**ANNA CAROLINA VILHENA DA CRUZ SILVA CANTO**

# Estratégias tecnológicas para valorização de matrizes cárneas

# Technological strategies to improve meat sources utilization

Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal Fluminense, como requisito parcial para obtenção do grau de Doutor. Área de Concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal.

Orientador: Prof. Dr. TEÓFILO JOSÉ PIMENTEL DA SILVA

Niterói

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2014

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## RESUMO

As perdas econômicas podem ocorrer em todas as etapas da cadeia produtiva de matrizes cárneas, principalmente, durante o processamento tecnológico das carcaças, onde há produção de retalhos, no qual a falta de aproveitamento adequado, ocasiona diminuição na lucratividade para a indústria. Em relação a carne de jacaré-do-pantanal (*Caiman yacare*), seu retalho de carne é caracterizado como uma carne magra com elevado valor nutricional, características estas que justificam esforços para seu aproveitamento para a elaboração de novos produtos com valor agregado. Além disso, a manutenção da qualidade bacteriológica durante a estocagem do produto cárneo na indústria, distribuição e ponto de venda, representam outro desafio e fonte de prejuízo para a cadeia de produção da carne. Existem diversas técnicas de conservação que promovem a extenção da validade comercial de produtos cárneos, sem alteração significativa do valor nutricional e sensorial dos produtos. Neste contexto, a alta pressão hidrostática (APH) representa uma alternativa eficaz porém, devido ao elevado custo de implementação, seu emprego se justifica em uma carne com alto valor econômico como a carne de jacaré-do-pantanal. Além da validade comercial, durante a estocagem no ponto de venda, outro parâmetro que pode ocasionar perda na lucratividade para a indústria é a aparência do produto, principalmente a cor, cuja falta de estabilidade representa importante fonte de rejeição pelo mercado consumidor. Desta forma, o objetivo do presente estudo foi avaliar o efeito de diferentes alternativas tecnológicas capazes de valorizar matrizes cárneas, reduzindo perdas econômicas durante a cadeia produtiva da carne, como elaboração de produto reestruturado acidionado de transglutaminase e substitutos de NaCl (experimento 1), aplicação da APH (experimento 2) e investigação dos componentes moleculares envolvidos na estabilidade de cor (experimento 3). A partir dos resultados do experimento 1 pode-se concluir que o sinergismo entre transglutaminase microbiana e os substitutos do NaCl (KCl e $MgCl_2$) melhorou as características sensoriais e instrumentais de textura do bife reestruturado de caiman. Além disso, no experimento 2 observou-se uma melhora na qualidade bacteriológica, principalmente das amostras submetidas à pressão de 400 Mpa, entretanto, a aplicação desta tecnologia, aumentou a razão n–6/n–3 e o índice aterogênico, demonstrando um comprometimento da composição lipídica de matrizes cárneas ricas em PUFA. Os resultados do experimento 3 indicaram que a alteração na estabilidade de cor proveniente da variação animal, pode ser atribuída ao aumento da abundância de proteínas relacionadas ao metabolismo glicolítico, o qual provavelmente contribuiu para a maior estabilidade de cor através da regeneração de NADH. Além disso, uma possível modificação pos-translacional da mioglobina nos bifes de LL instáveis para cor, potencialmente comprometeu a estabilidade de cor. Sendo assim, conclui-se que tanto o uso da transglutaminase associado a substitutos de NaCl, quanto a APH foram eficazes no aumento da valorização dos retalhos e melhoria da qualidade bacteriológica da carne de caiman. Ainda, o aumento do conhecimento em nível molecular das proteínas envolvidas com a estabilidade de cor do Longissimus potencialmente abre frentes de estudos para a melhoria e manutenção da qualidade da cor da carne.



Palavras-chave: Bife Reestruturado; *Caiman yacare*; APH; Proteoma; Longissimus.

## ABSTRACT

Economic loss may occur during several steps of meat production, primarily at carcass fabrication, where trimmings are generated and their sub utilization favors decrease on industry revenue. In terms of caiman carcass processing (*Caiman yacare*), the trimming from this specie is considered low in fat with high nutritional value, characteristics that justify efforts to utilize this matrix for development of new products with high added value. Furthermore, the bacterial spoilage prevention during industry storage, distribution and at retail point, represent another challenge to the meat production chain. There are several techniques that promote shelf-life extension of meat products, without negatively affecting its nutritional value and the sensory attributes. In this context, the high hydrostatic pressure (HHP) is an effective alternative however, due to the high implementation cost; its use is mostly justified in meat with high economic value such as caiman. Besides the shelf-life, during retail display, another parameter that also affects revenue loss is the product appearance, especially color, which decrease on stability is an important source for consumer rejection. Thus, the aim of the present research was to evaluate the effect of different techniques to enhance meat utilization, decreasing economic losses during meat chain processing, such as restructured caiman steak manufactured with transglutaminase and NaCl replacer addition (experiment 1), HHP (experiment 2), and investigation of the molecular components involved on beef Longissimus color stability (experiment 3). From the results of experiment 1 it can be concluded that the synergy between microbial transglutaminase and NaCl substitutes (KCl and $MgCl_2$) improved sensory and instrumental texture parameters of caiman restructured steak. In addition, in experiment 2, it was observed an improvement on bacteriological quality, especially in samples subjected to 400 MPa pressure level, however, the application of this technology has increased the n-6/n-3 ratio and the atherogenic index, suggesting deterioration of the lipid profile in this PUFA-rich matrix. Moreover, the results from experiment 3 indicated that the Longissimus color stability variation caused by animal variation is potentially attributed to an increase on the abundance of proteins related to glycolytic metabolism, contributing to greater color stability through NADH regeneration. Allied to this fact, a possible post-translational modification of myoglobin on color-labile LL steaks potentially compromised the color stability. Therefore, it is concluded that the use of transglutaminase in association with NaCl substitutes, as well as APH were effective to increase meat trimming profitability and to improve the bacteriological quality of caiman meat. Furthermore, the increasing knowledge on the molecular level of proteins involved on the color stability of Longissimus potentially represents substrate for further studies to improve and maintain the color quality of meat.



Keywords: Restructured steak; *Caiman yacare*; HHP; Proteome; Longissimus

## LIST OF FIGURES

## LIST OF TABLES

# TABLE OF CONTENTS

# 1 INTRODUCTION

Economic losses occur throughout the whole meat processing chain especially during carcass fabrication due to the production of low-value cuts that without proper use, decreases industry revenue. Furthermore, the increase on consumer demand for healthier food has prompted the meat industry to investigate muscle foods alternatives with increased nutritive value. Caiman meat (*Caiman yacare*) is considered low in fat and rich in polyunsaturated fatty acids. In addition, in Brazil, the sustainable caiman farming favors the production of leather with superior quality, as well as promotes a more environment-friendly animal handling. Nonetheless, the caiman meat processing chain is not yet well developed thus allowing the production of a great volume of meat trimming waste.

The caiman meat physico-chemical characteristics combined with the trimmings waste, support the development of novel products manufactured using low-value cuts. For the manufacture of meat products, sodium chloride is widely used to increase protein extractability favoring gel formation and matrix stability. Nonetheless, due to the negative impact of sodium on human health (e.g. blood hypertension and cardiac disease) decrease on the content of this salt is desirable. Furthermore, the enzyme transglutaminase (commercialized as microbial transglutaminase; MTG) catalyzes protein covalent cross-linking between glutamine and lysine residues favoring the aggregation between meat pieces. MTG represents a suitable processing alternative to improve texture and appearance on restructured products with low-sodium claim. The MTG influence on restructured meat products quality is already well characterized nonetheless, its effect on the sensory attributes of low-sodium restructured caiman steak is yet to be investigated.

In addition, the bacterial spoilage represents another source of economic loss for both meat industry and public health, due to product waste during manufacture, retail display, as well as with therapeutic and legal expenses. In this context, high hydrostatic pressure (HHP) is a non-thermal technology, in which packaged food is submitted to pressures above 100 MPa, using a liquid as pressure transmitter. This technology improves bacterial quality and extends the product shelf life, representing a reliable

technology to decrease the bacterial load of fresh caiman meat. However, due to the physical nature of this technology and the chemical characteristics of the lipid fraction of caiman meat, further studies are required to elucidate its influence on reactive molecules (namely fatty acids), as well as determine the appropriate pressure level for this type of meat.

Moreover, another source of economic loss on meat industry is related to the meat color during retail display. Beef color is an important attribute during the purchase decision; the consumers associate bright cherry-red color with beef freshness. The beef discoloration during retail display can be associated as a process of deterioration of the product, leading to rejection. Myoglobin is the main component of beef color, and its redox state is directly influenced by exogenous and endogenous factors. Recent researches developed on the USA, demonstrated an animal variation on color stability and discoloration during retail display (KING, et al. 2011a; KING et al., 2011b), however, molecular explanations are not completely elucidated. Thus, further studies are necessary to better understand the meat color stability.

Therefore, the objective of the present research was to investigate technological alternatives to improve the utilization of meat sources to decrease the meat industry loss during processing.

# 2 LITERATURE REVIEW

## 2.1 ECONOMIC LOSS IN MEAT CHAIN

Disposal of raw materials may occur throughout the whole meat processing chain which can be minimized using a variety of strategies. Carcass fabrication is considered an important step for meat loss and subsequent industry revenue decrease, due to underuse of meat trimmings (characterized as low value cuts) originated from this process (WRAP, 2011). The correct use of these co-products has a positive impact on economy and environment, avoiding the negative aspects of trimmings disposal. In beef and pork industry, co-products represent 11.4% and 7.5% of gross income, respectively. Moreover, 66.0%, and 52.0% of live weight consist of co-products. The edible meat co-products usually have a great content of nutrients and high amount of connective tissue (JAYATHILAKAN et al. 2012). In order to valorize this low value cuts produced by slaughterhouses, meat industry rely on alternative meat product processing such as restructured steaks with the objective to improve the trimmings sensory quality and consequently increase their value in food market (NIELSEN; PETERSEN; MOLLER, 1995).

