in protein stability and the aggregation pattern. When myofibrillar proteins are sufficiently soluble in salted meats, e.g., by the addition of at least 2% NaCl, the presence of phosphates tends to increase electrostatic repulsions between proteins, therefore, reducing myosin-myosin interactions and gel network development.

The phosphate-induced enhancement in protein solubility and gelation is responsible for the improvement in meat-binding in comminuted and restructured products (Fukazawa et al., 1961; Froning, 1965; Trout and Schmidt, 1986). However, the effect of phosphates is sensitive to pH and ionic strength. In general, phosphates contribute to meat particle-binding only if the NaCl concentration is less than 2% (equivalent to an ionic strength of approximately 0.5) under the typical pH condition (pH 5.50–6.0) in processed meats (Xiong, 2005). If the salt content exceeds 2% or the pH of the meat product is sufficiently high (>6.3), the effect of phosphates tends to diminish. With the combination of high levels of salt and pH, NaCl alone is capable of causing myofibrils to swell and myosin to solubilize; this would explain the lack of beneficial effect on meat-binding when polyphosphates are added under these conditions. Thus, the exact efficacy of phosphates in modifying protein functionality is directly related to the solubility status of myofibrillar proteins, which is influenced by the processing conditions as indicated above.

ALKALIS AND ACIDS

Alkalis

Sodium carbonates, similar to alkaline phosphates as described above, have been used to enhance the textural quality of meat products through alterations of their physiochemical properties. Chicken, beef, and pork products treated with alkaline carbonates, e.g., sodium bicarbonate ($NaHCO_3$) and carbonate (Na_2CO_3), show increased juiciness and overall palatability (Sheard and Tali, 2004; Sen et al., 2005). Sodium bicarbonate has also been used to alleviate the inferior-water-binding problem associated with pale, soft, exudative pork (Kauffman et al., 1998). The efficacy of bicarbonate is attributed to the ability to partially solubilize myofibrillar proteins and enhance their electrostatic repulsion primarily through elevation of the pH. As a result, carbonate treatments cause a transverse expansion of myofibrils, allowing more water pickup and retention. Traditional alkali marinades include the application of carbonate compounds in conjunction with sodium chloride to enhance hydration of raw meat and reduce the cooking loss (Sheard and Tali, 2004).

Bertram et al. (2008) compared the water pickup and cooking yield of pork samples marinated with various amounts and combinations of sodium chloride, sodium pyrophosphate, and sodium bicarbonate. The bicarbonate marinated samples produced the highest weight gain and yield and the lowest cooking loss (Figure 2). Such a superior processing yield of the bicarbonate-alone marinade was not seen in the other marinade treatments with single or combined sodium chloride and phosphate. Bicarbonate-marinated pork muscle had more extensive myofibril swelling and, consequently, reduced space between the myofibrils. Because sodium bicarbonate lowers the hydrogen ion concentration, it shifts the pH of intramuscular aqueous phase away from the isoelectric point of myosin (pH ~5.2). An enhanced electrostatic repulsion leads to the expansion of myofilament lattices. This expansion may increase the protein surface, hence, further promoting hydrogen bonding and electrostatic interactions between water and individual muscle proteins. Thus, swelling of the myofibrils promotes an osmotic compression of water molecules, thereby enhancing juiciness of cooked products and increasing consumer acceptance.

Other studies have also shown that the addition of sodium carbonates to meat and meat emulsions increased pH values and decreased thermal drip loss. Dolata et al. (1999) reported that sausages produced with carbonate additions (0.075–1.7%) had an increased cooking yield when compared with control sausages. Specifically, the addition of the carbonate preparation OPREN (0.075%) yielded the highest pH value and lowest thermal drip loss, indicating an inverse relationship between the 2 factors. The elevation of pH value by 0.5 would markedly improve water-holding capacity of meat batters. Sen et al. (2005) recorded a larger increase in pH values in chicken (broiler) breast meat marinated with 3% bicarbonate than the control treated with 2% NaCl or breast meat marinated with 3% tripolyposphate. Results also indicated a reduced cooking loss percentage for meats treated with bicarbonate. Thus, bicarbonate treated meat may improve moisture retention in commercial products.

Acids

Treatments of raw meat or isolated proteins with weak acid compounds, e.g., through marination or blending, can also improve the cooked product quality by influencing the protein functionality. Acid marination is especially desirable for lower-grade meat cuts. The marination creates a tenderized meat by affecting muscle proteins and fiber structures through a variety of processes. Examples include the pH reduction to a level far below the isoelectric point of myosin to create swelling of muscle fibers and

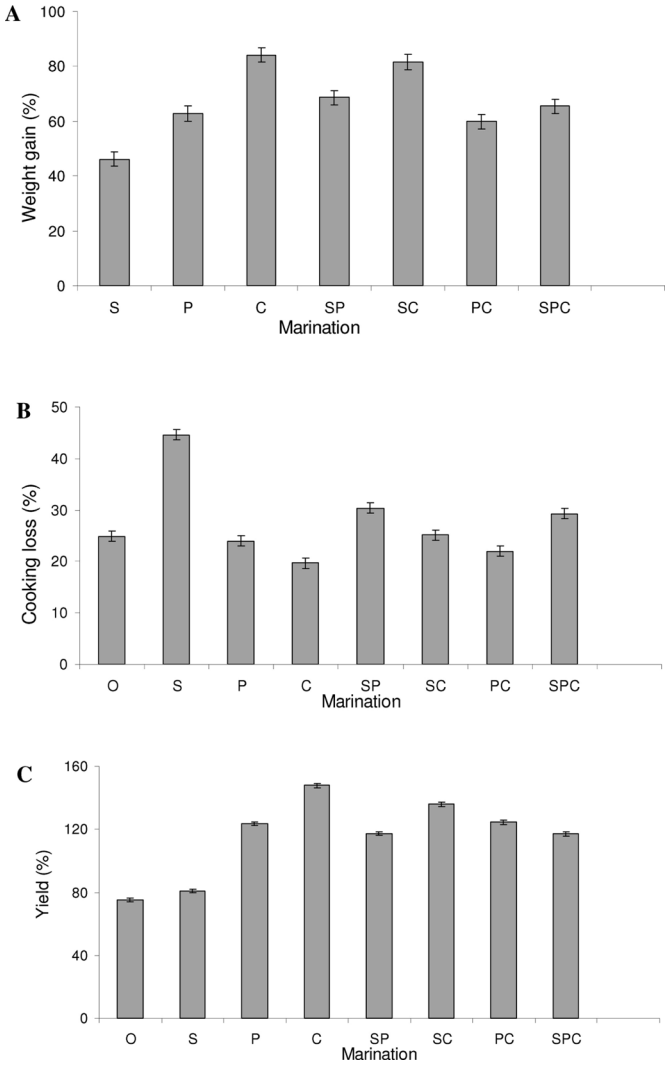

Figure 2. Water uptake (weight gain) (A), cooking loss (B), and cooking yield (C) of marinated pork strips. Marination solutions: O, control; S, 5% NaCl; P, 5% $Na_4O_7P_2$; C, 3% $NaHCO_3$; SP, 5% NaCl + 5% $Na_4O_7P_2$; SC, 5% NaCl + 3% $NaHCO_3$; PC, 5% $Na_4O_7P_2$ + 3% $NaHCO_3$; SPC, 5% NaCl + 5% $Na_4O_7P_2$ + 3% $NaHCO_3$. Adapted from Bertram et al. (2008).

connective tissue, enhanced proteolytic degradation of myofibrils by cathepsins, and increased solubility of collagen fibrils (Berge et al., 2001; Aktas et al., 2003). The type of acids is important in the overall pH reduction, moisture retention, and cooking yield of final meat products. For example, lactic acid yields a lower pH value compared with citric acid in marinades. This difference is attributed to the buffering properties of citric acid, a weak organic acid with 3 dissociation constants (pK_{a1} = 3.06; pK_{a2} = 4.74; pK_{a3} = 5.40). Therefore, lactic acid, with only one ionizable group (pK_a = 3.86), gives rise to stronger acidification of the muscle fibers, thereby producing greater moisture uptake. Because these carboxylic acids are capable of sequestering divalent cations such as Ca^{2+}, it is possible that they contribute to increased water-binding in meat also through the removal of divalent cations that otherwise might promote protein-protein interactions via the bridging effect.

Several studies have shown an improvement in water-binding capacity of meat upon exposure to acidic conditions below the typical pH of postmortem tissue. A direct correlation between the concentration of a specific acid and the final pH and tenderness of meat is generally observed. For instance, Ke et al. (2009) reported a significant increase in water-binding capacity of bovine muscle at pH 3.52 upon the addition of citric acid (0.2 M). Burke and Monahan (2003) reported increased moisture uptake, fiber swelling, and collagen solubility, along with a reduced cooking loss of shin beef, upon marination treatments with 31% citric juices (orange, lemon). The substantial reduction in the pH (from 5.7 to 3.1) was thought to be responsible for the improved water-binding capacity in the muscle tissue. The swelling of myofibrils may be due to the weakening of the Z-line, M-line, and possibly other regulatory proteins (Offer and Trinick, 1983). Orekovich et al. (1992) observed, through electron microscopy, a loss of the M-line at low pH and a loss of Z-line material at high pH.

Solubilization of collagen fibrils is also a likely factor that contributes to water-binding in acid-marinated meats. Collagen fibrils are comprised of highly cross-linked tropocollagen, a triple helix of polypeptide strands stabilized through hydrogen bonds. The mechanism by which collagen fibrils are rendered soluble by acids has not been fully elucidated; it may involve either the hydrolysis of peptide bonds or the breaking of covalent cross-linkages. The hydrolysis of peptide bonds suggests that the scission of peptides through acid marinades could solubilize collagen (Burke and Monahan, 2003). Another possibility for the acid-induced solubilization of collagen fibers is that the optimal pH for the proteolytic enzymes cathepsins is between 4.0 and 5.5. Cathepsins are endogenous proteases that are released from lysosomes and cleave myofibrillar and collagenous proteins. Therefore, lowering the net pH of meat will increase the rate of cathepsin activity. Ertbjerg et al. (1999) reported an increase in the release and activity of Cathepsin B and L with the addition of lactic acid.

Because electrostatic interactions are an important force in the aggregation of proteins, gelation of muscle proteins or meat homogenates at various pH conditions has been subjected to extensive investigations. The optimum pH for myofibrillar protein gel formation is slightly acidic (~6.0) for mammalian species and poultry and close to neutrality for fish (Xiong, 1997). However, at pH below the isoelectric point of myosin (5.2), strand-type myosin and mixed myofibrillar protein gels do form without heat treatment. For example, bovine myosin spontaneously forms a gel without cooking when the pH was slowly lowered to 4.0 (Hermansson et al., 1986). Ke and Hultin (2005) reported that chicken breast homogenate with 88% moisture formed a strong gel under acidic conditions with gels formed at pH 3.16 being more elastic compared with gels formed at pH 3.70 and 4.06. Gels at pH 3.16 and 3.70 had greater

water-holding capacity than gels formed at pH 4.06. The more positive charges in the more acidic gels ostensibly contributed to stronger water binding ability.

PROTEIN OXIDATION AND ANTIOXIDANTS

Oxidation is a common occurrence in meat processing. Process operations that disrupt the cell, e.g., fabrication, comminution, and mixing various salts in the presence of molecular oxygen, subject fresh meat to various endogenous (heme compounds, dehydroascorbic acid, hydrogen peroxide, singlet oxygen, etc.) as well as process-generated oxidizing compounds. The latter substances include hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), and thiyl radical ($RS^{\bullet}$). Because of their low sensory threshold, lipid and myoglobin oxidation can be readily detected. However, protein oxidation usually occurs unnoticed until it reaches an advanced stage where product structure hardening or softening and reduced water-holding capacity become evident. Nevertheless, protein research over the past decade has led to the recognition that proteins located in the muscle cell or exposed during food processing can be readily modified by active oxygen and nonoxygen species generated via lipid peroxidation, metal- or enzyme-catalyzed oxidation, and other chemical and biological processes. Most of these oxidants, though not intentionally generated or added in meat processing, are discussed here because they seem to be of greater significance than previously thought.

In the presence of molecular oxygen, iron (Fe) and copper (Cu), naturally abundant in muscle tissue, are strong catalysts of protein oxidation. The most common sites of protein oxidation by these transitional metal ions are the basic and sulfur-containing amino acid residues, e.g., His, Arg, Lys, Met, and Cys (Stadtman, 2006). In metal-catalyzed protein oxidation, His residues can be converted to Asp or Asn residues, Pro to Glu and γ-glutamicsemialdehyde, and Lys to 2-amino-adipicsemialdehyde. Oxidation of these amino acid residue side chain groups results in denaturation and formation of protein free radicals. Subsequent interactions of protein radicals lead to protein aggregation that can either impair or enhance protein functionality, depending on the extent of oxidative modification (Xiong, 1996). Furthermore, secondary products from lipid peroxidation, e.g., malonaldehyde, can modify protein structure and reactivity by forming complexes and aggregates (Li and King, 1999).

It has been shown that turkey breast myofibrillar proteins exposed to 0.025 mM Fe^{2+} or Cu^{2+} in the presence of 10 mM ascorbate had decreased functional properties (solubility, gelation, and water-binding), coinciding with the formation of protein carbonyl compounds and high-molecular-weight polymers or oligomers (Decker et al., 1993). Liu and Xiong (2000) reported that incubation of chicken myosin with 0.1 mM $FeCl_3$, 20 mM H_2O_2, and 1 mM ascorbate (a $^{\bullet}OH$-generating system) substantially decreased the gel-forming capability. Because ascorbate is an important reactant, it should be considered as a significant oxidant rather than an antioxidant in this particular oxidizing system. Hence, caution must be taken when it is intended as a natural additive to inhibit oxidative processes in muscle foods. Indeed, ascorbic acid can act both as a prooxidant and as an antioxidant depending on its concentration level and the chemical environment (Lee et al., 1992).

However, several studies have also generated evidence that mild oxidation can be beneficial for muscle protein functionality. Pacheco-Aguilar and Crawford (1994) observed a 2-fold increase in gel hardness and elasticity of Pacific whiting surimi by treatment with 0.25% potassium bromate ($KBrO_3$), an oxidizing compound. They attributed the functionality improvement to the oxidative inactivation of proteases responsible for gel-weakening (modori) as well as disulfide bond formation between myosin molecules. Srinivasan and Hultin (1997) reported that hydroxyl radicals, generated by mixing low concentrations of $FeCl_3$, H_2O_2, and ascorbate, were highly capable of inducing protein carbonyl production. The $^{\bullet}OH$-oxidized fish proteins exhibited decreased protein solubility by 14%, but it improved shear stress and true strain of cooked gels by 70% and 20%, respectively. The emulsification characteristics were also improved as a result of protein oxidation.

In our study, we found that washing beef cardiac muscle mince with water only (without added antioxidants) produced surimi of an improved gel-forming ability when compared with surimi prepared in the presence of added antioxidants, such as propyl gallate and α-tocopherol (Srinivasan and Xiong, 1996; Parkington et al., 2000). Storage of water- and ascorbate-washed surimi on ice for one week substantially enhanced its gel-forming ability and gel elasticity with a simultaneous increase in protein carbonyls from 4.5 to 14 nmol/mg protein. Forces involved in the stabilization of gels made from oxidatively modified proteins include disulfide linkages, dityrosine, and carbonyl-amine bonds.

Additional evidence of the involvement of protein oxidation in muscle protein functionality was recently obtained in our lab from a pork muscle myofibrillar protein study. We noticed that the improvement of protein gel rigidity upon heating was dependent upon the concentration of oxidants (hence, degree of protein oxidation) used to modify proteins. In general, mild oxidation, induced by $^{\bullet}OH$ (Blanchard et al., 2007) or ferryl [Fe(IV)-oxy] species from metmyoglobin (Figure 3), favored protein gel network formation when compared with antioxidant control. However, excessive oxidation resulted in extensive protein aggregation and, therefore, poor structure and rigidity of gels. It seems possible to utilize antioxidants to control the degree of protein oxidation so as to produce targeted protein structural changes or interactions for an optimum functionality.