In addition, during industry retail storage, and food distribution, meat products are susceptible to spoilage due to the contamination during carcass fabrication, cuts processing, storage, cold chain disruption, and others. Contaminated product represents a risk for food-borne disease in addition to the product deterioration, resulting in consumer rejection, shelf-life decrease and meat industry economic loss, due to product disposal, health expenses (around 12 billion per year), legal fees, consumer complaints and product recall. Therefore, food industry seeks efficient decontamination techniques to increase the bacteriology quality and the shelf-life of products. In addition, such techniques should not negatively affect the product freshness characteristics, allowing its distribution, retail storage, and improvement on the value-added price of final product (SKANDAMIS; NYCHAS; SOFOS, 2010).

Moreover, meat and meat product appearance are crucial for the consumer purchase intention, thus contributes to meat industry revenue. Meat color is influenced

by meat pigments such as myoglobin and hemoglobin, which are affected by exogenous and endogenous factors. The consumer market is used to a bright cherry-red for fresh meat, brown or gray for cooked meats, and pink for cured meats. Meats are divided in two color groups depending on pigment concentration; red meats (beef and lamb) have greater myoglobin concentration than white meats (pork and poultry meat) (JAMES; JAMES, 2010). In the USA, color deterioration generates an enormous loss of US$ 1 billion per year (SMITH et al., 2000).

## 2.2 CAIMAN YACARE

In Brazil the use of native species, as *Caiman yacare,* represents an important tool for biodiversity preservation in addition to the improvement on the country economy. Moreover, both the Brazilian domestic market and markets from developed countries are increasing the demand for exotic meats due to their exquisite characteristics and nutritional appeal. *Caiman yacare* pertains to the Crocodilidae family and in Brazil; their habitat is the Pantanal region with a population of 35 million individuals on average (MOURÃO et al., 2000b; SAADOUN; CABRERA, 2008; VICENTE-NETO et al., 2010).

| | |
|---|---|
| Kingdom | Animalia |
| Phylum | Chordata |
| Class | Reptilia |
| Order | Crocodilia |
| Family | Alligatoridae |
| Genus | *Caiman* |
| Especies | *Caiman yacare* |

Amongst the twenty three species belonging to the Crocodilia order (OAKS, 2011), six are present in Brazil (BÉRNILS; COSTA, 2012) and four of them, which are from the Alligatoridae family, are legally allowed for commercial exploitation: *Caiman crocodilus* (Linnaeus, 1758), *Caiman yacare* (Daudin, 1802), *Caiman latirostris* (Daudin, 1802) and *Melanosuchus niger* (Spix, 1825; KING; BURKE, 1989). *Caiman yacare* specie is found in South America (Argentina, Brazil, Bolivia and Paraguay), and reaches the reproductive age around 9—10 years, 80 cm length and 12 kg live weight

(COUTINHO et al., 2005). During spawning, 21—38 eggs are laid, usually at rainy season (CSG, 2013).

Brazilian caiman population was highly degraded between years 1960 and 1980, due to intensive hunting to feed leather market. However in the nineties, the caiman farming regulation (BRASIL, 1990), favored the development of commercial farming at Mato Grosso and Mato Grosso do Sul states. Nowadays in Brazil, it is strictically legal to retail the leathers obtained from caiman farming and ranching. In addition, the caiman rearing regulation favored the specie preservation, improved the leather quality, as well as improved the animal carcass use, and further increased the retail value of caiman meat (FERNANDES, 2011; MOURÃO, 2000a; CSG, 2013). The caiman is considered carnivorous (SILVA, 2009); in their natural habitat they feed on insects, small shellfish and vertebrates (SANTOS, 1997). However, in captivity they are fed on livestock viscera supplemented of vitamins and minerals (ARMENTEROS, 2009).

In terms of caiman rearing systems, three different methods are known: harvesting, ranching and farming. The harvesting consists of capturing adult animals from their natural environment, avoiding negative impacts on the ecosystem. In ranching type, the eggs are sustainably collected from the natural environment, followed by incubation, rearing and slaughter when the animals reach on average 5—6 kg of live weight. In addition, the reproduction occurs in captivity, but a portion of the spawned animals are returned to the natural environment to decrease the ecosystem impact. The third method of caiman rearing is farming, where all the caiman biological cycle steps are done in captivity thus, representing the most controlled type of caiman rearing (VERDADE, 2004). Caiman is considered ready to be slaughtered when it reaches 18 cm of abdominal width (1 year), nonetheless, in terms of leather production; the caiman is slaughtered with 2 years and 27 cm of abdominal width (FETT, 2005).

## 2.3 CAIMAN MEAT (*CAIMAN YACARE*)

### 2.3.1 **Proximate composition**

The proximate composition of caiman meat is influenced by cut, animal size and nutrition. The proximate composition of four major caiman cuts (seared sirloin, dorsum steak, tail steak and members) from animals raised under captivity with 2.5—3 kg of live weight ranged from 75.44 to 77.18% of moisture, 23.57 to 24.37% of protein, 0.58—0.99% of ash, and 0.29—0.54% of fat (RODRIGUES et al., 2007). In another study, Vicente-Neto et al. (2006) demonstrated differences on physico-chemical composition of meat from wild animals and those raised under captivity. Protein content from caiman raised under captivity (23.93%) was greater than the meat from wild caiman (21.88%). The same trend was observed for cholesterol, 51.23mg/100g for farmed caiman and 38.83 mg/100g for wild caiman. On the other hand, ash and lipid content were greater on wild animals (1.17% and 2.98%, respectively) than the farmed counterparts (0.66% and 0.95%, respectively). Generally, the meat from farmed caiman exhibits better eating quality, with greater protein content and less lipid content, than the meat from wild caiman (VICENTE-NETO et al., 2006). The summary of caiman proximate composition data is presented on table 1.

During caiman post-mortem period, the glycogen metabolism on ilio-isquiocaudalis muscle lasts for fifty hours however, at thirty six hours, the pH decreases from 6.7 to 5.6 (TABOGA et al., 2003; VIEIRA et al., 2012).

Table 1. *Caiman yacare* proximate composition

| Proximate composition | *Caiman yacare* |
|---|---|
| Protein | 19.8—24.4% |
| Lipids | 0.3—5.4% |
| Moisture | 61.2—77.2% |
| Ash | 0.6—1.4% |

FERNANDES (2011); RODRIGUES et al. (2007); VICENTE-NETO et al. (2006)

### 2.3.2 **Color**

Color is the most important attribute influencing the consumer purchase decision (SUMAN; JOSEPH, 2013). Morais (2013) reported values ranging from 71.48 to 75.97 for *L** values (lightness), 3.05 to 5.87 for *a** value (redness), 5.70 to 9.23 for *b** value (yellowness), 6.57 to 10.89 for C* value (Chrome) and 54.89 to 63.31 for Hue angle value in the caiman tail meat. Therefore, based on instrumental color attributes, the caiman meat can be considered a white meat.

### 2.3.3 **Texture**

Vieira et al. (2012) investigated the caiman tail meat shear force (SF) and observed that the caiman meat can be considered a soft meat (SF < 6.0 kgf). Moreover, another study examined caiman tail meat hardness and documented shear force values of 2.31 kgf at twenty four hours post-mortem and 4.50 kgf at thirty days of refrigerated storage (MORAIS, 2013). In addition, CANTO et al. (2012) analyzed caiman tail meat texture profile analysis (TPA) and reported hardness value of 17.41 N, springiness value of 0.52, cohesiveness value of 0.41, and resistance value of 0.24.

### 2.3.4 **Fatty acid profile**

The lipid fraction of caiman meat contains a sum of saturated fatty acids ranging from 34.04% to 36.20% (VICENTE-NETO et al., 2010), exhibiting lower values than beef and sheep meat (ALFAIA et al., 2007; JUÁREZ et al., 2009). On the other hand, the polyunsaturated fatty acids content in caiman meat is greater (23.6 to 32.6%) than in ruminant meat (ALFAIA et al., 2007; SANTOS-SILVA et al., 2002). Monounsaturated fatty acid content oscillated from 29.0 to 43.04%; n—3 from 1.99 to 5.09%, and n—6 from 18.54 to 29.53%. In addition, the major fatty acids observed on caiman meat are

oleic acid (18:1 n—9), linoleic acid (18:2 n—6), palmitic acid (16:0) and stearic acid (18:0) (FERNANDES, 2011; VICENTE-NETO et al., 2010). The caiman fatty acid profile is summarized on table 2.

Table 2. *Caiman yacare* fatty acid profile

| Fatty acids | *Caiman yacare* |
|---|---|
| Saturated fatty acids | 34.0—36.2% |
| C16:0 | 21.5—22.5% |
| Monounsaturated fatty acids | 29.0—43.0% |
| C18:1 n—9 | 21.1—30.6% |
| Polyunsaturated fatty acids | 22.1—32.6% |
| C18:2 n—6 | 8.3—17.6% |
| n—3 | 2.0—5.1% |
| n—6 | 18.5—29.5% |

VICENTE-NETO et al. (2010)

### 2.3.5 **Bacteriology**

Hoffmann and Romanelli (1998) investigated the characteristics of caiman *Longissimus dorsi*, tail and loin microbiology and observed unacceptable levels of *Staphylococcus aureus* and *Salmonella* sp. However, for the best of our knowledge there is limit information on the bacteriology quality of Caiman during storage.

### 2.3.6 **Meat product**

Some studies demonstrated that caiman meat is a viable protein source for the elaboration of meat products; Romanelli et al. (2002) evaluated the consumer acceptance of four different caiman meat products (hamburger, canned meat, smoked meat and sausage) and observed at least 50% of product acceptance. In another study, Paulino et al. (2011), elaborated five hamburger formulation using caiman trimmings, differing on fat content (5% and 10% of pork fat) and the addition or not of liquid smoke. The formulation containing 5% of pork fat and added liquid smoke exhibited better

physic-chemical and sensory parameters than the other treatments. This formulation was rated 7.9 for global acceptance. Furthermore, another caiman product exhibiting increased consumer acceptance is the smoked tail (FERNANDES, 2011).

Morais et al. (2013) investigated the pork fat substitution by soybean oil (25%, 50% and 100% of pork fat) on the manufacture of caiman mortadella (70% of caiman meat). The pork fat replacement with soybean oil promoted the manufacture of mortadella with lesser saturated fatty acid content than the control samples; moreover, increasing the percentage of soybean in formulation, increased the polyunsaturated fatty acids content and decreased the atherogenic and thrombogenic indexes. In addition, the sensory attributes ratings of the caiman mortadella were similar to the commercial mortadella. Although there are several studies investigating the fabrication of meat products from caiman meat, there is limited information on the use of co-products such as carcass trimmings. In this way, despite previous investigation for caiman products formulation, more studies are necessary to develop caiman products using caiman trimmings, however maintaining the nutritional value of this meat.

## 2.4 RESTRUCTURED PRODUCTS

Restructuring process of low-value meat cuts represents a viable alternative to increase meat industry revenue. This technology improves the meat product texture and appearance, providing the trimmings or low value cuts, a whole muscle aspect, increasing its market value (KURAISHI et al., 1997). In restructured steak, the meat pieces can be bound in three ways: hot binding where heat is applied (cooking) in association with myofibrillar protein extraction promoted by sodium chloride and phosphates addition; cold setting, where the meat cuts or trimmings are adhered through chemical gelation promoted by cold binders; or the combination between cold binders and protein extraction (LENNON et al., 2010).

Restructured steaks with hot binding are sold as cooked however; due to the consumer demand for chilled raw restructured meat the use of cold binders received more attention (SUKLIM et al., 2004). Allied to the aforementioned aspect, the cold set binding eliminates the use of sodium chloride and phosphates for protein extraction,

harmonizing with the consumer market expectations for healthy food (LENNON et al., 2010). The mainly used cold binder agents are polysaccharides, blood plasma fraction, and microbial transglutaminase. From these three types of cold binder agents, the microbial transglutaminase was considered the best one for binding, appearance, cooking yield and sensory quality of beef restructured steaks (LENNON et al., 2010).

### 2.4.1 **Microbial transglutaminase (MTG)**

Microbial transglutaminase (MTG) is an enzyme that promotes the cross-link between glutamine and lysine residues through ε-(γ-glutamyl)-lysine bonds, resulting on inter or intra connection amongst meat pieces and polymerization. The cross-linking occurs by an acyl-tranfer reaction between an acyl donor (γ-carboxyamide group of peptide-bound glutamine residue) and an acyl-acceptor (primary amines), especially ε-amino group of lysine residues (KUMAZAWA et al., 2001; MOTOKI & SEGURO, 1998). The MTG have a molecular weight of 38,000 Da, an isoelectric point of 8.9, 331 amino acid residues, and is excreted mainly by a variant of *Streptoverticillium mobaraense* (KUMAZAWA et al., 2001). In addition, the optimum pH for the MTG activity is 5—8, while the optimum temperature is 50°C. However, this enzyme stays active at the pH range from 4 to 9, as well as at 10°C (MOTOKI; SEGURO, 1998). Suklim et al. (2004) prepared restructured scallops (*Argopecten gibbys*) with 1% of microbial transglutaminase and observed a chemically induced gel formation, at 5°C from a 2h chilled incubation. Moreover, microbial transglutaminase reaction effectiveness on improving the texture attributes in meat system is species-specific (CARBALLO; AYO; COLMENERO, 2006). Confirming the earlier study, AHHMED et al. (2007) documented that the difference on glutamyl and lysine residues content depending on the meat source (chicken and beef), caused a variation on meat polymerization and subsequently differentially affected the texture parameters.

The most advantage of the MTG application on restructured meat product fabrication is the cold-bind capacity (MOTOKI; SEGURO, 1998). The cross-link amongst proteins in the meat matrix promotes a gel formation favoring (KURAISHI; YAMAZAKI;

SUSA, 2001) improvements on texture and functional properties (HERRERO et al., 2008) without negatively affecting the color parameters (PIETRASIK, 2003). Herrero et al. (2008) studied the effect of transglutaminase on meat systems and observed a modification on texture properties of meat systems through large polymeric protein molecules with a more stable gel structure formation, leading to an increase on hardness, springiness and cohesiveness. In addition, a study investigated the impact of microbial transglutaminase at different levels (0 to 0.8 units/g sample) on fish gel (*Saurida undosquamis*) and detected an improvement on gel property and freshness during 10 days of refrigerated storage (BENJAKUL et al., 2008). Moreover, Monteiro et al. (2014a) examined four different concentrations of microbial transglutaminase (0%, 0.1%, 0.5%, and 0.8%) on tilapia steaks (*Oreochromis niloticus*) and documented improvement on sensorial and textural parameters without negative changes on proximal composition and nutritional value of the product. Another advantage of microbial transglutaminase use as cold binder is the potential to formulate healthier restructured steaks with less sodium content meeting the consumer, meat industry and public health agencies expectations (RUUSUNEN; PUOLANNE, 2005).

### 2.4.2 **Sodium intake reduction**

Sodium content in meat products has been associated with health problems such as cardiovascular disease, the major cause of premature death in developed countries (COLMENERO; AYO; CARBALLO, 2005; RUUSUNEN; PUOLANNE, 2005). Thereafter, meat industry and consumer market increased their interest on alternatives to decrease the sodium intake in meat meals (COFRADES et al., 2011). The daily sodium ingestion recommended is <2 g/day sodium (5 g/day salt) (WHO, 2007). Sodium chloride is the major source of sodium and promotes an important role on final product flavor, texture and shelf life, fact that difficult the reduction of sodium content; however, there are some strategies such as decrease on the sodium chloride content in the product formulation, and replacing it totally or partly by another salts such as potassium chloride and magnesium chloride (RUUSUNEN; PUOLANNE, 2005).

The mostly used sodium chloride (NaCl) substitute is potassium chloride (KCl), although mixtures over 50:50 NaCl/KCl potentially increases product bitterness and decreases its saltiness (VERMA; BANERJEE, 2012). Armenteros et al. (2009) detected no difference between control loins (100% NaCl) and those formulated with up to 50% of KCl substitution on the sensory analysis; loins with 50% of each salt exhibited the highest sensory scores. Moreover, Tahergorabi and Jaczynski (2012) demonstrated that KCl is a viable salt replacer in low sodium seafood surimi without affecting gelation and texture. Another potential substitute that has been studied is the magnesium chloride ($Mg_2Cl$) (VERMA; BANERJEE, 2012). Horita et al. (2011) observed that a partial NaCl replacement by KCl or $Mg_2Cl$ in mortadella did not change the emulsion stability and texture attributes (hardness, cohesiveness, elasticity and chewiness). In addition, tri-salt blend containing NaCl, KCl and $Mg_2Cl$ improved texture, and sensory attributes, resulting in a good acceptability and purchase intention of restructured tilapia steaks, representing an effective sodium substitutes for the formulation of healthier meat products (MONTEIRO et al., 2014b).

Moreover, efforts are required to ensure that the meat product maintain its overall quality during storage at industry, distribution and retail market; allied to this fact, meat matrix is rich in nutrients thus, represents an ideal environment for meat spoilage and food-borne pathogens proliferation. In order to ensure the meat bacteriology quality and satisfy the consumer market demand for fresh products with high quality and extended shelf life, meat industry should investigate non-thermal technologies that are considered promising to meat decontamination, such as the High Hydrostatic Pressure (HHP) (AYMERICH; PICOUET; MONFORT, 2008).

## 2.5 ALTERNATIVE CONSERVATION METHODS

Despite this, an increased consumption of this meat is subjected to the use of preservation methods that retard its spoilage and ensure its safety. The use of preservation methods aiming at assuring quality and safety aspects of Caiman meat is novel (Vieira et al., 2012). Recently, high hydrostatic pressure was shown to improve the quality of refrigerated Caiman tail meat (Canto et al., 2012).

### 2.5.1 High hydrostatic pressure (HHP)

The HHP system (Figure 1) is composed by a pressure vessel, closure (vessel), pressure transmission fluid, HHP pumps (generate the pressure), monitoring system in order to control pressure and temperature, and a system to handle the product (CAMPUS, 2010). In HHP, prepackaged meat and meat products are conditioned in a steel cylinder and pressures of 100 MPa or above are applied through a liquid pressure transmitter such as water. The pressure is transferred uniformly to product independent of it size and geometry, using an instantaneous and isostatic transmission of pressure. The effect of this technique on food chemistry and bacteriology is based on the Le Châtelier principle, where reactions of decrease in volume are associated with an increased on pressure (SIMONIN; DURANTON; LAMBALLERIE, 2012; ZHOU; XU; LIU, 2010). In commercial application, the pressure level used varies from 100 to 600 MPa, depending on the meat product (CAMPUS, 2010).