The strong dependence of muscle protein gelation on sulfhydryl oxidation (to form disulfide bonds) serves as an example of the benefit of limited protein oxidation in

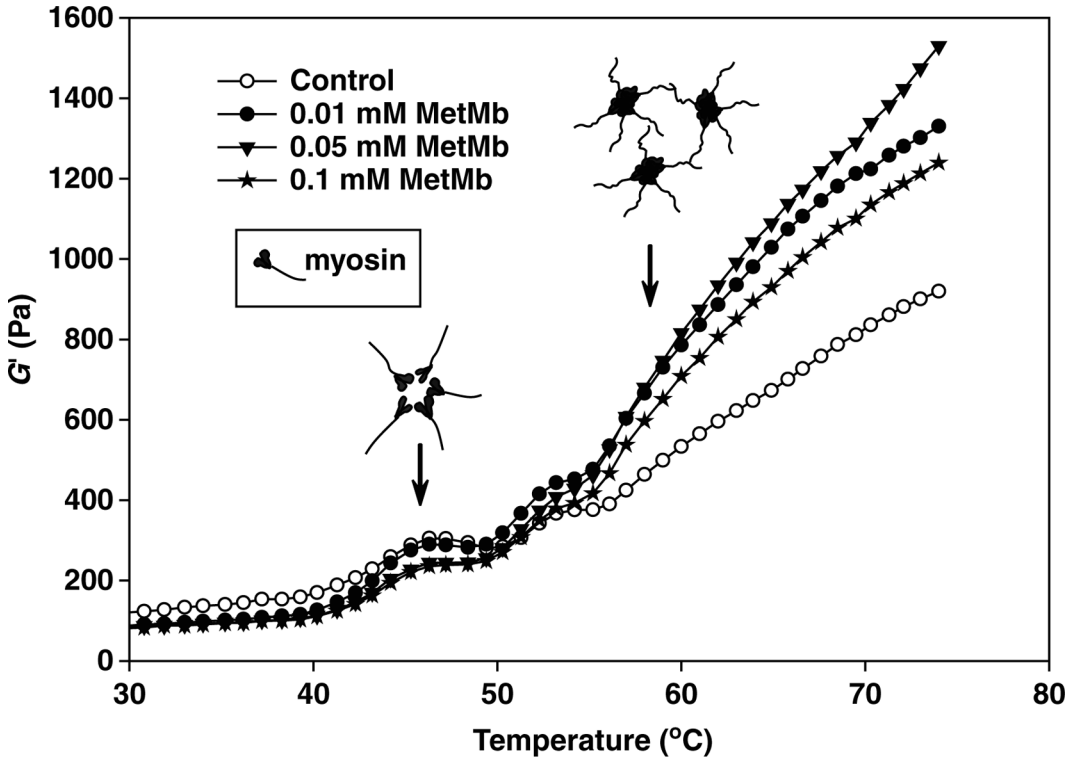

Figure 3. Dynamic rheological testing depicting the development of rigidity (storage modulus, G′) of myofibrillar protein gels (3% protein, 0.6 M NaCl, pH 6.0) during heating as affected by the oxidation of metmyoglobin (MetMb).

a controlled processing environment (Samejima et al., 1981; Lee and Lanier, 1995; Smyth et al., 1998). The aggregation of myosin at the initial stage of gelation involves disulfide formation between myosin heads. Although disulfide bond formation is not a prerequisite for the onset of myosin gelation, it plays an essential role in the continuous development of a tail-tail cross-linked, cohesive protein gel network during subsequent cooking. Moreover, in a finely comminuted product, oxidation-induced disulfide bonds are implicated in the interaction of fat globule membrane with protein gel matrix in the continuous phase. Such interactions may play a role in fat immobilization in cooked products.

Nonetheless, oxidation tends to reduce water-binding by proteins in the myofibrils and water-holding by protein gel matrices. For example, the exposure of porcine myofibrils to 1.47 mM of hemoglobin with or without 0.39 mM H_2O_2 resulted in a decreased water-holding capacity over broad pH (5.4–7.0) and ionic strength (0.29–0.71) ranges, as determined by low-field proton NMR (Bertram et al., 2007). Although not well understood, the reduced water-holding appeared to be related to dityrosine-dependent protein cross-linking. Recently, our lab has investigated the hydration properties of porcine myofibrils after exposures to $^{\bullet}OH$-producing solutions with oxidant concentrations close to in situ meat conditions (0.01 mM $FeCl_2$, 1 mM H_2O_2, 0.1 mM ascorbate) (Liu et al., 2009). Oxidation significantly lowered the hydration capacity (swelling) of myofibrils and decreased the extraction of myosin or the dissolution of the A-band (Figure 4). As time of exposure to the oxidants increased, the extent to which the thick filaments (myosin) slide past the thin filaments (actin) was substantially reduced, suggesting structural changes in myosin around the myosin-actin binding site. Similar phenomena were observed for pork stored in oxygen-enriched package systems where protein oxidation was notably accelerated compared with pork stored in a regular PVC packaging system (Delles et al., 2009).

NONMUSCLE PROTEINS

Processed meats are usually cooked to a final temperature of 65–73°C for palatability. However, these final temperatures are not sufficient to denature the main constituents of most plant proteins intended as meat binders or extenders. Consequently, interactions are limited between plant and animal proteins needed for the production of a viscoelastic composite product structure upon cooking. For example, 7S and 11S soy globulins denature around 75°C and 90°C (Scilingo and Anon, 1996). High NaCl concentrations (2–3.5%), usually present in processed meats, shift the denaturation of these soy proteins to even higher temperatures (Nagano et al., 1996). This might explain why native soy protein does not contribute to meat batter gelling properties (McCord et al., 1998). For this reason, commercial soy proteins for meat product applications are usually subjected to preheat treatment to dissociate protein subunits as well as induce partial structural unfolding. Preheat treatments of plant proteins

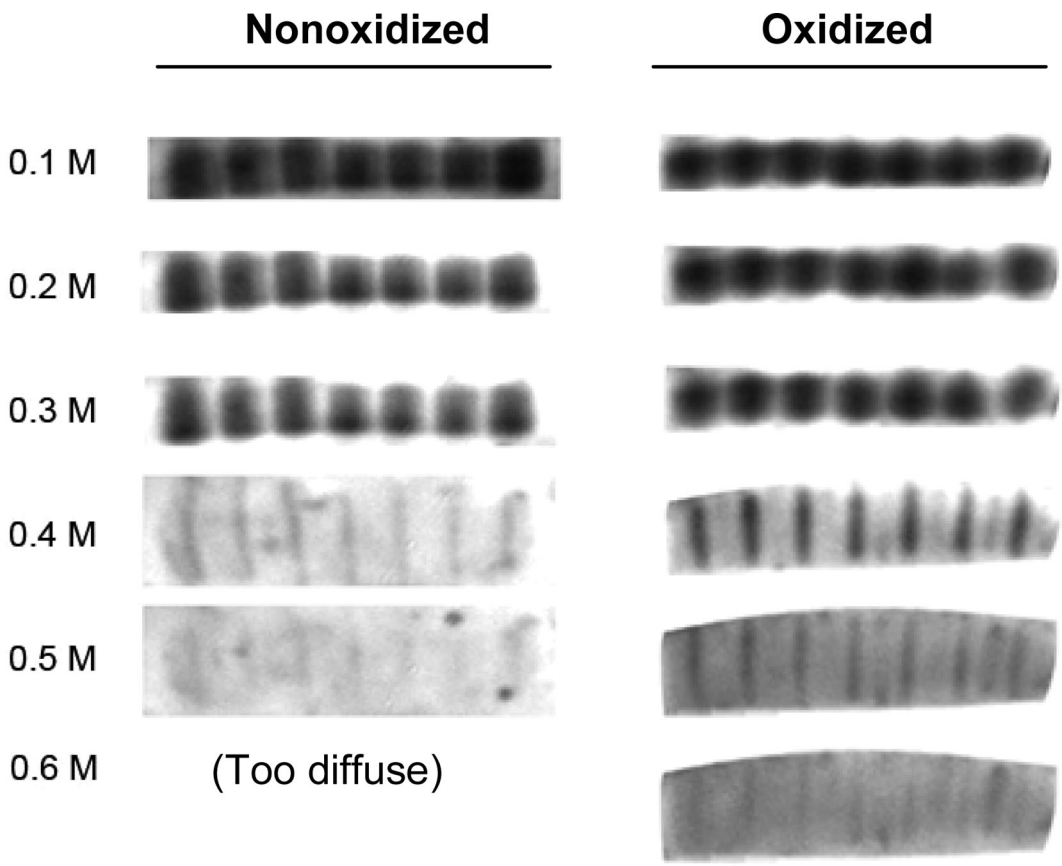

Figure 4. Phase contrast microscopy of control (nonoxidized) and oxidized (with 0.01 mM $FeCl_2$, 1 mM H_2O_2, 0.1 mM ascorbate) porcine myofibrils showing swelling and protein extraction during marination (irrigation) with various concentrations of NaCl (0–0.6 M) with 10 mM sodium pyrophosphate at pH 6.0.

would enhance their performance in meats, including meat batter emulsion stability and bind strength (Feng et al., 2003). Antagonistic interactions occur between myofibrillar proteins and native soy globulins. Conversely, when soy globulins are slightly destabilized by brief heat treatment, they can promote muscle protein gelaiton and water-holding capacity (Figure 5).

Similarly, untreated whey proteins, also shown to interfere with the gelation of myofibrillar proteins, can become significant promoters of myofibrillar protein gelation and emulsification when partially destabilized through heating (Hung and Smith, 1993). As much as 50% denatured protein can be found in commercial whey protein ingredients. This denaturation is usually achieved by heat treatment. Hence, by preheat treatment of the whey proteins to destabilize their structure, one would expect significant β-lactoglobulin-myosin interaction when a meat batter is cooked to 65–70°C. The ensuing hydrophobic aggregation and disulfide cross-linking will lead to a composite gel system of high rigidity and binding strength (Beuschel et al., 1992). It is also possible that heat-denatured whey proteins can act as active fillers in comminuted products, i.e., they can interact with surrounding meat proteins to reinforce the gel matrix (Barbut, 2006).

TRANSGLUTAMINASE

Transglutaminase is an enzyme that catalyzes an acyl transfer reaction (Glu → Lys) to form ε-(γ-Glu)-Lys isopeptide bonds within and between proteins, thus, promoting protein functionality (Kuraishi et al., 2001). The enzyme also catalyzes the hydrolysis of the γ-carboxyamide group in glutaminyl residues, resulting in deamidation. In muscle foods, where protein lysine residues (acyl acceptors) are abundant, the Gln-Lys cross-linking reaction prevails. While the enzyme can be extracted from a variety of natural sources, microbial transglutaminase (MTGase) produced from *Streptomyces mobaraensis* has attracted the most attention. MTGase was introduced initially by Ajinomoto Co. to meat processing to facilitate the production of muscle protein gels and the 'bind' in restructured raw meat. MTGase suits processed meats well as it maintains high activity over broad pH and temperature ranges (Kuraishi et al., 2001).

A soft gel that can bind meat particles in raw, restructured meat can be formed by incubation of myofibrillar proteins with MTGase at refrigerator temperatures. To obtain a stronger gel, a small amount of caseinate (an excellent MTGase substrate) is generally added to aid in meat

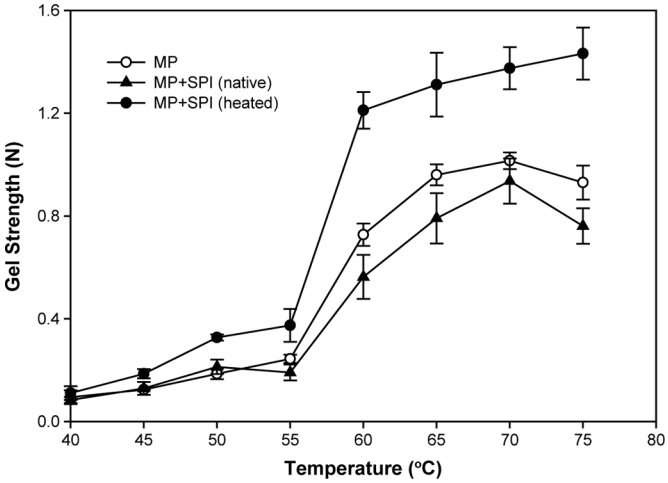

Figure 5. Penetration force (gel strength) of porcine myofibrillar protein (MP) gels (3% protein, 0.6 M NaCl, pH 6.0) containing 3% native (nonheated) and heated (90°C, 5 min) soy protein isolate (SPI). Neither native nor heated SPI formed a gel in the 40–75°C temperature range. Adapted from Feng and Xiong (2002).

particle binding. When heated to 30–50°C, MTGase becomes highly activated. Therefore, for cooked meat products, nonmeat protein additives are generally unnecessary as long as a small amount of salt is added to ensure a minimal amount of soluble proteins (substrate) is available for the enzyme. With MTGase, myofibrillar protein thermal gels can reach an overall rigidity of as much as 10 times that of control (MTGase-free) gels (Ramirez-Suarez et al., 2005) or MTGase-treated cold-set gels. This is true to both low- and high-salt protein or meat gels (Chin et al., 2009). The enhanced gel rigidity (storage modulus, G′) can be explained by the formation of thread-like cross-linking in MTGase-treated protein samples (Ahhmed et al., 2009). MTGase-treated myofibrillar protein and meat batter gels also exhibit improved water-holding capacity. Moreover, emulsifying activity of myofibrillar proteins, emulsion characteristics, and emulsion stability are markedly enhanced following MTGase treatment (Ramirez-Suarez et al., 2005). This would explain why MTGase treatments in situ improved meat emulsion firmness and stability (Kawahara et al., 2007).

CONCLUSIONS

Processed muscle foods, especially finely chopped products, are a heterogeneous, complex system in which proteins play a vital role in binding meat particles, retaining water, and immobilizing fat. As such, an integrated approach, instead of a simple ingredient treatment, is required to promote muscle protein functionality for desirable product quality characteristics. In order for muscle proteins to exert multiple functionalities within the same product, a combination of different ingredients and processing procedures is generally required. Many chemical ingredients can function synergistically during meat processing. In particular, phosphates are highly effective in enhancing muscle fiber hydration and myofibrillar protein solubility, making them extremely valuable for the production of reduced-salt products. However, phosphates tend to suppress protein aggregation, hence, the formation of a cohesive protein gel and a rigid fat globule membrane. Therefore, by combination with salts and proper alkali or acid compounds, phosphates at low concentrations can maximize the overall protein functionality for optimal meat product quality. Nonmuscle proteins and transglutaminase are other but large-molecule chemical additives that can be used in conjunction with small-molecule chemical ingredients to enhance the functionality of muscle proteins as well. Oxidative modification in a controlled redox environment (e.g., low concentrations of ascorbate, H_2O_2, and $FeCl_2$, with tocopherols as the regulator) could also be beneficial to muscle protein functionality. Because muscle foods are a complex, protein-based matrix structure, continuing research through a fundamental approach is needed to better understand the interactions of these chemical additives with proteins that are responsible for the yield, sensorial, and nutritional quality of the final product.

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Fish Protein Isolate and Its Superior Functionality

Jae W. Park

INTRODUCTION

Conventional Surimi Processing

Surimi is stabilized myofibrillar proteins refined from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants (Park and Lin, 2005). Conventional surimi processing (Figure 1) from white flesh fish utilizes typically 25–30% of the body mass including recovered insoluble particles from wash water. Fish flesh is ground into small particles (3–4 mm diameter) first before going through rigorous washing and dewatering using batch washing and rotary screens, respectively. Washing is an essential step in removing water soluble proteins (primarily sarcoplasmic proteins) and other impurities that reduce product quality. Sarcoplasmic proteins exist in the fluids within and between muscle fibers, and include many metabolic enzymes that diminish the stability of functional proteins during storage. Myofibrillar proteins, the primary components with the ability to form a 3-dimention gel networking, constitute approximately 70% of the total proteins in minced fish meat. Reduction in water soluble proteins, in turn, concentrates myofibrillar proteins. Thus it is known to enhance the functional property of surimi.

A proper washing process is vital in achieving high quality surimi with high recovery. An insufficient washing process could result in substantial loss of gel quality during frozen storage. On the other hand, over washing could cause substantial loss of fine particles and excessive moisture content. Maintaining water temperature near 5°C or below is critical for cold water species, such as Alaska pollock and Pacific whiting to maintain protein quality (Park and Lin, 2005).

Once washing is completed, washed meat will go through mechanical refining which removes scales, pin bones, and connective tissues. Then refined meat will be subjected to mechanical screw press to reduce the moisture content to 83–85%. This dewatered meat is then mixed with cryoprotectants to maintain frozen stability of myofibrillar proteins. It is typical that cryoprotectants consist of 4–5% sorbitol, 4% sugar, and 0.2–0.3% sodium triploy phosphate. Surimi packed in 10-kg block is then frozen using a contact plate freezer before storing at −20°C or lower for 2-yr shelf life.