The HHP have a variety of advantages as a mild preservation technique, preserve the environment due to less energy uptake, decreases or eliminates microorganisms content, process the products at ambient or lower temperature and maintain the fresh product characteristics (SIMONIN; DURANTON; LAMBALLERIE, 2012; ZHOU; XU; LIU, 2010). Nowadays, in seafood the HHP has been recognized to improve shelling, increasing the meat release (CAMPUS, 2010). Bert H. Hite in 1899 discovered the food preservation function of high pressure technology however, due to technological limiting factors, the first product introduced in market treated with this type of processing (HHP) was in Japan in 1990 (SIMONIN; DURANTON; LAMBALLERIE, 2012). Moreover, nowadays this technology has been used by different countries (Japan, US, Italy, Spain, Germany and Australia) with great acceptance as an alternative preservation processing for meat and meat products. Some examples present in market are chicken and pork cuts, cooked and cured ham, Parma ham, mortadella, bacon, salami and sausages (AYMERICH; PICOUET; MONFORT, 2008).

Figure 1. Industrial HHP equipment for HPP located at IRTA-CENTA (GARRIGA; AYMERICH, 2010)

#### 2.5.1.1 HHP effect on molecules and structures

In terms of chemical reactions, HHP does not affect covalent bonds of primary structure of protein and fatty acids with exception to the sulphydryl groups and thiol-disulphide interchange reactions (CAMPUS, 2010). Nonetheless, this technology disrupted hydrophobic interactions and ionic bonds, resulting on irreversible modification at the secondary, tertiary and quaternary protein structure (RENDUELES et al., 2011), and subsequently affecting membrane proteins and lipid conformation. Still, small compounds such as flavor and vitamins usually are not affected by HHP (CAMPUS, 2010). In addition, the pressure increase promotes an approximation amongst molecules, leading to water and lipid reversible phase transition (HUGAS; GARRIGA; MONFORT, 2002).

HHP can reversible affect the physico-chemical properties of water, the pressure also increases the separation of positive and negative charges, resulting in an increase from 10 to 100 fold of ionic product [H+] x [OH-] of water at 1000 MPa (temperature

dependent), due to water molecules rearrangement around electric charges (CHEFTEL; CULIOLI, 1997). The HHP can affect the protein structure in different levels depending on the pressure level, protein type, and processing conditions; this technique can promotes reversible protein unfolding as well as, partial or total denaturation (BAJOVIC; BOLUMAR; HEINZ, 2012). The protein modification usually is reversible in a pressure range from 100 to 300 MPa, and irreversible modification in pressures above 300 MPa. Furthermore, HHP favors the dissociation of oligomeric proteins into subunits, facilitating the proteolysis. The tertiary protein structure is modified using pressures around 200 MPa; while, the pressure level necessary to disrupt secondary protein structure are above 700 MPa. At this pressure level there is promotion of gel formation and agglomeration of proteins (BAJOVIC; BOLUMAR; HEINZ, 2012; RASTOGI et al., 2007).

Lipids submitted to HHP treatment using temperatures above 10°C and pressures above 100 MPa increases their melting point, favoring the crystallization of while in liquid state at room temperature (CHEFTEL; CULIOLI, 1997). In addition, the effect of HHP in fatty acid profile of lipid fraction is pressure level- and meat specie-dependent, high pressure can lead to oxidation of unsaturated fatty acids; decreasing the nutritional value of the product (MA; LEDWARD, 2013). Although some studies demonstrated that HHP did not alter the fatty acid composition of oysters, beef and salmon (CRUZ-ROMERO; KERRY; KELLY, 2008; MCARDLE et al., 2010; YAGI et al., 2009), He et al. (2012) observed a pronounced lipolysis of the phospholipids with a subsequently increase in free fatty acids content in pork muscle treated with HHP at pressure above 350 MPa. Moreover, these authors reported a decrease on the linolenic acid content from the triglycerides fraction, in samples treated with 500 MPa. During storage, the saturated fatty acids as well as the monounsaturated fatty acids content increased, while polyunsaturated fatty acids percentage decreased (linoleic, linolenic and arachidonic acids) (HE et al., 2012). Recently, Wang et al. (2013) studied the influence of HHP on yak (*Poephagus grunniens*) body fat and described that samples treated with 600 MPa exhibited a decrease on polyunsaturated fatty acids content and also demonstrated a decline on PUFA/SFA ratio and increase on n−6/n−3 ratio from yak body fat pressurized with 400 or 600 MPa during refrigerated storage.

#### 2.5.1.2 HHP effect on bacteriology

The HHP efficacy on bacteriological elimination or sub lethal injury to bacterial cells depends on pressure level, holding time, temperature, food composition, pH, water activity, bacteria type and microbiota resistance. HHP causes the bacteria elimination when the damage accumulated overlaps the bacteria repair capacity, culminating on cell death. The major damages in bacteria cell are separation of the membrane from the cell wall, lengthening of the cell, compression of gas vacuoles and nuclear material condensation. HHP also causes modifications on bacteria cell proteins, enzymes, and genetic mechanisms (AYMERICH; PICOUET; MONFORT, 2008). In membrane, the damage is caused by pore formation on the double phospholipids layer membrane during the decompression step, which leads to cytoplasmic material leaking (PATTERSON, 2005). The resistance of microorganisms is highly variable and depends mainly on the type and its growth phase; gram-negative bacteria and bacteria at the log phase have a greater susceptibility to HHP than gram-positive bacteria and cells at the stationary phase (AYMERICH; PICOUET; MONFORT, 2008). In addition, spores are pressure resistant and require at least two pressurization steps for their inactivation. On first step, in order to activate the spore, a specific combination of pressure and temperature is applied while during the second step, most of the bacteria cells are destroyed (PATTERSON, 2005; RENDUELES et al., 2011).

McArdle et al. (2010) submitted beef *Pectoralis profundus* muscles at 200, 300 and 400 MPa and reported that HHP decreased bacteria total viable counts levels on treated samples, improved the meat bacteriological quality and extended the meat shelf-life. In another study, Erkan and Uretener (2010) combined different temperature/processing periods/pressure in HHP treatment (temperatures at 3, 7, 15, 25˚C; for 5–10 min; at 220, 250 and 330 MPa) on sea bream (*Sparus aurata*). Pressurized samples exhibited an increased on shelf-life of 3 days, from 15 to 18 days while stored under refrigeration (ERKAN; URETENER, 2010). Moreover, important food pathogens such as *Salmonella typhimurium*, *Escherichia coli*, and *Listeria monocytogenes* inoculated in poultry breast were eliminated below detection levels at 600 MPa. Furthermore, at 450 and 600 MPa the bacteria count decreased 4—8 and 6—

8 log (CFU/g) and increased the shelf-life from 3 and 7 to 14 days, both (KRUK et al., 2011). In bay scallop (*Argopecten irradians*), treatments at 200 MPa for 3 min decreased the aerobic plate count (APC, 2.98 log cfu/g) and coliforms (75 MPN/g) on control sample to levels under detection (<1 log cfu/g for APC and 0.3 MPN/g for coliforms) (YI et al., 2013). Nonetheless, the influence of HHP on chemical and microbiological parameters of caiman is yet to be investigated.

Although the meat industry efforts to guarantee meat and meat products nutritional value and spoilage inhibition, during refrigerated retail display there are several factors that potentially affect the product color stability leading to economic loss due to consumer rejection (SUMAN; JOSEPH, 2013)

## 2.6 UNITED STATES BEEF PRODUCTION

In 2013, the US was the main beef producing country (11.2 million tons of carcass weight equivalents—CWE), and its domestic consumption was 11.2 million tons of carcass weight equivalents (CWE), followed by Brazil with 9.9 and 7.9 million tons of CWE, and EU with 7.6 and 7.7 million tons of CWE, respectively. In addition, although the US achieved the first place in beef importation (1.1 million tons of CWE), the county only reached the fourth place in beef exportation (1.1 million tons of CWE). The first country of beef exportation was Brazil (2.0 million tons of CWE), India and Australia reached the second and third places with 1.9 and 1.6 million tons of CWE each, respectively (USDA, 2013).

In the US, the commercial beef production system can be divided in three different phases: the first called cow-calf, consist of cows breeding, gestation, calving, and calve weaning (6—9 months); the second phase is the stocker, where the objective is to add 200—400 pounds on weaned calves; on the third phase (feedlot), the animals are finished using an association of forage and grains until reaching a live weight around 1,000 and 1,500 pounds) (McBRIDE; MATTHEWS, 2001). The seven most prominent US beef breed are *Bos Taurus* such as Angus, Red Angus, Hereford, Limousin, Charolais, Gelbvieh, and Simmental (KING et al. 2011a).

### 2.6.1 **Beef skeletal muscle fiber type**

Meat quality parameters is influenced by myofiber type composition; regarding contractile characteristic, there were characterized three major fiber types characterized in the skeletal muscle, namely type I, IIA, and IIB. However, the myosin heavy chain, which represents 35% of skeletal muscle protein content, is classified in four different types according to its contraction speed. The myosim heavy chain isoforms are devided into slow type or I, or fast type or II; the type II is further subdivided into IIa, IIx, and IIb isoforms. IIb isoform is exhibits the greatest contraction speed (fast twitch) (LEFAUCHEUR, 2010).

In terms of metabolic classification, the fibers are divided depending on the primary energy metabolism pathway. The oxidative and glycolytic pathway, separate the fibers as solely oxidative (fiber type I), oxidative-glycolytic (fiber type IIa and IIx), and solely glycolytic (fiber type IIb) (LEFAUCHEUR, 2010; PETER et al., 1972). In beef, an increase on the glycolytic metabolism in muscles improves the meat color stability (O'KEEFE; HOOD, 1982), potentially due to the decrease on the oxygen consumption, promoting a decrease on myoglobin autoxidation, and consequently decreasing the metmyoglobin accumulation (O'KEEFE; HOOD, 1982; RENERRE; LABAS, 1987).

### 2.6.2 **Beef color**

Color is the main appearance attribute, determinant on beef purchase decision (MEILGAARD; CIVILLE; CARR, 2007). The consumers associate a bright cherry-red color with beef freshness, in which the color deterioration (browning) can lead to rejection, resulting on economic loss for the meat industry (over US$1 billion per year in the US) (SMITH et al., 2000). Beef color is mostly governed by myoglobin, which is a sarcoplasmic monomeric protein with 153 amino acids, comprising a heme prosthetic group and a globin (SUMAN; JOSEPH, 2013). Myoglobin exists in four different redox forms (Figure 2) in beef, the deoxymyoglobin (purplish-red color), oxymyoglobin (bright cherry-red color), carboxymyoglobin (bright cherry-red color) and metmyoglobin (brown

color). The first three redox forms contains the heme iron on the ferrous state, on the other hand, metmyoglobin which is associated with unwholeness, is characterized by the oxidative reaction of the heme iron from ferrous ($Fe^{2+}$) to a ferric ($Fe^{3+}$) state (BEKHIT; FAUSTMAN, 2005; SUMAN; JOSEPH, 2013). Redox myoglobin forms have different absorbance spectras, the absorbance peaks for deoxymyoglobin is 557 nm, oxymyoglobin at 542 and 582 nm, carboxymyoglobin at 543 and 581 nm, and metmyoglobin 503 nm. However, total myoglobin concentration can be determined at 525 nm of absorbance (SUMAN; JOSEPH, 2013).

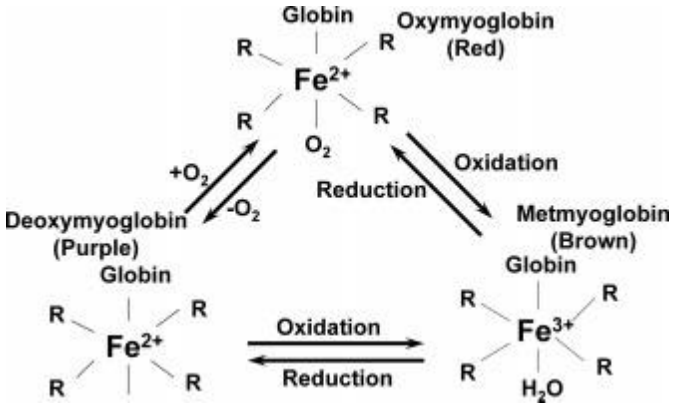


Figure 2. Myoglobin redox states (SUMAN et al., 2007)

Exogenous and endogenous factors affect myoglobin redox stability in beef (SUMAN; JOSEPH, 2013). Ligands, antioxidants, and pro-oxidants represent exogenous factors influencing the meat color (SUMAN; JOSEPH, 2013). Moreover, the main endogenous factors and live animal–related factors governing color stability already studied are breed, feed, management (LYNCH et al., 2002) and muscle-specificity (JOSEPH et al., 2012; MCKENNA et al., 2005). In addition, previous reports in the US documented an influence of animal-to-animal variation in color stability during retail display (KING et al., 2011a; KING et al., 2011b).

Beef discoloration during storage is determined by accumulation of metmyoglobin, which is affected by the reduction of the oxidized myoglobin form to deoxymyoglobin through muscle metmyoglobin reducing activity (KIM et al., 2006). Reduced nicotinamide adenine dinucleotide (NADH) plays an important role in

metmyoglobin reduction, controlling metmyoglobin accumulation and further meat browning (MANCINI; HUNT, 2005). In addition, Kim et al. (2006) observed that lactate improved beef color stability, through lactic dehydrogenase activity, promoting the conversion of lactate to pyruvate and NADH regeneration. In beef muscles considered color stable, the reduced redox ferrous form of myoglobin are favored by low oxygen consumption rate (O'KEEFFE; HOOD, 1982) and increased metmyoglobin reducing activity (LEDWARD, 1985).

## 2.7 PROTEOMICS AND MEAT COLOR TRAITS

Proteomics tools are applied to study the proteome (set of proteins present at a specific time in a sample), which is affected by endogenous and exogenous factors thus, is characterized as dynamic (a snap shot of certain treatment condition). In proteomics, techniques such as chromatography and electrophoresis are employed to promote protein separation followed by protein identification through mass spectrometry analysis (HOLLUNG et al., 2007). In meat science, the widely method is the two dimensional electrophoresis and its steps are: protein extraction, protein separation based on their isoelectric point, protein separation based on their molecular weight, gel image digitalization and analysis, selection of differentially abundant protein spots, proteins identification using mass spectrometry analysis, and results interpretation (HOLLUNG et al., 2007). The majority of the proteins related to meat color parameters and color stability in postmortem period are present in the sarcoplasmic proteome (HAMELIN et al., 2005; JOO et al., 1999; JOSEPH et al., 2012; KIM et al., 2006).

Proteomic tools were already successfully used to identify the beef myoglobin susceptibility to lipid oxidation-induced oxidation (SUMAN et al., 2006; 2007). The aforementioned studies documented that bovine myoglobin primary structure is more susceptible to 4-hydroxy-2-nonenal (HNE) nucleophilic attack with subsequent adduction than porcine myoglobin primary structure; allied to this fact, the authors reported that bovine oxymyoglobin is more susceptible to oxidation induced by lipid oxidation than in porcine counterpart. In addition, MS-based proteomic tools have been used to elucidate species-specific meat color stability in turkey, emu, ostrich, porcine, bovine and bison

(JOSEPH et al., 2010a; JOSEPH et al., 2010b; NAIR et al., 2014; SUMAN et al., 2007). Moreover, JOSEPH et al. (2012) documented muscle-specific color stability. In this study, the sarcoplasmic proteomes of *Longissimus lumborum* (color-stable) and *Psoas major* (color-labile) muscles were analyzed and correlated with beef color attributes. The proteins over-abundant in the color-stable *Longissimus lumborum* muscle were positively correlated to the surface color stability (peroxiredoxin-2, dihydropteridine reductase, and heat shock protein-27 kDa) and redness (aldose reductase, creatine kinase, and β-enolase) while the protein over-abundant in color-labile *Psoas major* muscle exhibited a negative correlation with redness (mitochondrial aconitase). The authors concluded that the over-abundance of antioxidant proteins and chaperones in color-stable group improved the color stability during retail display. Recently, Desai et al. (2014) evaluated the muscle sarcoplasmic and myofibrillar proteome of normal and reddish channel catfish (*Ictalurus punctatus*) fillets, employing two-dimensional electrophoresis followed by mass spectrometry and documented an over-abundance of the beta subunit of hemoglobin in the sarcoplasmic proteome of reddish fillets and concluded that the over-abundance of hemoglobin is the main cause of the red color defect in catfish fillets.

# 3 CHAPTERS

## 3.1 CHAPTER 1: PHYSICO-CHEMICAL AND SENSORY ATTRIBUTES OF LOW-SODIUM RESTRUCTURED CAIMAN STEAKS CONTAINING MICROBIAL TRANSGLUTAMINASE AND SALT REPLACERS. PUBLISHED ON *MEAT SCIENCE*, 96, 623–632, 2014 (PAPER 1)

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## ABSTRACT

Our objective was to examine the physico-chemical and sensory attributes of low-sodium restructured caiman steaks containing microbial transglutaminase (MTG) and salt replacers (KCl and $MgCl_2$). Trimmings from caiman carcasses were processed into restructured steaks with or without MTG and salt replacers; the five treatments were CON (1.5% NaCl), T-1 (1.5% NaCl + 1% MTG), T-2 (0.75% NaCl + 1% MTG + 0.75% KCl), T-3 (0.75% NaCl + 1% MTG + 0.75% $MgCl_2$), T-4 (0.75% NaCl + 1% MTG + 0.375% KCl + 0.375% $MgCl_2$). T-4 demonstrated the greatest ($P < 0.05$) succulence and the lowest ($P < 0.05$) values for cooked hardness, springiness, and cohesiveness. The greatest ($P < 0.05$) purchase intention was for T-3. Furthermore, T-3 and T-4 were similar ($P > 0.05$) to controls in salty flavor. Our findings suggest that the combination of MTG, KCl, and $MgCl_2$ can be employed as a suitable salt reduction strategy in restructured caiman steaks without compromising sensory attributes and consumer acceptance.


Keywords: Caiman crocodilus yacare; Sodium reduction; Low-salt; Salt replacers; Restructured meat

### 3.1.1 Introduction

The increasing costs of meat production have prompted the industry to develop novel processing strategies to utilize the carcasses, and value-addition of low-value meat cuts generates additional revenue. Meat restructuring allows for efficient utilization of low-value cuts and carcass trimmings. Restructured meats exhibit consumer-desirable texture and appearance, and thus have increased retail value (Marques, Marostica, & Pastore, 2010). Restructured meats are commonly manufactured using salt and mechanical processes to extract myofibrillar proteins, which form a protein matrix entrapping fat, water, and flavor compounds, resulting in a desirable texture and flavor (Pearson & Gillett, 1996).

The increase in consumer health consciousness has led to development of healthy meat products through reformulation and using muscle foods with high nutritive value (Trespalacios & Pla, 2007). Meat from caiman (Caiman crocodilus yacare) is low in fat and rich in polyunsaturated fatty acids (Romanelli, Caseri, & Lopes Filho, 2002; Paulino et al., 2011). Commercial caiman production (primarily for leather) is an emerging agricultural activity (Vicente Neto, Bressan, Faria, Vieira, Santana, & Kloster, 2007) in Brazil, where significant quantity of caiman meat is wasted due to lack of suitable processing technology to utilize the carcass trimmings.