Fish Protein Isolate (FPI)

A fish protein isolate (FPI) process (Figure 1) using acid or alkali extraction followed by isoelectric precipitation provides extremely high yields (35–45%) with the inclusion of sarcoplasmic proteins and it also demonstrates better functional properties (Hultin and Kelleher, 2000). This process consists of homogenizing fish tissue, solubilizing in acid (pH < 3.0) or alkali (pH > 10.0), recovering proteins by centrifugation (10,000 x g) after adjusting the pH to 5.5 using 1–2 N NaOH or 1–2 N HCl, respectively, and neutralizing with 1–2 N NaOH before freezing with cryoprotectants. Two approaches (surimi and FPI) in isolating fish proteins have a distinctive difference in their processing chemistry. Conventional surimi processing avoids any possible denaturation to prevent protein damage and maintain protein quality. In contrast, the FPI process induces chemical denatuation by adjusting pH to an acid or alkali condition and neutralizing by NaOH or HCl. Two acidic and alkaline chemicals during processing are basically neutralized as NaCl and H_2O. Therefore these chemicals can be categorized as a processing aid, but the resultants (salt and water) have to be labeled. Depending on the pH of fish (raw material) and the pH of FPI (end product), it should also be noted that unused sodium ions can be retained in FPI, possibly resulting in higher sodium content.

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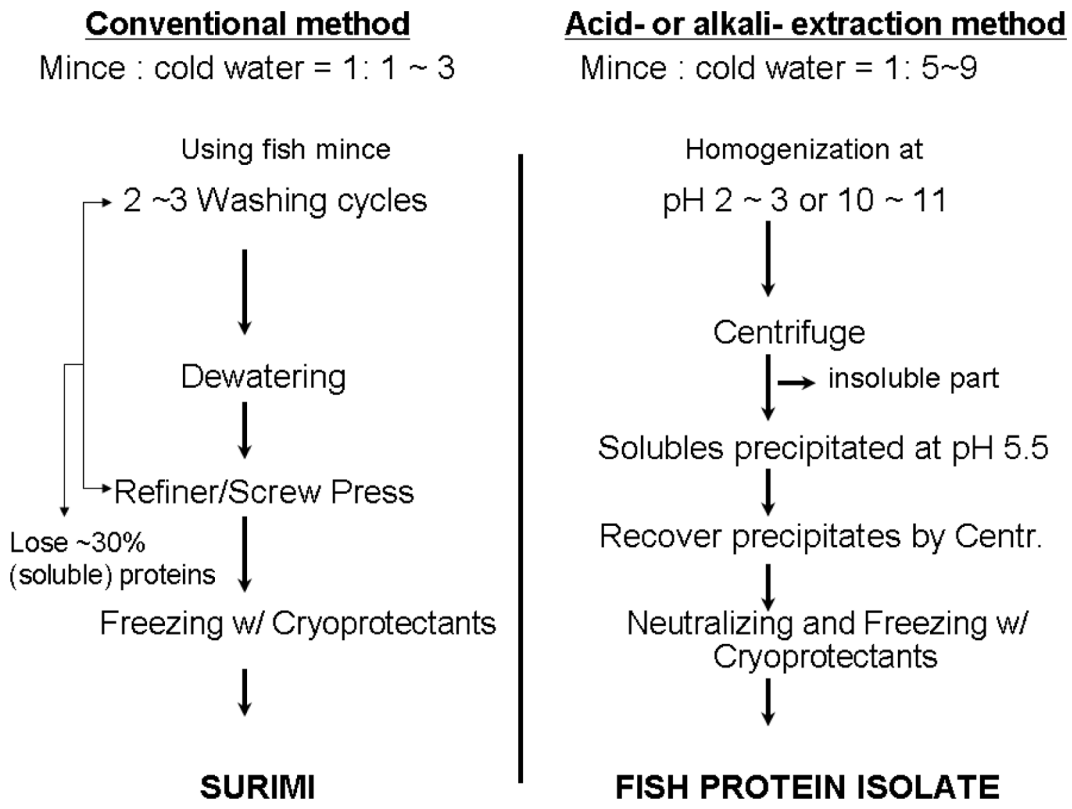

Figure 1. Processing flow for conventional surimi and fish protein isolate. Adapted from Park (2008).

PROCESSING CHEMISTRY OF FISH PROTEIN ISOLATE (FPI)

Extraction of FPI and Its Gelation

Several groups in the United States have lead numerous researches on functional fish protein isolate (FPI) made from various species (Pacific whiting, herring, catfish, Pacific sardine, Atlantic croaker, tilapia, jack mackerel, menhaden, rockfish, trout, krill, and giant squid) since 2003 following the original patent by Hultin and Kelleher (2000). A Sea Grant supported regional project (2000–2002) among 3 universities (University of Massachusetts, Oregon State University, and NC State University) played a major role in exploring this new technology in public views. Various physical and chemical properties of FPI were evaluated as fish muscle proteins were extracted using either acidic or alkaline extraction. Chemical-induced denaturation and chemical-induced refolding were also measured to determine the conformation changes of protein structure.

Alkaline extraction appears to give better gelling functionality (Kim et al., 2003; Yongsawatdigul and Park, 2004; Kristinsson and Liang, 2006; Chen and Jaczynski, 2007a; Thawornchinsombut and Park, 2007). According to Yongsawatdigul and Park (2004), the gelling properties of rockfish proteins at various pH extraction conditions were clearly demonstrated with a descending order of alkaline extraction, water washing, and acid extraction (Figure 2). It should be noted a distinct protein with MW of 120 kDa was found in acid-extracted paste, but it disappeared when made into a gel (Figure 3).

This protein band presumably resulted from degradation of myosin heavy chain (MHC) during acid extraction and probably disappeared to interact with other proteins during gelation. In alkali-extracted gel, protein bands with 120 and 42 kDa almost disappeared. Therefore gelation of alkali-extracted FPI could have been completed through interaction of myofibrillar and sarcoplasmic proteins via disulfide linkages. They also demonstrated the significant reduction of total sulfhydryl content as FPI formed a gel. It should also be mentioned that other interactions, such as hydrophobic interactions, have been reported to play an important role in gelation of fish muscle (Park et al., 1994), which could contributed to the rheological properties of acid-extracted and alkali-extracted FPI. Kristinsson and Hultin (2003a) reported an increase in surface hydrophobicity of cod myosin when it was treated in acidic or alkaline pH conditions, indicating the role of hydrophobic interactions in gel formation.

Yongsawatdigul and Park (2004) observed that actin bands of acid- and alkali-extracted FPI, under the SDS-PAGE without β-mercaptoethanol, were less intense than those of mince or washed mince (surimi), indicating actin may favorably interact with MHC of acid- and alkali-ex-

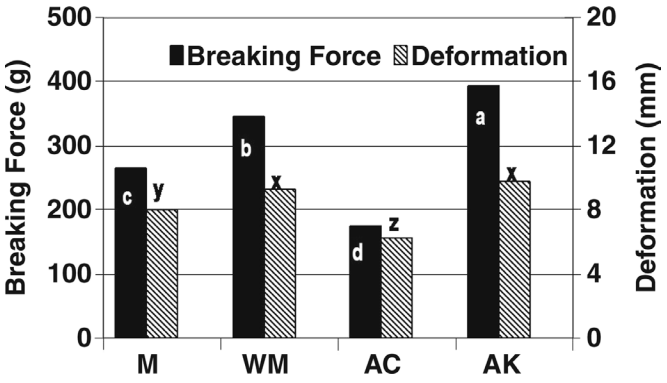

Figure 2. Breaking force and deformation of rockfish muscle proteins prepared by various treatments. Adapted from Yongsawatdigul and Park (2004). M: mince; WM: washed mince (surimi), AC: acid-extracted; AK: alkali-extracted. Different letters (a-d or x-z) denote a statistical difference ($P < 0.05$).

tracted FPI through disulfide linkages (Figure 3). Kristinsson and Hultin (2003a, 2003b) found that alkali- and acid-treated cod myosin had more exposed reactive SH groups, presumably promoting myosin head-to-head aggregation.

Kristinsson and Hultin (2003a) extensively examined the unfolding of acid- or alkali-treated myosin and gelling at a lower temperature, suggesting a less stable conformational structure of the refolded proteins. Improved functional properties were due to partial unfolding of myosin by acid or alkali. Thawornchinsombut and Park (2007) reported that salt solubility of proteins from FPI did not contribute significantly to their gelation properties. FPI prepared at pH 3 or 11 with NaCl could be partly refolded at pH 7.0. Nevertheless, some myosin fragments and actin did not refold. Kristinsson and Hultin (2003a) confirmed that the earlier onset of gelation correlated well with thermal unfolding/aggregation behavior of myosin (Figure 4). This is consistent with thermal unfolding being a prerequisite for gelation. Development of gel strength on heating has been found to correlate with increased turbidity (Samejima et al., 1981; Gill et al., 1992) and hydrophobicity (Wicker et al., 1986; Xiong, 1997).

Yongsawatdigul and Park (2004) showed a distinctively different storage modulus of AC and AK compared with washed and unwashed mince. G′ increased continuously from 25°C without a decline starting at 39°C and a sharp increase starting at 46°C (Figure 5). Since a decline of G′ was attributed to denaturation of light meromyosin (Xiong and Blanchard, 1994), it could be assumed that light

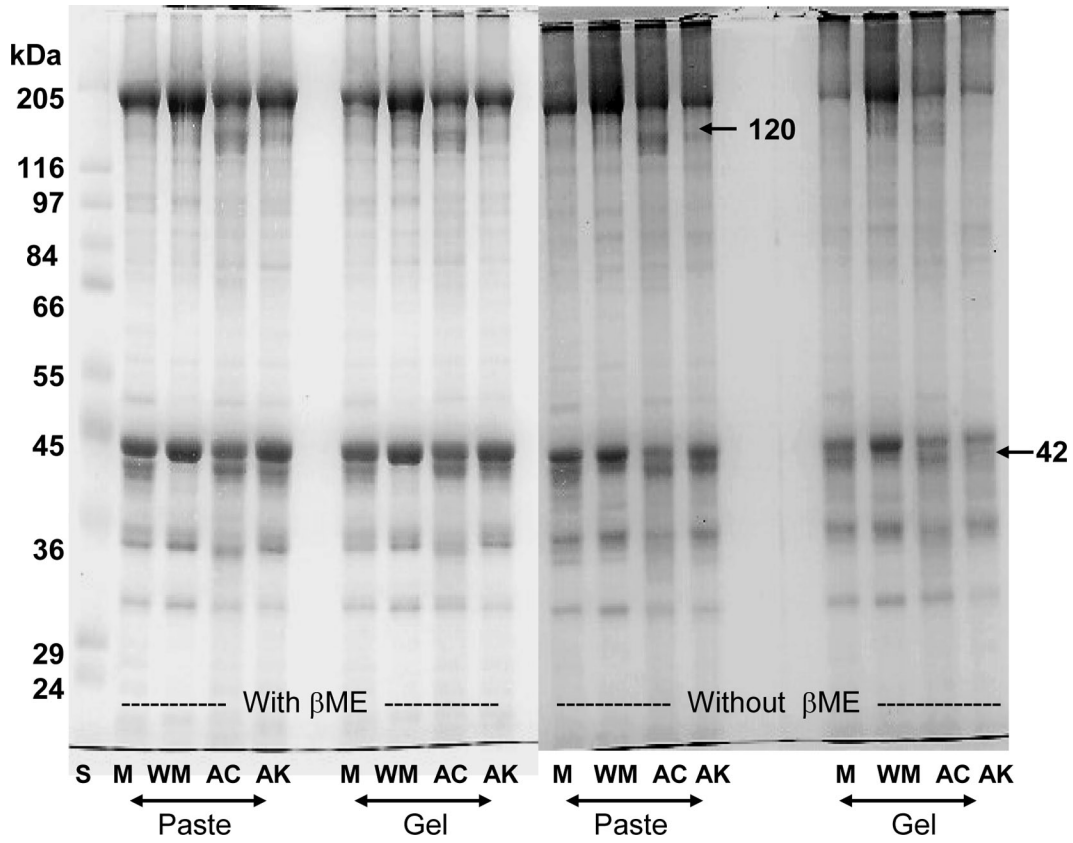

Figure 3. Sodium dodecyl sulfate PAGE (SDS-PAGE) patterns of rockfish muscle proteins prepared by various treatments solubilized in buffer with and without β–mercaptoethanol (βME). Adapted from Yongsawatdigul and Park (2004). S: standard molecular weight; M: mince; WM: washed mince (surimi), AC: acid-extracted; AK: alkali-extracted.

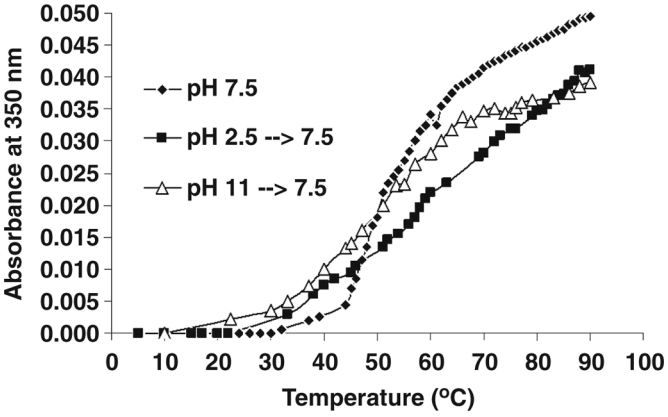

Figure 4. Thermal aggregation of native and refolded myosin as assessed by turbidity development at 350 nm. Protein concentration was ~0.45 mg/mL and samples were heated in a sealed cuvette at 1.5°C/min. Adapted from Kristinsson and Hultin (2003a).

meromyosin might have undergone denaturation during acid and alkali treatment. Therefore, viscoelastic properties of AC and AK were mainly contributed from aggregation of denatured muscle protein, which was previously induced by acid and alkaline conditions. Phase angle of these samples started to decrease at about 20°C, indicating the formation of an elastic material at relatively low temperature (Yongsawatdigul and Park, 2004). Recovering proteins using isoelectric precipitation at pH 5.5 could result in a zero net charge and promote protein aggregation. Morita et al. (1987) reported that chicken breast myosin had a long filamentous structure at pH 5.4 and formed a fine strand gel structure through myosin head interactions, exhibiting higher rigidity. Therefore, muscle proteins could readily aggregate to form elastic gel networks after isoelectric precipitation.

Kristinsson and Hultin (2003b) studied conformational and structural changes of cod myosin at pH 2.5 and 11 and after subsequent pH adjustment to 7.5. They suggested that on acid unfolding, the myosin rod may fully dissociate due to electrostatic repulsion within the coiled coil, while it does not dissociate at alkaline pH. Both pHs led to significant conformational changes in the globular head fraction of the myosin heavy chains, suggesting it takes on a molten globular configuration. A large part of the myosin light chains are lost on both pHs. On pH adjustment to neutrality, the heavy chain takes on a structural form similar to the native state with the coiled coil rod reassociating from acid pH while leaving the globular head less packed, more hydrophobic and structurally less stable. The irreversible change brought about in the globular head region leads to the failure of light chains to reassemble onto it, a drastic loss in ATPase activity, and more exposure of reactive thiol groups. Choi and Park (2002) also measured no detectable ATPase activities when Pacific whiting proteins were extracted at pH 2.5. Thawornchinsombut and Park (2007) indicated that gelation mechanisms of FPI were identical with the same NaCl concentration regardless of

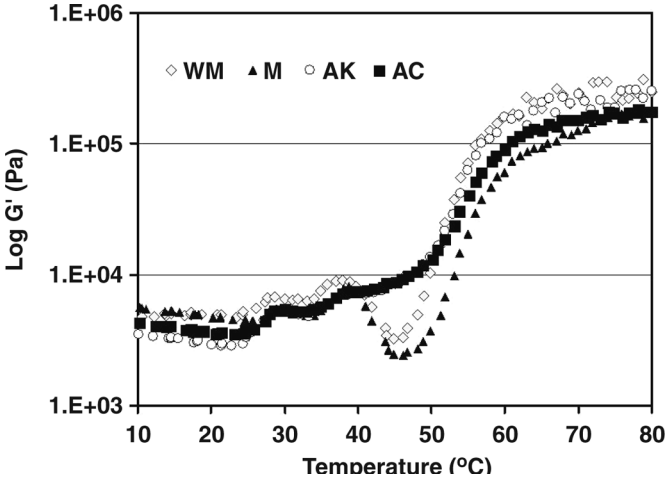

Figure 5. Dynamic rheology thermograms of rockfish mince prepared by various treatments: WM, washed mince; M, mince; AK, alkali-extracted FPI AC, acid-extracted FPI. Adapted from Yongsawatdigul and Park (2004).

pH. FPI prepared at pH 3 or 11 with NaCl could be partly refolded at pH 7. Nevertheless, some myosin fragments and actin did not refold.