The role of sodium in hypertension and cardiovascular diseases (He & MacGregor, 2008) is a major concern in food industry because processed meats are a major source for sodium in human diet (Engstrom, Tobelmann, & Albertson, 1997). Therefore, sodium reduction is a priority in meat industry. Sodium reduction can lead to obstacles because salt (NaCl) is a reliable protein extractor, which enhances flavor and palatability traits. Potassium chloride (KCl) can be used as a salt replacer, but it imparts bitterness and decreases saltiness. Magnesium chloride (MgCl2) has also been explored as a salt replacer (Hur, Ye, Lee, Ha, Park, & Joo, 2004; Ruusunen & Puolanne 2005). In general, salt reduction strategies compromise sensory and textural attributes of meat products (Ruusunen & Puolanne, 2005; Doyle & Glass, 2010) and negatively influence consumer acceptance (Desmond, 2006). In this perspective, sodium reduction strategies in restructured meat products need further research (Lee & Chin, 2011; Cofrades, Lopez-Lopez, Ruiz-Capillas, Triki, & Jimenez-Colmenero, 2011).

Microbial transglutaminase (MTG) is an enzyme promoting protein aggregation in muscle foods through covalent cross-linking between glutamine and lysine residues (Seguro, Kumazawa, Ohtsuka, Toiguchi, & Motoki, 1995; Lee & Lanier, 1995). MTG is active at the pH range 5–8 and temperature range 2–60°C. The efficiency of MTG is governed by the availability of the target amino acids, which depends on the species of meat (Lennon, McDonald, Moon, Ward, & Kenny, 2010; Ahhmed, Kawahara, Ohta, Nakade, Soeda, & Muguruma, 2007; Kawahara, Ahhmed, Ohta, Nakade, & Muguruma, 2007). In meat products, MTG is utilized to improve water-holding capacity and texture (Chin, Gob, & Xiong, 2009). Furthermore, MTG has been proposed as an ingredient to achieve protein gelation and matrix formation in low-salt meats (Colmenero, Ayo, & Carballo, 2005; Fulladosa, Serra, Gou, & Arnau, 2009).

Caiman carcass trimmings can be utilized for manufacturing heart-healthy, low-sodium processed meats through restructuring and salt reduction strategies. Nonetheless, investigations are yet to be undertaken to explore the use of MTG in combination with potassium chloride and magnesium chloride in low-sodium restructured caiman meat products. Therefore, the objective of the present study was to examine the sensory attributes, texture, and color of low-sodium restructured caiman meat steaks containing potassium chloride, magnesium chloride, and MTG.

## 3.1.2 **Materials and methods**

### 3.1.2.1.1 *Caiman meat processing*

Caiman carcass trimmings (tail, neck, legs, and back) were procured from a federally-inspected slaughterhouse at Caceres, Mato Grosso, Brazil. Trimmings from twenty caiman carcasses were obtained, and the meat (500 g) from each carcass was individually packaged and frozen immediately. Frozen trimmings were transported in dry-ice to the meat laboratory of the Universidade Federal Fluminense, where they were thawed at 4°C overnight before processing. Thawed trimmings from five carcasses (2.5 kg) were pooled, mixed well, and ground through a 10 mm plate. The 2.5 kg meat was

further divided into batches of 500 gram representing five different treatments. The experiment was repeated four times providing four replicates (n = 4).

The formulations for the five treatments are presented in Table 1. The ingredients included caiman meat, chilled water, sodium tripolyphosphate, sodium chloride, potassium chloride, magnesium chloride, garlic powder, onion powder, and MTG (Active WM, Ajinomoto Co. Inc., Kawasaki, Japan). Active WM contained 99% (w/w) maltodextrin and 1% (w/w) MTG from Streptoverticillium sp. with activity of 100 U/g.

The ingredients for 500 g batch were hand-mixed for five minutes, during which MTG was sprinkled. The meat mixture was bulk-packaged in cylindrical shape (7 cm diameter and 20 cm length) with polyvinylchloride film. Several holes were punctured on the meat tubes to remove the trapped air, and the tubes were stored at 4°C for 18 h for cold binding. Ten 2-cm thick steaks were cut from each tube, and the steaks were individually vacuum packaged and frozen at –18°C for 24 h.

#### 3.1.2.2 Meat pH and proximate composition

The pH of caiman trimmings and raw restructured steaks was measured using a digital pH meter (Digimed, Sao Paulo, Brazil) after homogenizing 10 g sample in 90 mL distilled water (Conte-Junior, Fernandez, & Mano, 2008). Proximate composition (moisture, protein, fat, and ash) of caiman trimmings and raw restructured steaks was determined according to AOAC (2005).

#### 3.1.2.3 Cooking yield

The raw frozen steaks were weighed and grilled in a pan until the internal temperature reached 35°C. Then they were flipped to the other side and cooked to an internal temperature of 70°C. Internal temperature was monitored using thermocouples inserted to the geometric center. Cooked steaks were cooled for 30 min at 25°C and weighed. Cooking yield was calculated from the difference in the weight of raw and cooked steaks and expressed as percentage of initial weight (Boles & Swan, 1996).

#### 3.1.2.4 Instrumental color evaluation

CIE L* (lightness), a* (redness), and b* (yellowness) values were measured at two random locations on each steak using Konica Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan) with illuminant D65, 8 mm diameter aperture, and 2° standard observer (AMSA, 2012). The colorimeter was calibrated with a standard white plate (Y = 94.2; x = 0.3160; y = 0.3326). The raw color measurements were taken on the surface immediately before grilling. The cooked samples were bisected parallel to the grilling surface and maintained at 25°C for 30 min, at which time the internal color was determined.

#### 3.1.2.5 Texture analysis

The raw and cooked steaks were subjected to Texture Profile Analysis (Bourne, 1978) using a TA.XT Plus texture analyzer (Stable Micro System, London, United Kingdom) equipped with a 75 mm diameter cylindrical metal probe. Steaks at 25°C were cut into cubes (2 cm x 2 cm x 2 cm). Samples were compressed to 70% of their original height in two cycles at pre-test speed of 5 mm/s, test speed of 1 mm/s, and post-test speed of 5 mm/s. The time between the compressions was 2 s. The load cell used was 250 N. Eight repetitions were used per sample, and the readings were averaged before statistical analyses. The data obtained were processed by Texture Expert Software (Stable Micro System, London, United Kingdom) and expressed as hardness, springiness, cohesiveness, and resistance (Bourne, 1978). Hardness is defined as the force (N) necessary to attain 70% deformation and is determined as the peak force required for the first compression. Springiness is a dimensionless parameter, which is defined as the ratio of the height the sample returns after the first compression to the maximum deformation. Cohesiveness is defined as the strength of the internal bonds making up the body of the sample; it is also a dimensionless parameter calculated as the ratio of active work done under the second compression curve to the work done

under the first compression curve. Resistance characterizes the ability of the product to regain its original shape after deformation and is also a dimensionless parameter. It is defined as the ratio of work returned by the sample as compression force is removed to the work required for compression.

#### 3.1.2.6 Quantitative Descriptive Analysis

Sensory profile of raw and cooked restructured caiman steaks was determined by eight experienced and trained panelists through Quantitative Descriptive Analysis (QDA) as previously described (Therkildsen, Stolzenbach, & Byrne, 2011). The panelists were regular consumers of meat products and were recruited from the graduate students of the Department of Food Technology at the Universidade Federal Fluminense. The panel consisted of eight members (three men and five women between 23 and 32 years age). During training, the samples were offered to the panelists, and the attributes (appearance, aroma, flavor, and texture) were identified through an open discussion amongst the panel members moderated by a leader. After identifying the attributes, the panel further met for eight 2-hour sessions to establish, by consensus, the definitions and references to elaborate the scorecard. The panelists generated eleven clearly defined attributes with suitable references (Table 2) through an open discussion moderated by the leader. After identification of the attributes and definition of the references, the training with the descriptive terms was carried out using the perception intensity scale with anchor points of “light” or “dark” for color and “slight” or “a lot” for the other attributes. Before carrying out the QDA, the performance of the panel was evaluated to verify the ability to discriminate samples, repeatability, and agreement amongst the members (Damasio & Costell, 1991).

Visual color was evaluated in both raw and cooked samples. Steaks were presented to panelists on disposable white plastic plates under white light in individual booths constructed according to the specifications of the International Standards Organization (ISO, 1985) to evaluate the color attributes. Palatability attributes were evaluated only in cooked steaks. Cooked samples were cut into cubes (1 cm x 1 cm x 1 cm) and presented to the panelists as described above. Unsalted crackers and drinking

water at room temperature were offered to clean the palate between samples. The panel completed the QDA under laboratory conditions and evaluated the samples based on previously determined references in four replicates for all attributes per panelist, using a scorecard with a non-structured 9 cm-long perception intensity scale. For color attributes 0 represented “light” and 9 represented “dark”, while for the other attributes (appearance, aroma, flavor, and texture), 0 represented “slight” and 9 represented “a lot”.

#### 3.1.2.7 Consumer sensory testing

For cooked samples, consumer analysis was conducted with 60 panelists (age 18–63 years) recruited from the students, faculty, and staff of the Universidade Federal Fluminense. Panelists had no previous training in sensory analysis of meats. Acceptance test was used to evaluate the degree at which consumers like or dislike the products regarding the appearance, aroma, flavor, texture, overall acceptability, saltiness, firmness, and purchase intention. The samples were presented in randomized blocks in a sequential monadic way. Using a nine-point hedonic scale (1 = dislike completely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like completely), the consumers evaluated appearance, aroma, flavor, texture, and overall acceptability (Cruz et al., 2013). In addition, saltiness and firmness were evaluated using a nine-point Just-About-Right (JAR) scale (1 = extremely too little salty or firm than ideal; 2 = much less salty or firm than ideal; 3 = moderately less salty or firm than ideal; 4 = slightly less salty or firm than ideal 5 = just about right; 6 = slightly saltier or firmer than the ideal; 7 = moderately saltier or firmer than ideal; 8 = much saltier or firmer than ideal; and 9 = extremely too much salty or firm than ideal) according to Cervantes, Aoki, Almeida, Nepomuceno, and Pulzatto (2010).

The samples were evaluated at the sensory laboratory. The sensory evaluation was performed under individual booths, and necessary precautions were taken to ensure that the panelists made independent judgments. The samples were coded with random three-digit numbers, and the order of presentation was determined by random

permutation. Samples of each treatment were provided to the panelists along with unsalted crackers and drinking water at room temperature to clean the palate between samples (Meilgaard, Civille, & Carr, 1999).

#### 3.1.2.8 Statistical analysis

The experimental design was a randomized complete block design. Using the trimmings from five caiman carcasses in each trial, the experiment was repeated four times to provide four replicates (n = 4). One-way ANOVA was used to analyze cooking yield, instrumental data (texture and color), QDA, and consumer acceptance. Data were analyzed with XLSTAT (Addinsoft, Paris, France). Tukey's test was used to compare treatment means at 5% significance level ($P < 0.05$). Multivariate methods were also performed. The results of the QDA were also evaluated by principal component analysis in a correlation matrix with the data centered and scaled on the variable average. A matrix was elaborated with five rows and twenty-six columns, with the rows representing the treatments and the columns the instrumental and sensory descriptors. Descriptive information obtained from the trained panel was related to the consumer preference data using partial least squares regression (PLS). The PLS regression was used to model the acceptance test through instrumental color, textural analyses, and yield as well as the sensory data (van Schalkwyk, McMillin, Booyse, Witthuhn, & Hoffman, 2011). In addition, Penalty analysis (PA) was used to analyze JAR data to identify possible alternatives for product improvement. This method is based on multiple comparisons to verify if the JAR scaling is significantly related to parameters in the acceptance test (Cervantes et al., 2010). In addition, the correlation between the instrumental and sensory data (for color and texture parameters) was determined using Pearson's correlation at 5% significance level ($P < 0.05$).

### 3.1.3 **Results and discussion**

#### 3.1.3.1 Meat pH and proximate composition

The pH of caiman trimmings was 5.71, which was close to the values reported in fresh beef (De Marchi, Penasa, Cecchinato, & Bittante, 2013), pork (Holmer et al., 2009), and chicken (Basaran, Basaran-Akgul, & Rasco, 2010). The caiman trimmings contained 76.13% moisture, 18.94% protein, 1.02% fat, and 2.35% ash. The moisture and protein contents of caiman trimmings were comparable to chicken (Trespalacios & Pla, 2007; Basaran et al., 2010) and pork (Kim et al., 2008). On the other hand, the lipid content in caiman trimmings observed in the present study was lower than the values reported for beef (Nayak, Kenney, Slider, Head, & Killefer, 1998b), chicken (Trespalacios, & Pla, 2007), and pork (Herrero, Cambero, Ordonez, de la Hoza, & Carmona, 2008). The observed ash content in caiman trimmings was greater than the values reported for chicken (Trespalacios & Pla, 2007) and beef (Nayak et al., 1998b).

The pH and proximate composition of raw restructured steaks are presented in Table 3. Control (CON) and T-1 samples demonstrated similar ($P > 0.05$) pH values, which were lower ($P < 0.05$) than the low-sodium products. The low-sodium products exhibited similar ($P > 0.05$) pH values. Our results indicated that MTG alone did not influence the pH of the product. In agreement with our findings, previous research documented that MTG alone did not influence the pH of restructured cooked pork shoulder (Dimitrakopoulou, Ambrosiadis, Zetou, & Bloukas, 2005), restructured ham (Lee & Chin, 2011), and restructured chicken meat (Basaran et al., 2010). In addition, the results of the present study are similar to those of Horita, Morgano, Celeghini and Pollonio (2011), who observed that reduced-fat mortadella containing 1% of NaCl and 1% of KCl had greater pH than 2% of NaCl product. Protein extractability in meat depends on the ionic strength, pH, and type of the salt (Franks, 1993). The size of ions can also influence the gel strength (Tang, Tung, & Zeng, 1996). An increase in the concentration of chloride ions can increase the pH of food matrix, and this property is attributed to the negative charge provided by the chloride ions, which neutralizes the

positive charges in matrix generated by an acidic pH (Rocha-Estrada, Cordova-Murueta, & Garcia-Carreno, 2010). On a molar basis, the chloride ion concentrations in the treatments were in the order: T-3 (0.39 M) > T-4 (0.35 M) = T-1 (0.35 M) = CON (0.35 M) > T-2 (0.32 M). The increased chloride ion concentration can partially explain the observed greater pH in T-3 than CON and T-1. The greater ($P < 0.05$) pH of T-2 and T-4 than CON and T-1 might be due to the differences in the size of the cations; hydrated sodium ion has a radius of 0.276 nm, while that of potassium ion is 0.232 nm (Helmke & Sparks, 1996). The smaller size of hydrated potassium ion can possibly increase diffusion into the muscle food matrix (Sperelakis, 1995; Barat, Baigts, Alino, Fernandez, & Perez-Garcia, 2011) increasing protein extractability and substrate availability for MTG. The action of MTG on protein substrates leads to generation of ammonia (De Jong & Koppelman, 2002), which increases the pH of food matrix.

The raw restructured caiman steaks did not exhibit differences ($P > 0.05$) in moisture, fat, and ash contents (Table 3). However, protein content was greater ($P < 0.05$) in CON than in the other treatments, possibly due to its greater proportion of caiman meat used in the formulation (Table 1). Our data on moisture, fat, and ash contents are in agreement with those reported by Cofrades et al. (2011) on low-salt restructured poultry products containing only MTG. In addition, Horita et al. (2011) observed that reduced-fat mortadella prepared with sodium replacers had similar proximate composition as that of regular salt product. Furthermore, incorporation of MTG did not influence the proximate composition of restructured cooked pork shoulder (Dimitrakopoulou et al., 2005).

#### 3.1.3.2 Cooking yield

The data on cooking yield are presented in Table 4. T-1 demonstrated greater ($P < 0.05$) cooking yield compared to CON, indicating that MTG alone increased processing yield. Furthermore, low-sodium restructured caiman steaks containing MTG and salt replacers (T-2, T-3, and T-4) had greater ($P < 0.05$) cooking yield than CON and T-1 suggesting the effectiveness of the combinations of MTG, KCl and/or $MgCl_2$ in improving product yield. The improved cooking yield in low-sodium treatments (T-2, T-3,

and T-4) could be partially attributed to the increased pH, which was further away from the isoelectric point (pH 5.0 – 5.2) of major muscle proteins. As the pH moves away from the isoelectric point, muscle proteins become densely charged resulting in a concomitant increase in hydrophilicity and water retention (Puolanne and Halonen, 2010). Among low-salt products, the steaks containing KCl (T-2 and T-4) exhibited the greatest values ($P < 0.05$) indicating a possible synergistic effect of KCl and MTG. While all low-sodium products exhibited similar pH, the improved cooking yield in KCl-containing formulations (T-2 and T-4) could be partially due to increase in protein extractability induced by the smaller-sized hydrated potassium ions in the matrix (Sperelakis, 1995; Barat et al., 2011).

Our results indicated that incorporation of KCl and $MgCl_2$ in low-sodium restructured meats can increase the cooking yield. Divalent and monovalent cations promote changes in water-holding capacity through different mechanisms (Barat, Perez-Esteve, Aristoy, & Toldra, 2012). In agreement with our findings, previous reports documented that the replacement of sodium chloride by magnesium chloride or potassium chloride increased the extractability and solubility of myofibrillar proteins favoring gel formation in beef batter model systems (Nayak, Kenney, Slider, Head, & Killefer, 1998a; Pigott, Kenney, Slider, & Head, 2000). MTG catalyzes intra- and inter-protein cross-linking between glutamine and lysine, promoting network formation, and turning the muscle food matrix into a high molecular weight polymer entrapping water molecules, which improves processing yields. Moreover, MTG promotes glutamine deamination during which water acts as a nucleophile and leading to the covalent attachment of the water molecule to the protein chain (Ohtsuka, Umezawa, Nio, & Kubota, 2001). All these mechanisms increase the water content in the meat products and effectively contribute to the increased cooking yield in MTG-containing restructured caiman steaks.

In agreement with our findings, Tseng, Liu, and Chen (2000) reported increased yield in low-salt chicken meat-balls containing MTG. Several researchers (Pietrasik & Li-Chan, 2002; Pietrasik, Jarmoluk, & Shand, 2007) observed that MTG addition decreased the cooking loss in pork batter gels. Moreover, Pietrasik (2003) documented that MTG positively influenced the parameters contributing to the water binding

properties of beef gels. In contrast, other researchers observed no effect of MTG on cooking loss in porcine myofibrillar protein gel (Chin et al., 2009) and low-salt restructured poultry meat (Cofrades et al., 2011). The differences between our observations and these reports could be, in part, due to the variations in the concentration of MTG used.