Enzymes in FPI

ATPase activity is basically not measurable on acid and alkali extraction because a large portion of the myosin light chains are lost in both pH treatments (Choi and Park, 2002; Kristinsson and Hultin 2003b). The protein does not regain its native configuration as the pH is readjusted to 7.0–7.5. They also confirmed that a native configuration and ATPase activity are not a prerequisite for the gelation of FPI.

Choi and Park (2002) found significant cathepsin L activity in Pacific whiting muscle proteins subjected to acid extraction. However, Yongsawatdigul and Park (2004) noted that both acidic and alkaline extraction did not significantly promote proteolysis of myosin heavy chain of rockfish. This is probably species dependent. Kim et al. (2003) reported the best textural properties were obtained from Pacific whiting fish proteins treated at pH 11 followed by pH 2. Pacific whiting fish proteins treated pH 12 demonstrated the worst texture.

Cathepsins are very reactive enzymes causing gel softening in many types of fish species, including Pacific whiting. The control of cathepsin activities is a key factor to obtain high quality gels. A pH shift was thought to be an effective ways to inactivate cathepsins. Cathepsin L-like activities were found in all samples (pH 2–12) (Kim et al., 2003). Fish proteins-treated at pH 10.5 showed the highest activities of cathepsin L-like enzymes when the

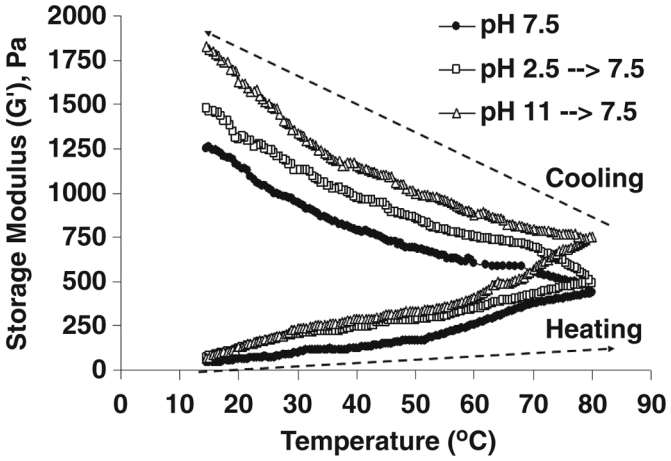

Figure 6. Viscoelastic behavior of cod myosin on heating and cooling. Protein concentration was 35 mg/mL. Samples were heating at 1.5°C/min and cooled subsequently when the temperature reached 80°C. Adapted from Kristinsson and Hultin (2003b).

activities were measured using the pH 5.5 buffer, which is known as the optimum pH for L-like enzymes. Dramatic reduction in activity was distinctively observed at pH 11 and 12. A possible explanation for this sudden change is that cathepsin L-like enzymes might be largely inactivated at pH 11 or higher.

In general, sarcoplasmic proteins, including proteolytic enzymes, are quite stable under mild alkaline conditions. Cathepsin B-like enzymes appeared to be highly activated at acid treatment (Kim et al., 2003). However, the alkaline process removed them dramatically. Especially at pH 12, no activities were detected. Most lysosomal proteinases are active at acidic pH. Cathepsin B has maximal activity at pH 6.0 and is unstable above pH 7.0 (Kang and Lanier, 2000). Cathepsin B-like could therefore not tolerant alkaline condition. Cathepsin H-like activities were not detected in any samples (no data reported). This enzyme was likely damaged by acid or alkali treatment.

Transglutaminase, which is unique to most fish, making a covalent link between lysine and glutamic acid upon setting or slow heating, is also apparently affected by acidic or alkaline extraction. The reduced effect of setting was observed in acid- and alkali-extracted FPI (Kim and Park, 2008). This observation indicates that endogenous transglutaminases could have been damaged during acid or alkali extraction.

Lipid in FPI

Both acid and alkali extraction can remove lipid through the centrifugation step. Better lipid removal, lower oxidation during storage, and reduced proteolytic degradation are advantageous results from alkaline extraction (Undeland et al., 2002; Kristinsson and Liang, 2006; Chen and Jaczynski, 2007a, 2007b). During oil processing to obtain a soap stock, free fatty acids are more readily removed with alkaline treatment than acidic treatment (Nawar, 1996).

Conventional surimi made from white flesh fish like Alaska pollock or Pacific whiting does not give any fishy odor. However, when surimi (average moisture content at 75%) is dried, fishy odor is clearly detected. This is due to

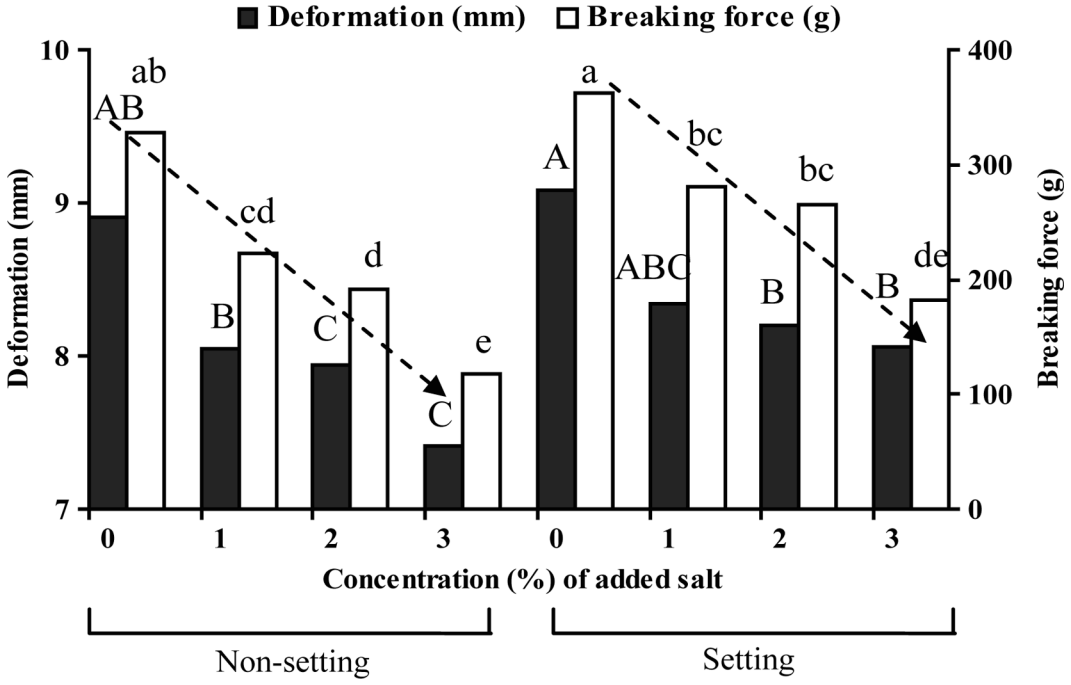

Figure 7. Negative effect of salt addition on texture properties of FPI. Adapted from Kim and Park (2008). Different capital letters indicate significantly different deformation values, while different lower cases denote significantly different breaking force values.

the presence of membrane lipid. During the processing steps of FPI, high speed centrifugation (100,000 x g) is applied (Hultin et al., 2005). The centrifugation step will produce 3 layers consisting of: a) upper layer with neutral lipid, b) sediment with membranes, insoluble proteins, bones, and skin, and c) middle layer with soluble protein. Therefore FPI should give desired stability to oxidative rancidity. Hultin et al. (2005) suggested the addition of divalent cations, such as calcium and magnesium, together with organic acid (i.e., citric acid), which contains more than one carboxylate group (COO^-) and has a hydroxyl group (-OH), allow much easier separation of the membranes from the proteins.

Color of FPI Gels

Slightly whiter color for alkali-extracted FPI gels was reported by Kim et al. (2003) with Pacific whiting, Kristinsson et al. (2005) with catfish, Chaijan et al. (2006) with sardines, and Perez-Mateos and Lanier (2006) with menhaden. However, acid-extracted FPI demonstrated slightly whiter gel by Yongsawatdigul and Park (2004) with rockfish and Chen and Jaczynski (2007a, 2007b) with rainbow trout and krill. For gel color, it appears that fish species influences the color effect rather than acid or alkali preparation of FPI.

SUPERIOR GELLING PROPERTIES OF FPI

Various research groups demonstrated superior gelling properties of alkali-extracted FPI. It was thought, in general, that improvements in functionality are directly linked to the extent of partial unfolding of myosin in alkali followed by refolding at neutral pH (Kristinsson and Hultin, 2003a; Thawornchinsombut and Park, 2007). The change of pH could have lead to conformational changes, resulting in better charge distribution (Kristinsson and Hultin, 2003b). Yongsawatdigul and Park (2004) observed that a greater extent of disulfide linkages in gel prepared by alkaline extraction resulted in higher gel breaking force and deformation. Superior gelling properties of FPI are likely due to combined effects of conformation changes, sarcoplasmic proteins, and homogeneous dispersion of disrupted muscle tissues.

Conformational Changes

In classic muscle chemistry, it is generally understood that salt-soluble protein extractability has a direct impact on the textural properties of muscle tissue. However, it is not likely the case with FPI. The myofibrillar proteins in FPI were not extracted well when NaCl was added perhaps due to protein aggregation caused by acid or alkali extraction. Salt solubility of FPI was not closely related to their textural properties (Choi and Park, 2002; Kristinsson and Hultin, 2003b).

Kristinsson and Hultin (2003a) reported non-cooperative thermal unfolding/aggregation was similar for proteins in the molten globular state (Dill and Shortle, 1991). The head region of myosin was likely in a molten globular conformation after refolding (Kristinsson and Hultin, 2003b), suggesting that much of the modified functionality of myosin may be due to this head region. The rod section of myosin has been found to be predominantly responsible for the rigidity (i.e., formation of a strong gel network) development of the gel (Samejima et al., 1981). The substantial increase seen in the storage modulus on cooling was therefore likely due to the interaction between myosin rods. The ability of refolded cod myosin to form a strong gel on cooling was thus likely due to the fact that its rod was in the native configuration after refolding (Kristinsson and Hultin, 2003b) and still would have the ability to form a strong and stable protein network. The increased gel strength after cooling for the pH 2.5 and pH 11 treated protein compared with native myosin, respectively, can possibly be explained by the improved ability of the head groups to initiate the protein network (Figure 6; Kristinsson and Hultin, 2003a).

Role of Sarcoplasmic Proteins

Sarcoplasmic proteins are removed in conventional surimi manufacture to concentrate the content of myofibrillar proteins, while they are retained in the FPI process. Do sarcoplasmic proteins enhance the gelling properties of FPI? Even though the removal of sarcoplasmic proteins would increased the proportional concentration of myofibrillar proteins, thus enhancing the gelation properties of surimi (myofibrillar proteins), various studies confirmed that sarcoplasmic proteins positively contributed to the gelation of myofibrillar proteins (Morioka et al., 1992; Kim et al., 2005; Park and Park, 2007). Yongsawatdigul and Park (2004) reported that disappearance of 120 kDa protein when acid-extracted paste was formed as a gel and disappearance of 42 and 120 kDa proteins when alkali-extracted paste was formed as a gel indicate their interaction with myofibrillar proteins via disulfide linkages.

Kim et al. (2005) studied sarcoplasmic proteins treated at various pH conditions. Addition of sarcoplasmic proteins appeared to delay thermal denaturation of myosin and actin. The least amount of proteins was lost when sarcoplasmic proteins were treated at pH 2 or 3 followed by precipitation at pH 5.5. Gelation properties of sarcoplasmic proteins were not as good as myofibrillar proteins, but positively contributed to gelation with myofiblillar proteins as judged by breaking force. Therefore, the retained sarcoplasmic proteins are likely to be one of reasons for superior gelling properties of FPI.

Disintegration of Muscle Tissue

Sato and Tsuchiya (1992) observed stronger, more deformable meat gels correlated with more homogeneous dispersion of proteins when viewed by transmission electron microscope (TEM). Recently Wright and Lanier (2008) reported that stronger, more deformable gels were made from alkali-extracted FPI, possibly due to more homogeneous dispersion as viewed by TEM. Wright (2007)

evaluated the slurries produced by acid or alkali extraction and gels made from the slurries based on the effect of disruption and dispersion using transmission electron microscopy. Disruption of ultrastructure and better dispersion of proteins positively affected gel forming ability of the meat. The maximum effect of disruption and dispersion occurred when the pH was raised to 11, likely because of the removal of ultra structural proteins, so called solubility inhibitory proteins.

Wright (2007) also tested calpain, a naturally occurring endopeptidase to measure the effect of disruption/dispersion in isolated chicken myofibrils without conformational change. Calpain pretreatment produced the strongest gelling properties in subsequently comminuted myofibrils. Calpain's ability to disrupt the Z-disk and titin might have better dispersed the myosin and actin.

COMMERCIAL APPLICATIONS OF FPI

Two inventors are commercially practicing their patents under MPF Inc. and Proteus Industries, respectively. MPF (www.succulence.com) is marketing its business under the Succulence System by injecting FPI into high valued fish steaks in the form of a marinade. They claim it will increase yield, restore succulence to frozen meats or fish, reduce or eliminate phosphates, and recover meats from trimmings. This company primarily exercises alkaline extraction. Marinade is made by mixing isolated fish protein with a certain portion of water while the pH is adjusted to neutral.

Proteus Industry (www.proteusindustries.com) is marketing its business under the Nutrilean "The Fat Blocker" label by spraying isolated protein solution on to the surface of battered and breaded chicken, fish, or beef before frying in oil. They claim health benefits (reduced fat), consumer attributes (great taste), operational benefits (increased yield), and application flexibility (spraying, dipping, marinade, or injecting). According to Kelleher and Williamson (2005), aqueous acidic protein solution, which is an aqueous solution of myofibrillar proteins and sarcoplasmic proteins is derived from animal muscle tissue and has a pH between about 2.5 and about 3.5.

FUTURE OF FPI

Can it replace surimi or can it be used like surimi? The development of FPI will undoubtedly play a major role in replacing surimi with its higher recovery yield and better gelling properties. However, further research will be needed to demonstrate how FPI can be easily used. One of the major issues related to gelling properties is the negative effect of salt on FPI (Figure 7). Interestingly, salt addition to FPI gave a negative effect on gel texture, indicating fish proteins were chemically unfolded during pH-driven extraction. Textural properties of acid- or alkali-extracted FPI decreased as NaCl increased, especially at 2–3%, regardless of setting treatment (Kim and Park, 2008). Gels made with salt and acid- or alkali-extracted FPI were generally of poorer quality than those made with CS, added salt and cold setting. However, under non-setting conditions, both acid- and alkali-extracted FPI without salt exhibited stronger gels (breaking force of 305 g and 325 g, respectively) than CS with 2% NaCl (230 g breaking force). This was concomitant with several reports of pH-shifted protein isolates (Undeland et al., 2002; Kristinsson and Hultin, 2003b; Pérez-Mateos et al., 2004; Yongsawatdigul and Park, 2004).

During acidic or alkaline treatment, salinity did not increase, indicating HCl and NaOH were chemically neutralized and salt was released with water. In fact, the salinity values of FPI were significantly lower than that of surimi. This may indicate better frozen stability of FPI (Thawornchinsombut and Park, 2007).

Marinade made from FPI can make a significant contribution to lowering the omega-6 to omega-3 ratio effectively toward catfish, tilapia, poultry, and red meats (Lanier, 2009). Eating too much omega-6 and too little omega-3 causes clots and constricts arteries to increase risk for heart attacks, increases swelling to worsen arthritis, and aggravates a skin disease called psoriasis (Mirkin, 2009). In fact omega-3 oil purified from fish (salmon or menhaden) can be added into FPI marinade to form an emulsion. Omega-3 oil in FPI as emulsion would be better distributed and retained much better in meats upon injection. This application can ameliorate public perception of the dangerous high omega-6 levels in the meats by injecting emulsion marinade made with isolated meat protein and fish oil. It should give no detrimental effects to taste or texture while promoting yield gain.

CONCLUSIONS

Fish protein isolate (FPI) made from various types of fish, including by-products or pelagic fish, can be used as a functional ingredient as surimi replacement, marinade, or fat blocker. Superior gelling properties of alkali-extracted FPI were probably due to a combined effect of better charge distribution through conformational changes, partial refolding at neutrality, homogeneous dispersion of myofibrillar proteins disintegrated through mechanical homogenization, and retained sarcoplasmic proteins. This technology can be easily extended to poultry and red meats by extracting functional proteins from their by-products. Whether it is used as a gelling protein or injected as marinade in an emulsion with omega-3 oil, it will play a significant role in developing values added products.

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Cattle Welfare and Beef Quality

Neville G. Gregory

PROCURING CATTLE

Most abattoirs have preferred types of animal for the markets they supply. Large cattle are often preferred for manufactured meats because of the greater processing efficiency. Heifers and light weight feedlot cattle can be more prone to dark cutting, because they are more active during the preslaughter period, which makes them less appropriate for retail as steaks. (Table 1; Kreikemeier and Unruh, 1993; Kreikemeier et al., 1998). Heifers also have a poor dressing % when pregnant.

Double muscled (DM) cattle are very common in Europe, and their advantages and disadvantages are worth noting in case semen from double muscled bulls becomes more widely used in USA dairy herds. They have 3 animal welfare problems; dystocia and the need to perform caesarians, large tongues and feeding difficulties in calves, and exercise-induced metabolic acidosis (Gregory, 2007). The benefits in terms of meat quantity and quality in the crossbreds (DM × Holstein Friesian [HF]) are conflicting. Cutlet size is larger and lean meat yield is superior in the crossbred compared with purebred HF steers. However, the meat has less marbling, a higher shear force and more total water (Mojto et al., 1998). They are not prone to high pH_{ult} as they have more difficulty walking and running, and are less active before slaughter.

EFFECTS OF HEAT STRESS ON MEAT QUALITY

Heat stress can affect meat quality in 2 ways. First, acute heat stress before slaughter can lead to dark cutting when there is muscle glycogen depletion. Second, long-term heat exposure during the rearing period can influence carcass and muscle composition. For example, it can favor greater muscle marbling and greater deposition of fat in internal depots, in place of the subcutaneous depot (Mader and Davis, 2004; Nardone et al., 2006). This could be an advantage for some specialized markets.

The overall frequency of dark cutters is highest during the second half of summer (Figure 1; Kreikemeier et al., 1998). Providing shade in the feedlot can reduce the frequency of dark cutters (Mitlöhner et al., 2002). In the absence of shade it is good practice to sprinkle cattle with water to relieve heat stress and control aerial dust in the feedlot. In a study in southern US, daily sprinkling for 2 min every hour between 1100 and 1700 h, when ambient temperature exceeded 30°C, did not increase the prevalence of *Salmonella* or *E. coli* O157:H7 in feces or on the hides (Morrow et al., 2005). So, veterinary public health concerns about water sprinkling may not be warranted.

Heat tolerance can be improved by introducing tropically-adapted composite breeds such as *Bos indicus* Brahman crosses, but it will lead to tougher meat through raised calpastatin activity in the meat (Müller et al., 1982). Brahman crosses also have less marbling and reduced meat juiciness (Wheeler et al., 2001). The toughness can be avoided either by limiting the proportion of *Bos indicus* in the slaughter generation composites, or by increasing the post mortem aging period in the abattoir, or by using a heat adapted *Bos taurus* breed such as the Tuli instead of the Brahman. Tuli-sired cattle also deposit fat internally instead of in the carcass which may be an advantage for both roasting and manufactured meat markets (Sprinkle et al., 1998).

TRANSPORT CONDITIONS

Deaths during transport are relatively uncommon in cattle, but there have been isolated cases of heat stress-related deaths during long journeys (McQueen, 1972). Those that survived these journeys, but had to be promptly slaughtered because they were in a heat stress crisis, were passed as fit for human consumption at meat inspection.

Bruising can be worse at high densities especially in cattle that go down during the journey (Tables 2 and 3). Hard cornering was the main reason for falls in the second study (Table 3), and bruising in the 'fallers' was re-

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Table 1. Relationship between live weight in US feedlot cattle and the frequency of dark cutters

	Cattle weight (kg)			
Item	<454	454–498	499–544	>544
Number of lots	259	1,089	1,459	852
Cattle per lot	203	215	207	162
% dark cutters	0.94	0.89	0.75	0.57
Dressing %	63.8	63.7	63.5	63.0

flected in higher CK activities in blood samples taken after unloading. These animals also had higher plasma cortisol concentrations. Dividing cattle into small pens within a vehicle can reduce the hazards associated with rapid changes in vehicle motion (Eldridge et al., 1988).

Putting these 2 studies together, bruising could be minimized by setting the maximum stocking density at no more than 450 kg per m^2. However, this would not allow for large animals traveling long distance, which would require more space. To simplify things, a general equation is used by some authorities

$$\text{Space required} = 0.021 \times \text{Liveweight}^{0.67}.$$

PRESLAUGHTER HOLDING PERIOD

The potential advantages and disadvantages of preslaughter holding periods at an abattoir include the following:

Advantages

- Opportunity to rehydrate
- Easier to handle after a settling period
- Likely to be less pressure on stockmen putting animals up for slaughter

Disadvantages

- Greater risk of high pH or intermediate pH meat if held for too long
- Greater opportunity for bruising if there is mounting behavior
- Greater risk of fecal soiling if pen floor is not cleaned regularly

In practice, the effects on meat quality are not large, but they can be commercially significant. For example, cattle held at an abattoir over the weekend (and fed) were more likely to be dark cutting (1.64%) than cattle slaughtered the day after arrival (0.77%).

In general, holding cattle without feed but in a relaxed state for up to 24 h has had limited effect on meat pH. For example, there were no differences in *triceps brachii* pH_{ult} when *B. indicus* crossbred cattle were trucked 200 km from a feedlot to an abattoir in South Africa, and slaughtered either on arrival, or after a 3 h holding period or the following day (approximately 24 h holding) (Grosskopf et al., 1988). If they fight when held at the abattoir, the situation is quite different. McVeigh et al. (1982) showed that 6 h fighting when bulls were mixed, was sufficient to reduce *l. dorsi* glycogen by 59%. It took 4 to 7 d for muscle glycogen to recover when they were separated and fed a barley-based ration. The type of feed will determine the rate of muscle glycogen recovery. For the *semimembranosus*, the relationship between the rate of repletion (Y – mg glycogen/100 g muscle over 72 h) and energy intake (X – MJME intake/d during the 72 h) based on work by Gardner et al. (2001) was

$$Y = -0.24 + 7X.$$

Glycogen depletion and repletion depend on the muscle which is being assessed, through the function it serves. For example, muscles which are not taxed when maintaining a standing posture are virtually unaffected by a journey, and so glycogen loss is not much concern. This was shown by Shorthose et al. (1972) when Shorthorn steers were trucked 320 km to a railhead, rested for 16 h and then given a 970 km rail journey to an abattoir in Australia. By the time they arrived they had been without feed for 4 d. At the abattoir they were rested, watered and given limited feed according to the schedule shown in Table 4. There were virtually no effects from resting and

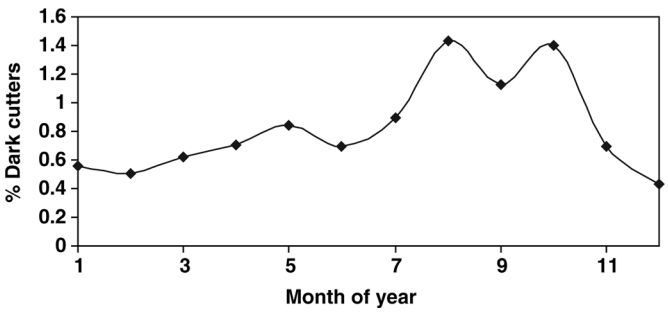

Figure 1. Frequency of dark cutting beef carcasses according to month of the year in the United States.

Table 2. Effect of stocking density during transport on carcass bruising and meat pH_{ult}[1]

Item	Stocking density during transport		
	Low	Medium	High
Number of animals	16	16	16
Floor space per animal (m^2)	1.39	1.16	0.87
Liveweight (kg/m^2)	288	345	459
Animals went down during journey (%)	0	0	38
Number of aggressive moves	3.0	2.3	0.3
Bruises per carcass	2.3[a]	1.2[b]	3.2[a]
Bruise score	4.6[a]	1.9[b]	8.2[a]
L. dorsi pH_{ult}	5.67	5.41	5.54

[a,b]Means within a row without a common superscript letter were significantly different at $P < 0.05$.
[1](Eldridge and Winfield, 1988).

re-feeding on *l. dorsi* pH_{ult}, but there were pronounced effects in the *flexor profundus* which contributes to standing posture. The pH_{24h} of *f. profundus* is normally 5.7.

In this study there were no treatment differences in Instron *l. dorsi* measurements, but the relationship between peak shear force and pH_{ult} was curvilinear, with highest values at a pH_{ult} of 6. Intermediate pH_{ult} beef is often tough, because it is outside the optimum pH ranges for calpain, caspase and cathepsin-induced tenderization and because it has reduced sarcomere length. The pH_{ult} range associated with increased toughness is usually between 5.8 and 6.2 when shear force measurements are made in cooked *l. dorsi*, and 6.2 to 6.4 when not cooked (Purchas and Aungsupakorn, 1993; Jeleníková et al., 2008). If intermediate pHult beef is given sufficient time to age it will lose its toughness, but this conditioning takes more than 7 d which may be too long for some distribution chains (Purchas, 1990). Situations that can lead to a higher prevalence of intermediate pH_{ult} beef include sourcing cattle from sale yards, excessive exercise before slaughter, and slaughtering cattle following transport without providing a rest period at the abattoir (Table 5; Jones et al., 1988; Kuzmanović and Elabjer, 2000; Jeleníková et al., 2008).

Preslaughter exercise depletes muscle glycogen and causes high pH meat in those muscles that work during the exercise. For example, mounting and fighting behavior results in high pH meat in the *L. dorsi*, *trapezius* and *semitendinosus* (Warriss et al., 1984). Running has very little effect on the *l. dorsi* even when plasma lactate concentrations are 5-fold higher than normal (Apple et al., 2006), but it depletes glycogen in the *semimembranosus* and *semitendinosus* (Gardner et al., 2001; Table 6). *Semitendinosus* is more refractory to muscle glycogen repletion than *semimembranosus*. Making assumptions that *l. dorsi* pH is representative of other muscles in the carcass is an over-simplification when exercise stress is involved.

Nevertheless, emotional stress, can lead to generalized adrenaline-induced glycogen depletion and dark cutting. This occurs in all muscles, including the *l. dorsi* (Apple et al., 2005). Loin muscle was darker, more tender and had a higher pH_{ult} when cattle were slaughtered promptly after a restraining-isolation stress period (6.35 versus 5.65).

Feedlot cattle that are grown rapidly have small feet, and are prone to getting foot-tired, and this can impact on meat quality. Animals that sat down in the abattoir holding yards overnight were likely to have brighter-red meat

Table 3. Effect of stocking density during transport on carcass bruising[1]

Item	Stocking density during transport		
	Low	Medium	High
Number of animals	24	30	42
Floor space per animal (m^2)	1.75	1.40	1.05
Liveweight (kg/m^2)	196	312	591
Animals went down during journey (%)	4	7	98
Bruise score	2.9	3.4	8.7*

[1](Tarrant et al., 1988).
*Bruise score significantly increased with stocking density ($P < 0.01$)

Table 4. Effect of four different management procedures at an abattoir on pH in shin and loin muscle

		At the abattoir: holding period, feed available, water available			
Muscle	Time postmortem	2 d, 0 d, 1 d	2 d, 1 d, 1 d	4 d, 2 d, 3 d	4 d, 3 d, 3 d
	70 min	$7.06^{a} \pm 0.07$	$7.09^{a} \pm 0.05$	$6.84^{b} \pm 0.05$	$6.85^{b} \pm 0.06$
F. profundus	24 h	$6.31^{a} \pm 0.02$	$6.40^{a} \pm 0.02$	$6.53^{b} \pm 0.03$	$6.57^{b} \pm 0.02$
L. dorsi	24 h	$5.77^{ab} \pm 0.04$	$5.88^{a} \pm 0.06$	$5.72^{b} \pm 0.07$	$5.63^{b} \pm 0.02$

[a,b]Means within a row without a common superscript letter were significantly different at $P < 0.05$.

(more acceptable for the Japanese kobi market) compared with those that did not sit down (Figure 2). The implication is that darker meat is more common in cattle that are overcrowded and do not rest adequately.

One of the main problems with high pH beef is that it spoils easily (Nortjé et al., 1985). It takes only one cut or one primal to develop an off odor to cause rejection of a whole box or consignment of beef. Trying to hide or 'lose' a dark cutting primal in a delivery of normal beef can ruin the perception of the entire consignment if the customer is struck by a bad smell on opening the door of the refrigerated van. Dark cutting beef deteriorates because it does not produce any glucose from glycogen post mortem. As a result, glycolytic microorganisms are replaced by putrefactive proteolytic bacteria which produce spoilage odors and ammonia. Adding glucose to the surface of dark cutting meat will counteract this effect if it is applied early enough after carcass dressing or boning.

PUTTING CATTLE UP FOR SLAUGHTER

When cattle are drafted for slaughter, each feedlot pen is destocked over a period of weeks until there are too few animals in the pen. The remaining animals are then put with another group of cattle, and one might think that regrouping could cause stress-related meat quality problems. However, Colditz et al. (2007) found that regrouping 2 weeks before slaughter had little effect on physiological stress indicators or meat quality after slaughter.

One of the hazards when handling cattle in the holding yards at the abattoir is that they become excited and develop a metabolic acidosis just before they are slaughtered. This happened when feedlot cattle were given 6 prods with a Hot Shot electric goad (Warner et al., 2007). The resulting *l. dorsi* meat had poorer tenderness, juiciness and flavor and released more drip and purge before cooking (Table 7). It was suggested that the poorer flavor was due to less production of free amino acids that normally occurs in meat during aging. Previous work in the USA had shown that electric goading can deplete glycogen in the *quadriceps femoris* and the *psoas major* as well as the *l. dorsi*, and so this is evidently a systemic adrenergic effect.

Breeders in Australia have started selecting bulls for better temperament, based on flight speed on release from a crush (chute). So far, there has been no relationship between temperament and pH_{ult}, but flighty *Bos indicus* crossbred steers tend to have lower early post-mortem muscle pH values (Petherick et al., 2002).

STUNNING AND SLAUGHTER

The stunning and slaughter methods used in cattle have few effects on meat quality, but it is important to perform them in a competent and professional way to minimize animal suffering. In the case of captive bolt stunning, the main concerns are with

Table 5. Effects of three preslaughter management systems on meat quality measurements ± SE

	Treatment		
Item	1	2	3
Transport distance (km)	3	320	320 twice
Time without feed before slaughter (h)	24	48	72
Gender mixing	No	Yes	Yes
L. dorsi pH_{24h}	5.68 ± 0.03	5.63 ± 0.03	5.72 ± 0.03
L. dorsi shear force (kg)	6.0 ± 0.4^{a}	6.8 ± 0.4^{a}	8.5 ± 0.5^{b}
L. dorsi L-value	38.1 ± 0.6^{a}	36.1 ± 0.6^{b}	35.7 ± 0.6^{b}

[a,b]Means within a row without a common superscript letter were significantly different at $P < 0.05$.
[1]Treatment 3 simulated marketing through a sale yard before slaughter.

Table 6. Effect of 9 km/h running for up to five 15-min intervals with 15-min rest periods in between on muscle glycogen loss % in 19-mo-old Angus steers

	Exercise duration		
Muscle	Short (2×)	Medium (4×)	Long (5×)
Semimembranosus	31 ± 5[b]	34 ± 7[b]	57 ± 4[a]
Semitendinosus	20 ± 6[b]	24 ± 7[b]	48 ± 4[a]

[a,b]Means within a row without a common superscript letter were significantly different at $P < 0.05$.

- occasional failure to stun an animal, and
- an imperfect stun, with a risk of the animal regaining consciousness.

The most common faults are

- using underpowered cartridges or pneumatic pressure relative to the class of cattle, and
- inaccurate shooting, especially when using low-powered guns.

In a study on 1608 cattle in the UK, the frequency of failure to collapse with the first shot was 4.2% in young bulls, 1.5% in steers and 2.3% in heifers (Gregory et al., 2007). In 12.1% of bulls, 5.9% of steers and 3.8% of heifers, the animals collapsed but there was not a deep level of concussion. This was assessed from signs that would indicate residual brainstem function and included normal rhythmic breathing, corneal reflex and eyeball rotation. Slaughtermen usually give an animal a second shot if they suspect that it is lightly stunned, and this decision is based on physical signs of the animal and soft-sounding shots ('fizzers'). Soft sounding shots are associated with a greater risk of a shallow depth of concussion, and this can be due to either under-filling of the cartridge with gunpowder during manufacture or incomplete discharge of the gunpowder during firing, but the latter is unusual. If un-discharged gunpowder collects in the bore of the gun, it may be detonated during a subsequent shot, and this excessive discharge can be alarming for the operator. The ideal shooting position in the head is the crossover point between 2 imaginary lines drawn between the corner of an eye and the base of the position of the horn on the opposite side of the head. Striking the head within 2 cm of this shooting position is important when using low powered gun-cartridge combinations, but is less so when using higher velocities. It is recommended that cattle must not be stunned in the poll position (back of the head) because there is a greater risk of a shallow depth of concussion (Daly, 1986). An exception may be made for animals such as water buffalo which have a large horn structure at the front of the head (Gregory et al., 2008a). They can be shot in the back of the head, while ensuring that the brain is hit, not the spinal cord.

Electrical stunning in cattle can lead to dangerous kicking during the epileptiform phase if the animal is not promptly stuck or electrically immobilized. It can also cause blood splash in the meat, which can be monitored in the whole carcass by examining the diaphragm. When a cardiac arrest is not induced electrically either at or immediately following a head-only stun (e.g., halal beef), cattle can regain consciousness remarkably quickly (Gregory et al., 1996; Wotton et al., 2000). It is essential to stick quickly to avoid staff injuries and recovery of consciousness in the animal. Ways in which electrical stunning can be misapplied include poor initial contact and interrupted current flow at the start of stunning (Gregory, 2001). When a cardiac arrest is induced at stunning, there are few commercial problems associated with poor bleeding provided the animal is stuck before blood starts to clot (Gregory et al., 1985; Gregory et al., 1988).

Slaughter without stunning has 3 welfare hazards. First, it requires controlled restraint and this can be stressful (Gregory 2005). Second, the cut could be painful. Third, there could be distress during bleeding out and before loss of consciousness. The likelihood of pain during the cut has been assessed from the electroencephalogram (EEG), and it was concluded that the spontaneous EEG in calves showed patterns that resemble those during other procedures that are normally considered painful (Gibson et al., 2009). Distress during bleeding out could arise in several ways, and it will be minimized if the animals quickly lose consciousness. There is a risk that animals will not lose consciousness promptly if they develop false aneurysms in the severed ends of the carotid arteries, and this could apply to about 7% of cattle (Gregory et al., 2008b). In addition some animals have blood in their respiratory tracts

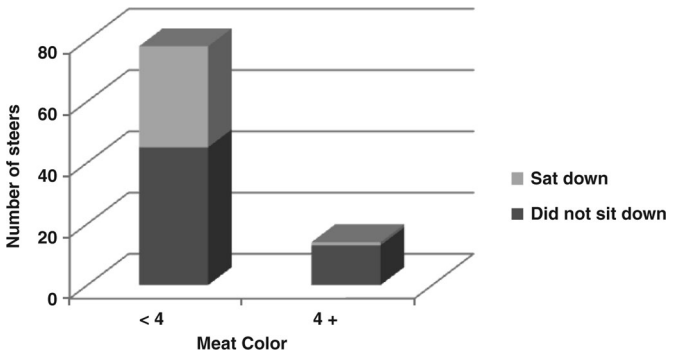

Figure 2. Effect of sitting down overnight in the holding yards at an abattoir on meat color.

Table 7. Effect of electric goad stress before slaughter on meat quality

Item	Control	Electric goad
Plasma lactate at slaughter (mol/L)	4.29[b]	7.12[a]
L. dorsi		
pH fall (units/h)	–0.139	–0.139
pH_{ult}	5.46	5.38
Drip loss at 2 d (%)	1.7[b]	2.3[a]
Purge on aging at 21 d (%)	3.5[b]	5.4[a]
Consumer score[1]		
Tenderness	59.5[a]	55.1[b]
Juiciness	56.9[a]	53.5[b]
Flavor	61.0[a]	57.2[b]
Liking	59.6[a]	55.9[b]

[a,b]Means within a row without a common superscript letter were significantly different at $P < 0.05$.
[1]Visual analog scale 1–100.

and this could be irritating if not painful before they lose consciousness (Gregory et al., In press).

BLOOD SPLASH

Blood splash (ecchymoses) is an occasional problem at beef abattoirs. The blood spots are obvious in the uncooked meat and can cause customer rejection of wholesale and retail cuts. The hemorrhages are in the venous side of the capillary network and can be common in cattle stunned electrically without inducing a cardiac arrest, and in cattle slaughtered by shechita. During shechita the way the animals are restrained may be critical. At one abattoir, the overall prevalence of grade 2 + 3 blood splash in kosher beef was 9.4%, when using a 0 to 4 point grading scale. The cattle were restrained with a neck yoke, chin lift, rump pusher and belly plate which rose from the floor to prevent the animal going down. In small animals the belly plate lifted them off the floor, with the back arched. Blood splash was only present in the hindquarters, and it is suspected that restriction of venous return in the abdomen through upward pressure from the belly plate contributed to venous capillary rupture distal to the compression. Cattle with a short body length or muscular conformation, such as the Aberdeen Angus and Belgian Blue, were more prone to the condition (15.3 and 13.3% versus 9.2% for other breeds). Similarly, heifers were more prone than steers (19.8% versus 14.3%). It is thought that these associations might be linked to greater stenosis of the vena cava within the abdomen of the susceptible types.

CONCLUSIONS

Meat distributors can help maintain their position in the competitive marketplace by ensuring sound welfare standards and consistently high beef quality standards.

In most situations the hazards that contribute to high pH_{ult} beef are understood and they can be managed accordingly. The same cannot be said for intermediate pH_{ult} beef, which is a much more common quality problem. Relatively minor preslaughter stresses can precipitate intermediate pH_{ult} beef, and with the rapid turnover of wholesale product, insufficient time may be allowed for aging and allowing this type of beef to become tender. Intermediate pH_{ult} beef is likely to be a quality feature that will attract more attention over the next decade. Reducing preslaughter stress and ensuring adequate feeding before slaughter may prove to be 2 of the most effective ways of managing this feature.

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Potential Roles of Lactate, Pyruvate, Succinate, and Citrate in Beef Color

Richard A. Mancini and Ranjith Ramanathan

INTRODUCTION

An increased demand for central packaging of case-ready meat products has given processors the opportunity to utilize numerous ingredients that add value and lengthen product shelf life. Exploring both food- and biochemistry-based research to identify potential pathways by which these ingredients effect beef color can provide meat scientists with new insights into fundamental myoglobin chemistry. In particular, investigations into the role of lactate (as an ingredient) in beef color suggests that other metabolites may be involved in myoglobin redox stability and thus, could be viewed as the impetus for this review. Our objective is to address the possible roles of lactate, pyruvate, citrate, and succinate in postmortem processes associated with myoglobin redox stability. Both meat science and biochemistry-based research suggests that metabolites can influence color via 3 possible mechanisms (Figures 1 and 2):

1. Metabolites can improve color via the production of NADH and electrons.
2. Metabolites can darken color by influencing mitochondrial oxygen consumption.
3. Metabolites can promote myoglobin redox stability by serving as antioxidants.

NADH AND ELECTRON PRODUCTION

Metmyoglobin Reduction

The role of metabolites in NADH generation could have significant implications on color stability. Metmyoglobin reduction involves the transfer of one electron from a donating source to the ferric heme of myoglobin (Taylor and Morgan, 1942). Early work noted that pigment reduction needed pyridine nucleotides and electron transferring compounds (Huennekens et al., 1957). It is now widely accepted that the pyridine nucleotide that donates electrons to metmyoglobin is NADH, which is involved in both enzymatic and nonenzymatic mechanisms of metmyoglobin reduction (Stewart et al., 1965; Brown and Snyder, 1969; Hagler et al., 1979; Livingston et al., 1985; Renerre and Labas, 1987; Bekhit and Faustman, 2005). Furthermore, Brown and Snyder (1969) suggested that the limiting factor in nonenzymatic metmyoglobin reduction is NADH. However, the location of the NADH pool as well as mechanisms involved in its replenishment in postmortem muscle are unclear.

NADH Production

Numerous postmortem processes will utilize NADH and this can limit the amount available for metmyoglobin reduction. Watts et al. (1966) and Saleh and Watts (1968) suggested a pathway for NADH regeneration whereby electrons are transferred from glycolytic and tricarboxlic acid metabolites to NAD, eventually being used for metmyoglobin reduction. In addition, Andrews et al. (1952) and Bodwell et al. (1965) noted that enzymes involved in glycolysis, the tricarboxylic acid cycle, and the electron-transport chain, remain active in postmortem muscle, and thus could be possible sources of reducing equivalents. More specifically, Stewart et al. (1965) hypothesized that lactate dehydrogenase catalyzes the production of reduced pyridine nucleotides (NADH) from lactate and diphosphopyridine (NAD). These authors believed this mechanism was feasible because both lactate and lactate dehydrogenase (LDH) are present and active in postmortem muscle. Nevertheless, Bodwell et al. (1965) concluded that the limiting factor in postmortem metabolism is the amount of substrates available to dehydrogenase and diaphorase enzymes.

Kim et al. (2006) reported that nonenzymatic metmyoglobin reduction occurred effectively in a model system containing lactate, LDH, and NAD, whereas deletion of one of the 3 components from the system limited met-

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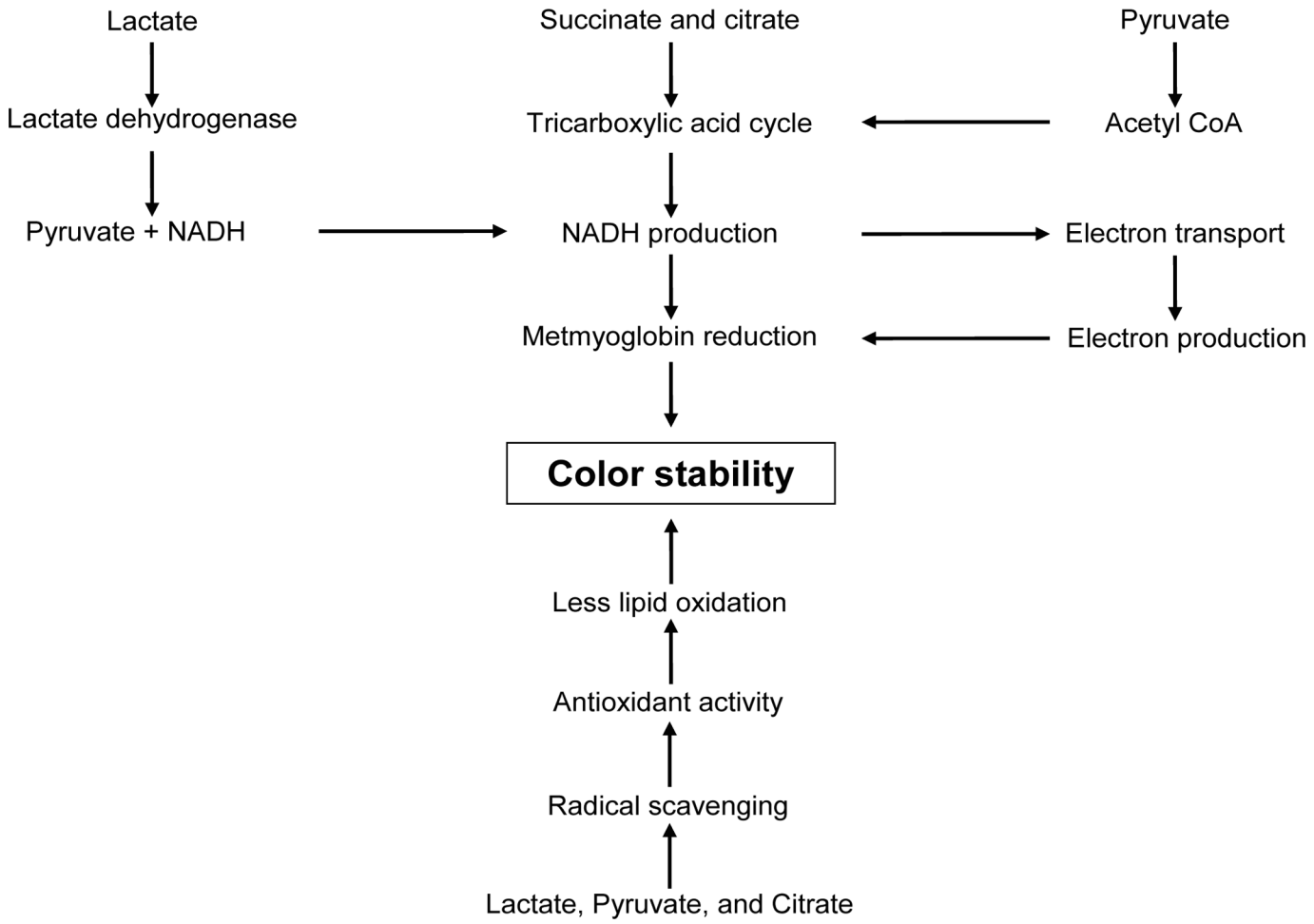

Figure 1. Potential roles of lactate, pyruvate, succinate, and citrate in beef color stability.

myoglobin reduction. Kim et al. (2006) also reported that beef *longissimus* steaks enhanced with 2.5% lactate exhibited more LDH and metmyoglobin reducing activity and contained significantly more NADH than treatments without lactate. These researchers concluded that lactate-mediated improvements in LDH activity likely contributed to increased NADH and metmyoglobin reduction. Ramanathan et al. (2009) reported that combining lactate, LDH, and NAD with bovine mitochondria resulted in an increase in oxygen consumption that was both significant and comparable to the direct addition of NADH to isolated mitochondria. This suggests that lactate addition to beef cardiac mitochondria can generate NADH via LDH, and this NADH can be utilized for beef mitochondrial oxygen consumption. Current research suggests that electrons generated by lactate-induced mitochondrial respiration can be used to reduce metmyoglobin (Ramanathan et al., 2009).

Jerez et al. (2003) suggested that inhibition of postmortem glycolysis could alter NADH content and therefore, effect color stability. In their study, beef muscles treated with citrate tended to have more NADH than control samples. This should produce postmortem conditions that are conducive to improved color stability (increased NADH and oxygen uptake; Jerez et al., 2003). Holmer et al. (2009) reported that replacing sodium chloride with sodium citrate could minimize discoloration by limiting myoglobin oxidation in beef *Infraspinatus, Longissimus,* and *Triceps brachii* steaks. These authors suggested that chemical reactions similar to those associated with lactate-induced color stability (i.e., NADH production) might also be responsible for the effects of citrate on beef color life. The potential role of other metabolic pathways in NADH-mediated color stability has been proposed. For example, Saleh and Watts (1968) indicated the possible involvement of glyceraldehyde-3-phosphate, fructose-1,6-diphosphate, malate, and glutamate in metmyoglobin reduction via their participation in pathways that regenerate NADH. Jerez et al. (2003) noted that NAD-NADH metabolism and color stability could be related to glycolytic components such as glyceraldehyde 3-phosphate oxidation, pyruvate reduction, and enolase activity.

Electron Transport Chain Mediated Metmyoglobin Reduction

Giddings (1974) and Lanier et al. (1978) suggested that electron transport chain intermediates may be involved in metmyoglobin reduction via reversed electron

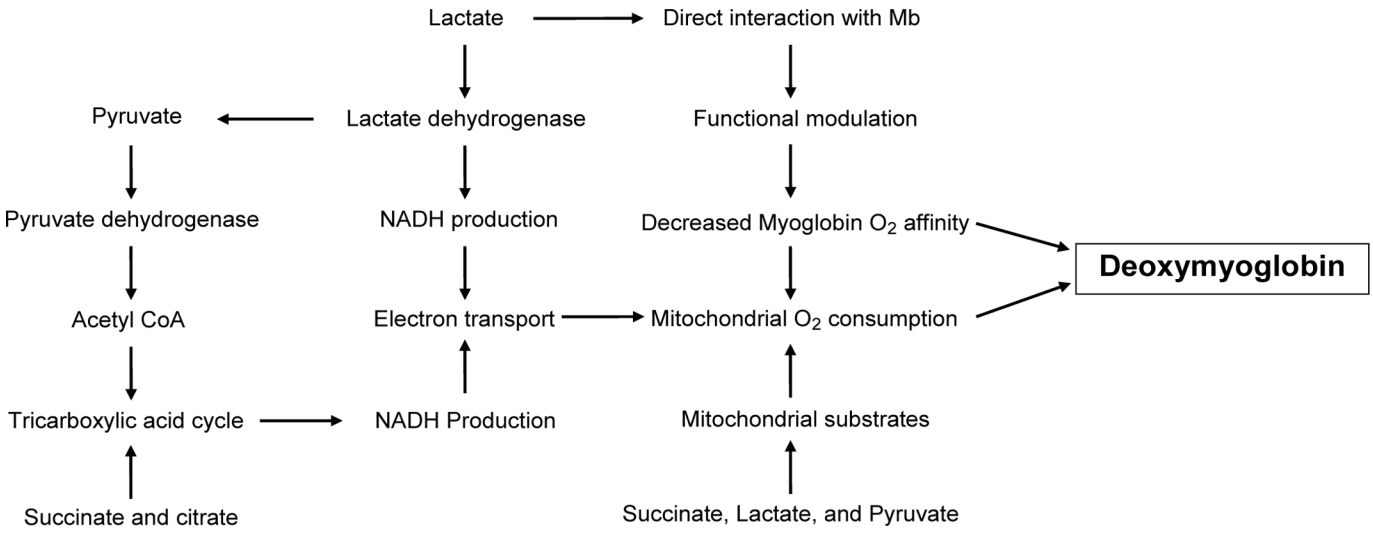

Figure 2. Potential roles of lactate, pyruvate, succinate, and citrate in beef color darkening.

transfer from succinate to NAD. More specifically, Tang et al. (2005a, 2005b) reported that succinate added to bovine mitochondria can be used by Complex II within the electron transport chain; producing electrons that can be donated to metmyoglobin via cytochrome c. Watts et al. (1966) proposed that adding succinate to ground beef can reduce metmyoglobin indirectly by creating anaerobic conditions resulting from succinate-mediated oxygen consumption. Lactate may also be involved in electron transport chain mediated color stability. More specifically, NADH resulting from lactate dehydrogenase activity might enter into the electron transport chain, generating electrons used to reduce metmyoglobin. Thus, NADH produced by lactate dehydrogenase has the potential to improve myoglobin redox stability either directly (where NADH reduces metmyoglobin) or indirectly (where NADH helps to generate electrons that nonenzymatically reduce metmyoglobin).

METABOLITES AND MITOCHONDRIAL RESPIRATION

The role of lactate in beef color is unique because although the ingredient minimizes surface color change and browning, it also darkens meat products. Whereas it is generally accepted that color life is related to maintenance of a bright-red muscle color, lactate-mediated color stability involves a dark-colored pigment that is stable during both storage and display. Holmer et al. (2009) reported that citrate darkened beef *Infraspinatus, Longissimus*, and *Triceps brachii* steaks, and suggested that further investigation into the mechanism by which citrate effects color is needed. No research has assessed the effects of pyruvate and succinate on beef darkening.

Recent meat science research suggests that the darkening effect of lactate is partially due to limited myoglobin oxygenation, presumably resulting from the ability of bovine mitochondria to use lactate as a substrate for respiration. Ramanathan et al. (2009) reported that lactate resulted in measurable State III and IV oxygen consumption by mitochondria isolated from bovine cardiac muscles. Similar results were reported in studies by Mole et al. (1978) and Baldwin et al. (1978), both of which concluded that the addition of lactate to rat muscle homogenates resulted in oxygen consumption via lactate metabolism.

Ramanathan et al. (2009) also reported that pyruvate increases bovine mitochondrial oxygen consumption. In support of this, Gallina et al. (1994) concluded that the addition of pyruvate to rat mitochondria resulted in oxygen consumption and noted that pyruvate uptake is approximately 22 times greater than that of lactate. Tang et al. (2005a, 2005b) concluded that the addition of succinate to a bovine mitochondria-myoglobin system resulted in both oxygen consumption and deoxymyoglobin formation. Thus, metabolite-induced effects on mitochondrial oxygen consumption could influence beef darkening.

Lactate also may be able to influence beef darkening through functional modulation of myoglobin. Giardina et al. (1996) reported a significant decrease in oxygen affinity for sperm whale and equine myoglobins in the presence of 5 mM lactate at pH 6.5. These researchers proposed 2 possible mechanisms for lactate-induced functional modulation of myoglobin, including (1) lactate physically blocks oxygen-heme interaction and (2) a lactate-induced 3 dimensional structural change in myoglobin alters the oxygen binding pathway.

The physiological implications of lactate-induced alterations in both mitochondrial oxygen consumption and myoglobin oxygen affinity are important in times of high energy demand and temporary muscle hypoxia, 2 conditions that occur during diving and running. During these activities, decreased muscle oxygen generates lactate via a change in metabolism from aerobic to anaerobic glycolytic energy production. Giardina et al. (1996) speculated that an increase in lactate concentration would provoke a

defensive mechanism whereby oxygen release from myoglobin is increased in an attempt to maintain both oxygen diffusion to mitochondria and aerobic energy production.

Lactate's functional modulation of myoglobin can have a 2 fold effect on darkening. First, a lactate-induced decrease in myoglobin's oxygen affinity and concomitant formation of deoxymyoglobin would support the observed color darkening associated with lactate-enhanced beef products. Second, an increased release and transfer of oxygen from myoglobin to mitochondria would further promote postmortem oxygen consumption by factors other than myoglobin, a process that darkens muscle color.

ANTIOXIDANT ACTIVITY

The interrelationship between lipid and pigment oxidation has received recent attention. Metabolite-mediated effects on lipid oxidation can translate into improved myoglobin redox stability. The antioxidant activity of pyruvate has been reported in various model systems such as rat renal tissues, guinea pig cardiac muscle, and knee joint synovial fluid (Herz et al., 1997; Bassenge et al., 2000; Valentovic and Minigh, 2003). These authors attributed pyruvate's antioxidant capacity to (1) its reactive keto-enol group, which can directly scavenge hydrogen peroxide and other ROS and (2) pyruvate's entry into the TCA cycle, which produces NADH that is subsequently used by glutathione reductase to regenerate glutathione (a potent antioxidant). The antioxidant effect of lactate has been reported in pork (Tan and Shelef, 2002), ground ostrich (Seydim et al., 2006), and beef *Longissimus* (Kim et al., 2009). Like pyruvate, lactate's antioxidant activity in various model systems has been attributed to its ability to scavenge OH^- and O_2^- radicals (Groussard et al., 2000). Ke et al. (2009) attributed citrate's antioxidant activity in ground beef to its ability to bind prooxidant metals. However, these authors also noted that the formation of citric acid-metal complexes can have both positive and negative effects on lipid oxidation. In addition to binding pro-oxidant metals, sodium citrate reduced pink color in cooked ground turkey rolls, possibly by chelating the heme iron of myoglobin and therefore, limiting the binding of nitrogenous ligands (Sammel and Claus, 2003).

CONCLUSIONS

Central packaging of case-ready beef products provides an opportunity to utilize numerous ingredients that improve color life. Future research is needed to better understand the biochemical mechanisms and pathways by which lactate, pyruvate, citrate, and succinate effect myoglobin redox stability. Indentifying and exploring these potential pathways can offer novel insights into fundamental myoglobin chemistry and provide a foundation for the development of strategies that improve beef color stability.

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Effects of Packaging and Injection Enhancement on Beef Quality

Jeannine P. Grobbel

INTRODUCTION

Many meat quality traits are involved in consumers' overall purchase decisions and satisfaction of meat products. Color is the major factor affecting consumers' purchasing decisions, while tenderness, juiciness, and flavor contribute to the overall eating satisfaction and palatability. Eilert (2005) indicated case-ready packaging is being used at an increasing rate. The type of packaging used, the different atmospheres associated with modified atmosphere packaging (MAP), and injection-enhancement can alter the color of meat and sensory attributes. McMillin (2008) published an excellent review of the use of MAP for meat.

EFFECTS OF PACKAGING ON TENDERNESS AND SENSORY TRAITS

To provide consumers with consistent and desirable products, it is necessary to understand the effects that packaging atmospheres have on important beef quality traits, including palatability factors of tenderness, juiciness, and flavor. Providing consistent, high quality beef to consumers is of utmost importance to maintain customer satisfaction and allow for repeat purchases.

Warner-Bratzler Shear Force

Warner-Bratzler shear force (WBSF) values from longissimus lumborum steaks indicate that, as a system, high-oxygen (HiO_2) MAP (d 18 postmortem) resulted in steaks being less tender than those packaged in ultra-low oxygen (ULO_2) with carbon monoxide (CO) MAP or vacuum packaging (VP) (d 28 postmortem) (Grobbel et al., 2008a). On d 14 postmortem there were no differences in WBSF and all treatments were more tender on d 14 postmortem than d 7 postmortem. It is probable that steaks packaged in HiO_2 MAP were less tender than other treatments at the end of display as a result of 10 d less aging time (d 18 vs. 28 postmortem) because of a shorter dark storage period (4 d) for HiO_2 MAP than ULO_2CO MAP and VP packaging treatments (14 d). Steaks packaged in all packaging treatments used for 14 d postmortem WBSF were held for 7 d in the dark and then cooked for WBSF measurement. Dark storage times for HiO_2 and ULO_2 atmospheres were developed to mimic what would happen in industry. There was a trend for steaks packaged in VP to be more tender than steaks packaged in ULO_2CO MAP on d 28 postmortem.

When comparing WBSF differences in enhanced and non-enhanced steaks from different muscles, tenderness increased with time postmortem (d 14 to 18/28) in enhanced longissimus lumborum (LL) and triceps brachii (TB) steaks but not in semitendinosus (ST) steaks (Grobbel et al., 2008b). They reported non-enhanced steaks were similar in tenderness on d 7 and 14 postmortem but were more tender on d 18/28 postmortem for all muscles. Enhanced LL steaks were more tender than non-enhanced steaks on d 7 postmortem, which was d 0 of packaging. This indicates that injection-enhancement has an immediate effect on tenderness. Injection-enhancement may increase tenderness through a dilution effect or through physical manipulation of the muscle structure with the injection needling process; however, the exact method of action is currently unknown.

Tenderness and Sensory Attributes

Tørngren (2003) found that steaks aged and packaged in HiO_2 MAP had more off-odor and off-flavor, including warmed-over flavor, with increased duration of storage in HiO_2 MAP. Steaks aged in HiO_2 MAP also were less tender and juicy than steaks aged in vacuum packaging and then packaged in PVC overwrap. Sørheim et al. (2004) found that steaks packaged in HiO_2 MAP were less tender than vacuum packaged steaks according to both instrumental and sensory tests. They also found that steaks packaged in HiO_2 MAP had more rancid taste and were less juicy than steaks vacuum packaged.

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Clausen (2004) found that beef loin steaks aged in 100% nitrogen (N_2) were equally as tender and juicy as those aged as a whole loin in vacuum packaging but more tender than those aged in vacuum skin packaging as steaks or those steaks packaged in 50% O_2/50% CO_2. They also found steaks packaged or aged in HiO_2 MAP to be less juicy and have more warmed over flavor than steaks packaged and displayed in air or vacuum skin packaging.

Madsen and Clausen (2006) evaluated beef loin steaks packaged in HiO_2 MAP, ULO_2 MAP with and without carbon monoxide (CO), and vacuum packaging. They reported that steaks packaged in HiO_2 MAP were less tender, had more warmed-over flavor, and less meat flavor than steaks packaged in vacuum skin packaging and ULO_2 with and without CO. They also found that steaks packaged in HiO_2 MAP had lower cook loss than steaks packaged in MAP with high levels of CO_2 (60%) with or without 0.4% CO.

Jackson et al. (1992) evaluated the volatile compounds from the headspace of beef strip loins vacuum packaged or in: 100% CO_2 MAP; 40% CO_2/ 60% N_2 MAP; or HiO_2 (80% O_2/ 20% CO_2) MAP and evaluated microbial changes. Steaks packaged in HiO_2 MAP developed strong off-odors and had methyl thiirane, ethyl acetate, benzene and 1-heptene in the packages after 7 and 14 d of storage but not in the vacuum packaged steaks or other MAP atmospheres.

POSTMORTEM PROTEOLYSIS AND PROTEIN OXIDATION

Both Clausen (2004) and Sørheim et al. (2004) attribute the reduction in tenderness of steaks in HiO_2 MAP to be caused by the O_2 and not by CO_2 or N_2. Clausen (2004) believes that the detrimental effects of O_2 on tenderness could be caused by protein oxidation. Rowe et al. (2004) found that oxidation of beef steak proteins early postmortem inactivated μ-calpain and decreased myofibrillar proteolysis and thus limited the extent of tenderization.

Grobbel et al. (2008b) reported desmin degradation was not affected by type of packaging but was affected by time postmortem, as expected. They found no difference in desmin degradation between non-enhanced and enhanced steaks. Longissimus lumborum desmin degradation increased from d 7 to d 14, regardless of enhancement treatment. Longissimus lumborum steaks had more degradation of desmin at d 14 than the ST or TB, regardless of enhancement treatment. Protein oxidation may be associated with muscle in the early stages after harvest, and muscles utilized by Grobbel et al. (2008b) were aged for 7 d in vacuum before exposing steaks to different packaging treatments.

FRESH MEAT COLOR STABILITY

There are several factors that contribute to a muscle's color stability and ability to maintain good color in the display case, and Mancini and Hunt (2005) thoroughly reviewed factors contributing to muscle color and stability. Seyfert et al. (2004a) compared color of injection-enhanced beef from hot- or cold-boned quadriceps muscles packaged in HiO_2 MAP or ULO_2 MAP and found that steaks packaged in HiO_2 MAP were brighter, more cherry-red and had more color stability than steaks packaged in ULO_2 MAP, even with increased display times for steaks in HiO_2 MAP.

Behrends et al. (2003) reported steaks packaged in HiO_2 MAP were redder than steaks packaged in PVC. They also found differences among muscles and packaging types, with biceps femoris and semitendinosus steaks packaged in HiO_2 MAP having redder lean color scores than steaks packaged in PVC.

Ultra-low oxygen MAP allows for extended shelf life; however, the display color is not appealing to many consumers in such environments. The use of CO has been approved by the FDA and USDA for levels up to 0.4% in retail MAP (USFDA, 2004). Products in MAP that include CO have improved beef color stability with extended display time (Luño et al., 1998; Sørheim et al., 1999; Hunt et al., 2004). Jayasingh et al. (2001) exposed meat to 5% CO for 24 h and then vacuum packaged the meat and it remained red in vacuum packaging for at least 5 wk. Brewer et al. (1994) had previously obtained similar results by packaging meat in 100% CO for 1 h before vacuum packaging. Sørheim et al. (1999) found the formation of off odor and discoloration was faster in meat packaged in HiO_2 MAP than in ULO_2CO MAP.

Modified atmosphere packaging of steaks from 5 different beef muscles using atmospheric O_2 (20%) and HiO_2 (80%) levels with and without 0.4% CO were evaluated and CO had no effect on color, reducing activity or oxygen consumption (Seyfert et al., 2007).

Vacuum packaged steaks were the most consistent in display color throughout display (Grobbel et al., 2008a), however, many consumers find the purplish red color of VP meat undesirable. They reported steaks packaged in HiO_2 MAP were an undesirable reddish tan by d 7 of display, whereas steaks packaged in the ULO_2CO MAP treatments were either dull red or slightly dark red by the end of display. Steaks packaged in HiO_2 MAP discolored faster and to a greater extent than those packaged in any of the ULO2 MAP or VP treatments, which had little or no discoloration (Figure 1). Steaks packaged in HiO_2 MAP discolored by d 4 of display and had 56% more metmyoglobin discoloration than those packaged in any other packaging treatment.

Grobbel et al. (2008b) reported non-enhanced TB steaks packaged in HiO_2 MAP became dramatically darker than those packaged in ULO_2CO MAP and VP. In general, they found TB steaks packaged in HiO_2 MAP discolored at a faster rate and to a greater extent than LL and ST steaks packaged in HiO_2 MAP, regardless of enhancement treatment.

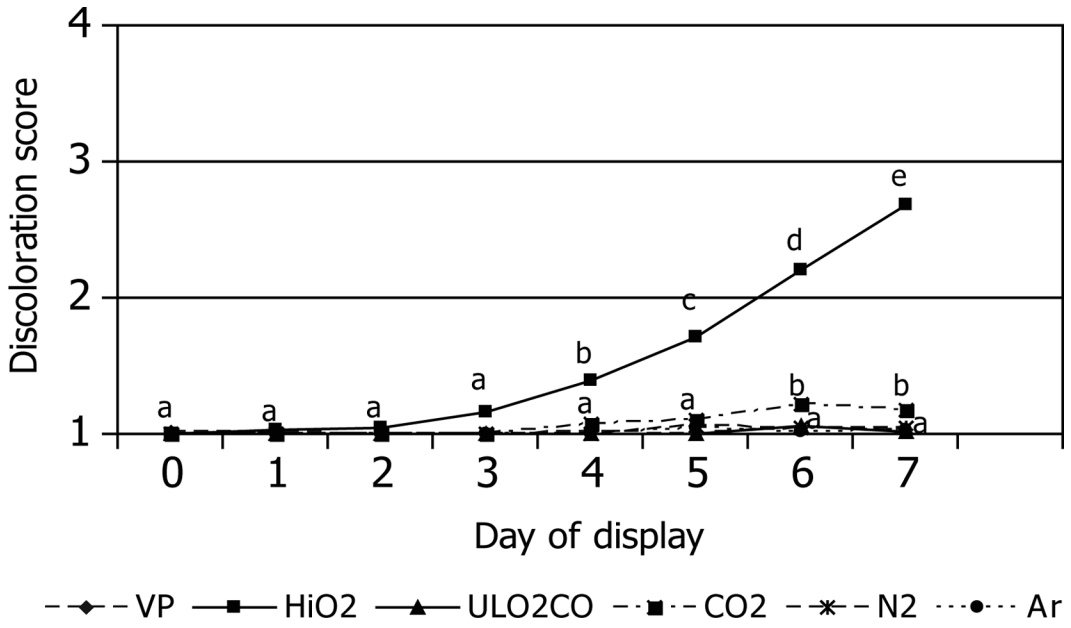

Figure 1. Discoloration score (1 = 0%, 2 = 1–19%, 3 = 20–39% metmyoglobin) means for longissimus lumborum steaks packaged in ULO_2COAr (99.6% Ar, 0.4% CO); ULO_2COCO_2 (99.6% CO_2, 0.4% CO); HiO_2 (80% O2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); ULO_2CON_2 (99.6% N_2, 0.4% CO); or VP (vacuum packaging) and displayed (Grobbel et al., 2008a [AU1: A permissions letter has been requested from the Journal of Animal Science to reprint Figs 1, 2, and 3.]). [abcde]Means with different letters differ ($P < 0.05$). Figure 1 reprinted from Grobbel, J. P., M. E. Dikeman, M. C. Hunt, and G. A. Milliken. 2008. Effects of packaging atmospheres on beef instrumental tenderness, fresh color stability, and internal cooked color. J. Anim. Sci. 86:1191–1199.

PREMATURE BROWNING

Premature browning is the internal appearance of well-done meat that has been cooked to temperatures lower than what is necessary to kill harmful pathogens (and when the meat should still appear red to pink internally) and has been found in ground-beef patties (Hague et al., 1994; Seyfert et al., 2004c) and whole muscle steaks (John et al., 2004; Seyfert et al., 2004b; Grobbel et al., 2008a). Different types of packaging also can change internal cooked color. Seyfert et al. (2004b, 2004c) determined that beef packaged in HiO_2 MAP was prematurely brown at a medium degree of doneness. They found that steaks packaged in HiO_2 MAP had a well-done appearance at 71.1°C whereas steaks packaged in ULO_2 MAP and cooked to 71.1°C had a pinkish-red interior as expected of meat cooked to a medium degree of doneness. They also reported that steaks packaged in HiO_2 MAP had 93.8% denatured myoglobin after cooking, whereas steaks packaged in ULO_2 MAP had 57.8% thermal denaturation of myoglobin.

Seyfert et al. (2004c) looked at ground beef patties packaged in HiO_2 MAP or vacuum packaging and reported that nearly 100% of patties made from ground beef packaged in HiO_2 MAP were prematurely brown at 71.1°C. Cooked color is not an indicator of doneness in ground beef or meat packaged in HiO_2 MAP and could pose safety risks if consumers do not properly use a thermometer to monitor and determine endpoint temperature.

Grobbel et al. (2008a) reported steaks packaged in HiO_2 MAP had the lowest a* values (brownest) for internal cooked color when compared with steaks packaged in ULO_2CO and VP and cooked to a medium degree of doneness. Tørngren (2003) found steaks packaged in HiO_2 MAP had more premature browning than steaks aged in VP and displayed in PVC overwrap.

John et al. (2005) compared top sirloin steaks packaged in 80% O_2, 0.4% CO, or vacuum at internal temperatures of 49, 57, 66, 71, or 79°C. They found that steaks packaged in 80% oxygen had the most oxidation, according to TBARS values, and greatest myoglobin denaturation at all cooking endpoint temperatures and storage times. Steaks packaged in 80% oxygen also became prematurely brown at 57°C although internal cooked color did not turn completely brown until steaks were cooked to 66°C. Steaks packaged in 0.4% CO or vacuum packaging maintained some pinkish red internal cooked color through 79°C.

INJECTION ENHANCEMENT OF MEAT

The use of 'injection enhancement' has been shown to improve beef tenderness and juiciness and typically is used in conjunction with case-ready packaging. Beef round muscles injected with a 10% solution containing salt, phosphate, and natural flavoring solution had less oxidation but more non-typical beef flavors than muscles injected with a 6% enhancement solution (Seyfert et al., 2005). They found that beef quadriceps muscles packaged

in HiO_2 MAP were less tender and had more off-flavors than those packaged in ULO_2 MAP. Steaks packaged in ULO_2 MAP had increased beef flavor and had less off flavors. Common descriptors of off flavors they found in muscles packaged in HiO_2 MAP were oxidative and rancid. Muscles packaged in both ULO_2 and HiO_2 MAP were also described as salty, sour, and bitter. They reported higher TBARS values for injection-enhanced steaks packaged in HiO_2 MAP than steaks packaged in ULO_2 MAP. According to trained sensory panelists, steaks packaged in ULO_2 MAP had increased myofibrillar and overall tenderness as well as decreased perception of connective tissue than steaks packaged in HiO_2 MAP. They suggest some of the differences in tenderness may be due to the differences in aging time, as steaks packaged in HiO_2 MAP were frozen for sensory analysis at 14 d postmortem and steaks packaged in ULO_2 MAP were frozen at 22 d postmortem.

Knock et al. (2006b) found that steaks packaged in HiO_2 MAP from muscles injected with potassium lactate with or without sodium acetate had increased color stability but were darker than control steaks. They also reported increased TBARS values with increased time steaks were displayed in HiO_2 MAP, with a greater increase from d 9 to d 14 of display. In a similar study looking at injected beef strip loin steaks, Knock et al. (2006a) found that adding potassium lactate to injection-enhanced beef packaged in HiO_2 MAP limited rancid flavor development while increasing brown-roasted and beef flavors. They also reported that steaks enhanced with sodium acetate had lower shear force than control steaks or steaks enhanced with potassium lactate but the mechanism for this tenderness difference is unknown. Wicklund et al. (2005) reported that enhanced beef strip loin steaks had lower WBSF values than non-enhanced steaks when loins were aged before enhancement and when loins were enhanced before aging. Vote et al. (2000) and Robbins et al. (2003) reported lower shear force in enhanced beef strip loin steaks than in non-enhanced steaks.

Sensory Analysis

According to sensory panelists, non-enhanced steaks packaged in HiO_2 MAP were less tender, had less beef flavor, and had more off-flavors than those packaged in ULO_2CO MAP and VP (Figures 2 and 3; Grobbel et al., 2008b). They found enhanced steaks packaged in VP had more beef flavor than enhanced steaks packaged in HiO_2 MAP. They reported enhanced steaks were juicier and had less perceptible connective tissue than non-enhanced steaks. Steaks packaged in HiO_2 MAP were less juicy and had more perceptible connective tissue than steaks packaged in ULO_2CO MAP, whereas steaks packaged in VP were intermediate and not different in juiciness from steaks packaged in HiO_2 and ULO_2CO MAP. The most common off-flavors associated with steaks packaged in HiO_2 MAP were oxidative or rancid. Enhanced steaks had more off-flavors than non-enhanced steaks, with typical descriptors of salty and metallic or chemical. Trained panelists also made comments on many of the enhanced

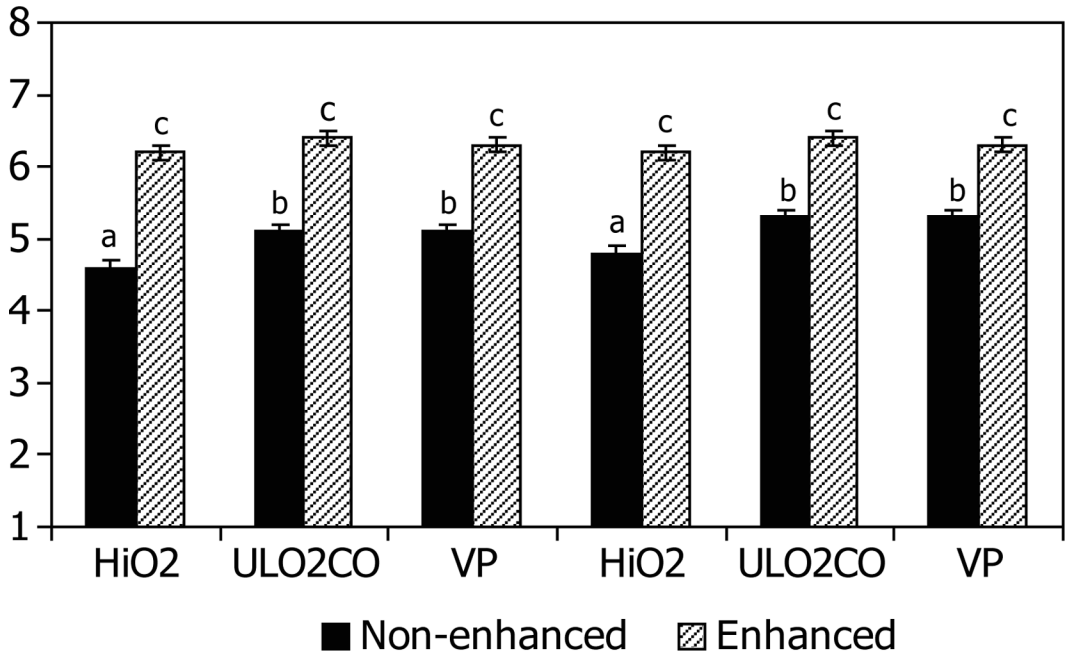

Figure 2. Enhancement x packaging treatment (HiO_2 = 80% O_2, 20% CO_2; ULO_2CO = 0.4% CO/35% CO_2/64.6%N_2; VP = vacuum packaging) myofibrillar tenderness and overall tenderness (1 = extremely tough, 4 = slightly tough, 6 = moderately tender, 8 = extremely tender) means and SE for longissimus lumborum, semitendinosus, and triceps brachii steaks (Grobbel et al., 2008b). [abc]Means with different letters within sensory traits differ ($P < 0.05$). Grobbel, J. P., M. E. Dikeman, M. C. Hunt, and G. A. Milliken. 2008. Effects of different packaging atmospheres and injection enhancement on beef tenderness, sensory attributes, desmin degradation, and display color. J. Anim. Sci. 86:2697–2710.

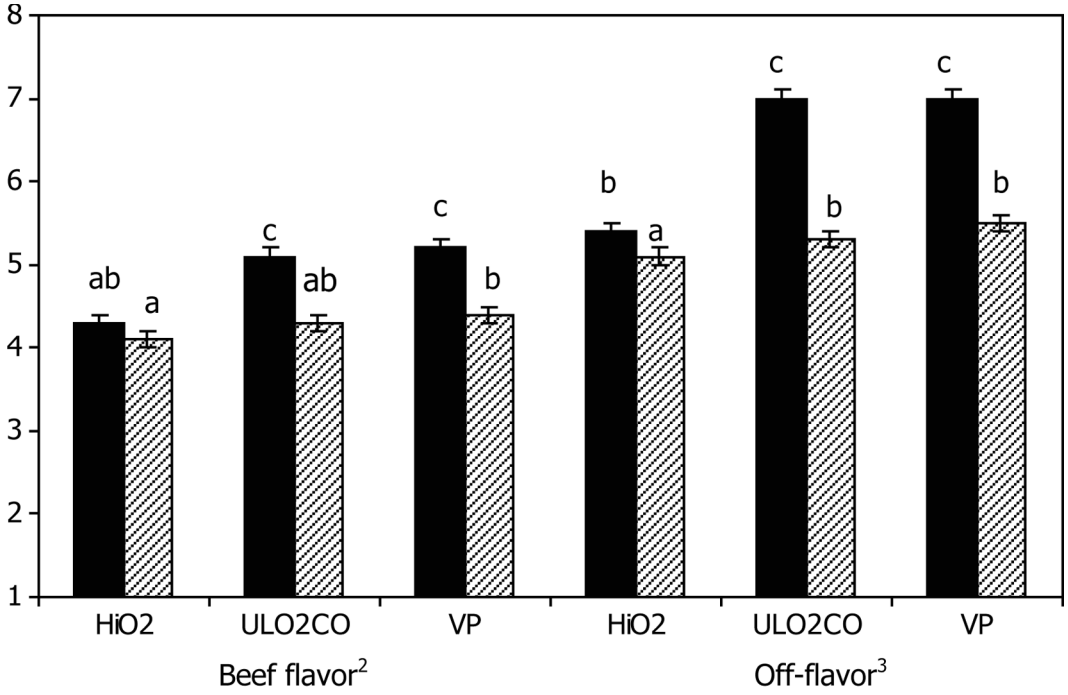

Figure 3. Enhancement x packaging treatment (HiO_2 = 80% O_2, 20% CO_2; ULO_2CO = 0.4% CO/35% CO_2/64.6%N_2; VP = vacuum packaging) beef flavor (1=extremely bland, 4=slightly bland, 6=moderately intense, 8=abundant) and off-flavor (1=abundant, 5=slight, 6=traces, 7=practically none, 8=none) means for longissimus lumborum, semitendinosus, and triceps brachii steaks (Grobbel et al., 2008b). [abc]Means with different letters within sensory traits differ ($P < 0.05$). Grobbel, J. P., M. E. Dikeman, M. C. Hunt, and G. A. Milliken. 2008. Effects of different packaging atmospheres and injection enhancement on beef tenderness, sensory attributes, desmin degradation, and display color. J. Anim. Sci. 86:2697–2710.

steaks that indicated an undesirable, mushy texture. Enhanced TB steaks had more beef flavor than enhanced ST steaks. Oxidative off-flavors associated with steaks packaged in HiO_2 MAP were expected because the O_2 present in the package atmosphere allows for more rapid and a greater extent of oxidation of proteins and lipids found in meat. Eliminating O_2 from the package environment, as with VP or ULO_2CO MAP, drastically decreases the rate and extent of oxidation, thus resulting in fewer off-flavors and increased beef flavor.

SUMMARY

Generally, enhanced beef is found to be juicier and more tender; however, there appears to be inconsistencies in the effects of enhancement on beef flavor intensity and off flavors, with some studies showing off-flavors in enhanced products. Packaging of enhanced beef in HiO_2 MAP tends to increase oxidative and/or rancid off flavors. With the goal of consistently providing consumers with tender, juicy, and flavorful beef, current enhancement strategies may increase tenderness and juiciness but, at the same time, may not adequately represent good beef flavor and, in some cases, typical beef texture.

Steaks packaged in ULO_2CO MAP and VP treatments result in better fresh color stability and had equal or better tenderness than steaks packaged in HiO_2 MAP. Packaging atmospheres alter internal cooked color, with steaks packaged in HiO_2 MAP exhibiting premature browning. Packaging type did not affect desmin degradation. Packaging beef in ULO_2CO MAP provides beef with a bright red color with extended color stability and provides for a longer aging time and increased tenderness while resulting in an internal cooked color that is expected when cooked to a medium degree of doneness, both of which would be beneficial to the meat industry. Although steaks packaged in VP did not discolor throughout display and had good color stability, they have a purplish red color that is not acceptable to most consumers. It is important for the beef industry to provide and promote good tasting beef that results in a pleasurable eating experience that will, in turn, ensure repeat purchases and consumption of beef. It is also important that the right muscles are enhanced in the right way and that value is increased but not costs.

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