#### 3.1.3.3 Instrumental color

##### 3.1.3.3.1 *Raw restructured caiman steak*

Neither MTG nor salt replacers influenced ($P > 0.05$) L* values in raw restructured caiman steaks (Table 4). On the other hand, a* and b* values were different ($P < 0.05$) among the treatments (Table 4). T-1 and T-4 exhibited a* values similar ($P > 0.05$) to that of controls, whereas T-2 and T-3 demonstrated greater ($P < 0.05$) redness than the controls. The observed lack of difference in a* values of control, T-1, and T-4 caiman steaks indicated that the incorporation of MTG alone or in combination with KCl and $MgCl_2$ may not affect the surface redness during retail. Based on surface redness, which influences consumer purchase decisions (Mancini & Hunt, 2005), the low-sodium product T-4 can be retailed in a manner similar to the controls. The b* values of T-1 steaks were lower ($P < 0.05$) than that of the controls indicating that MTG decreased the yellowness. In addition, T-4 also exhibited lower ($P < 0.05$) b* values than CON. However, T-3 demonstrated greater ($P < 0.05$) b* values than CON.

The color of uncooked meat products is dictated by myoglobin concentration, fat and water contents, and non-meat ingredients (Pietrasik & Janz, 2009). When myoglobin content and redox state remain constant, the color of comminuted products is mostly governed by the parameters such as fat content, non-meat ingredients and added/lost water during the processing (Trespalacios & Pla, 2007). In agreement with our results, several previous studies reported that the L* values were unaffected by the addition of MTG in catfish patties (Min & Green, 2008) and pork (Nielsen, Petersen, & Moller, 1995). However, Nielsen et al. (1995) documented a decrease in a* values in MTG-treated pork. Min and Green (2008) observed that in catfish patties a* values did

not change and b* values increased with the addition of MTG. These findings suggested a species-specific effect of MTG on meat color. The differences between our results and the previous reports could be attributed to the species-specific variations in the concentration and biochemistry of myoglobin in raw meat (Suman & Joseph, 2013).

#### 3.1.3.3.2 *Cooked restructured caiman steak*

The dull-brown color of cooked meat is due to the heat-induced denaturation of myoglobin (King & Whyte, 2006), and the process of cooking results in a decrease in redness and an increase in lightness and yellowness (Cofrades et al., 2011). The instrumental color parameters (L*, a*, and b* values) of cooked restructured caiman steaks were similar ($P > 0.05$; Table 4). These results indicated that cooked low-sodium and control restructured caiman steaks have similar color and may appear similar at the point-of-consumption. In support of the findings in the present study, Tseng et al. (2000) observed no differences among treatments in their study on low-salt chicken meat-balls. Furthermore, dicationic salts have been reported to have no effect on L* values in cooked beef batter (Pigott et al., 2000). Additionally, Horita et al. (2011) documented that salt reduction did not contribute to variations in L*, a*, and b* values of bologna. Furthermore, other researchers also reported that MTG levels had no effects on cooked color of restructured pork shoulder (Dimitrakopoulou et al., 2005) and chicken kebab (Kilic, 2003).

### 3.1.3.4 Instrumental texture

#### 3.1.3.4.1 *Raw restructured caiman steak*

Hardness and springiness were greater ($P < 0.05$) for MTG-containing steaks than controls, except for T-3 which was comparable ($P > 0.05$) to control (Table 4). In addition, incorporation of MTG increased ($P < 0.05$) resistance in the raw restructured caiman steaks (Table 4). Among the MTG-treated samples, hardness was similar ($P >$

0.05) for T-1, T-2, and T-4 (Table 4). On the other hand, T-2 exhibited greater ($P < 0.05$) values for springiness, cohesiveness, and resistance than the other MTG-containing treatments as well as control (Table 4). The increase in hardness, springiness, and resistance observed in restructured caiman steaks containing MTG can be attributed to the enhanced protein cross-linking. The formation of large polymeric protein aggregates improves the gel structure between meat particles (De Jong & Koppelman, 2002). In agreement with our results, Herrero et al. (2008) concluded that hardness, springiness, and cohesiveness of pork increased with the incorporation of MTG. In addition, MTG has been suggested as an ingredient to improve functional and textural properties of food products (Yokoyama, Nio, & Kikuchi, 2004). Nayak et al. (1998a) reported that in low-fat beef batters $MgCl_2$ lowers actin solubility, which decreases the substrate availability for MTG; this phenomenon partially explains the lower value of hardness observed in T-3 than other MTG-treatments. In contrast to our results, Nielsen et al. (1995) observed no effect of MTG addition on the textural attributes of pork. In the present study, the low values for textural parameters in meat systems without MTG indicated a pseudoplastic fluid behavior possibly due to low myofibrillar protein gelation.

3.1.3.4.2 *Cooked restructured caiman steak*

After cooking, the MTG-treated steaks demonstrated greater ($P < 0.05$) values for instrumental texture parameters than CON, with exception of springiness in T-4 (Table 4). Within the MTG treatments, T-4 had lowest ($P < 0.05$) values for hardness, springiness, and cohesiveness, whereas T-1 had the greatest ($P < 0.05$) resistance. In general, replacing NaCl partially with KCl and/or $MgCl_2$ in the presence of MTG decreased ($P < 0.05$) resistance, cohesiveness, and hardness. The observed increase in texture parameters can be attributed to the formation of glutamyl-lysyl bonds between myofibrillar proteins (Lee & Lanier, 1995). Protein-protein binding is enhanced by MTG, and a strong protein network among meat particles increases the breaking strength and results in a firm product (De Jong & Koppelman, 2002). Heat processing of MTG-incorporated restructured meat products promotes denaturation of protein molecules and leads to exposure of buried reactive groups, which ultimately improves

cohesiveness (Tellez-Luis, Uresti, Ramirez, & Vazquez, 2002). Several previous investigations supported the findings from the present study. Hammer (1998) reported that finely comminuted sausages containing 0.2% MTG were harder and firmer than the controls. Furthermore, Tseng et al. (2000) observed that the gel strength of low-salt chicken meat balls increased with the level of added MTG. Pietrasik (2003) reported that MTG increased hardness, cohesiveness, and springiness of beef gels.

#### 3.1.3.5 Quantitative Descriptive Analysis

QDA differentiated cooked restructured caiman steaks based on cooked color, spicy odor, salty flavor, bitter flavor, tenderness, succulence, and cohesiveness (Table 5). Among the MTG-treatments, T-2 demonstrated the lowest ($P < 0.05$) values for cooked color, which was similar ($P > 0.05$) only to controls. T-4 exhibited lower ($P < 0.05$) spicy odor than CON, T-1, and T-3. However, spicy flavor was not different ($P > 0.05$) among the treatments. With respect to salty flavor, the data indicated that T-1 had the greatest scores ($P < 0.05$). T-3 and T-4 steaks were lower ($P < 0.05$) in salty flavor than T-1 possibly due to the partial replacement of NaCl with KCl and/or $MgCl_2$. However, T-3 and T-4 steaks were similar ($P > 0.05$) to controls in salty flavor. Overall, MTG-treated steaks had greater ($P < 0.05$) bitter flavor than controls. Among the low-sodium MTG-treatments, bitter flavor was greatest ($P < 0.05$) for T-2, which contained highest level (0.75%) of potassium chloride. Potassium chloride, at a replacement level above 50%, is known to impart bitterness in low-salt foods (Desmond, 2006).

Control steaks demonstrated greater ($P < 0.05$) tenderness than T-1, T-2, and T-3. However, T-4 was rated similar ($P > 0.05$) to control with respect to tenderness. The least tender steaks were T-1 ($P < 0.05$), whereas T-3 and T-2 were intermediate. The most succulent steaks ($P < 0.05$) were T-2 and T-4, whereas the least ($P < 0.05$) succulent ones were T-3. For cohesiveness, T-1 exhibited the greatest ($P < 0.05$) scores, whereas controls and low-sodium restructured steaks (T-2, T-3, and T-4) demonstrated similar values ($P > 0.05$). QDA did not identify any difference ($P > 0.05$) in raw color, product uniformity, spicy flavor, and overall texture. The results of QDA analysis indicated that the low-sodium T-4 steak has several sensory properties (salty

flavor, tenderness, and cohesiveness) comparable to the controls, indicating the market potential for low-salt meats containing KCl and $MgCl_2$ as partial replacers for NaCl.

The results of previous sensory studies on low-sodium meat products were in conflict with our findings in QDA. Matulis, McKeith, Sutherland, and Brewer (1995) examined the influence of salt on sensory characteristics of frankfurters and concluded that salt increased saltiness and decreased tenderness. Gimeno, Astiasaran, and Bello (1999) observed lower cohesiveness and greater tenderness for low-sodium dry-fermented sausages (containing KCl and CaCl2) than the traditional ones. Furthermore, Dimitrakopoulou et al. (2005) reported no differences in juiciness for the control and MTG-containing cooked pork shoulders. The differences between our results and the previous reports could be attributed to the differences in the combinations of salt replacers used in the present study, which could have minimized the differences between control and the low-sodium products (T-2, T-3, and T-4).

### 3.1.3.6 Principal Component Analysis (PCA)

PCA explained 67.3% of total variance (Figure 1). The principal component-1 contributed 35.7% in defining the treatments into 2 groups (CON and T-2; T-1, T-3, and T-4). For this division, cooked instrumental texture (hardness, springiness, cohesiveness, and resistance), overall texture, and spicy flavor were the relevant parameters. CON and T-2 demonstrated lower values for all these parameters, except spicy flavor, than the other treatments. The principal component-2 contributed 31.6% in characterizing the treatments. Despite having low percentage, this component clearly divided the treatments based on raw instrumental texture (hardness, springiness, cohesiveness, and resistance), bitter flavor, and product uniformity. The high values for these factors separated T-2 from the other treatments. Additionally CON, T-1, T-3, and T-4 were rated high for cooked color. The combination of principal components 1 and 2 resulted in three groups – CON; T-2; and T-1, T-3 and T-4.

Pearson correlation analyses of product attributes (instrumental and sensory) indicated existence of strong correlation between several attributes. Positive correlation

(P < 0.05) was observed between sensory cooked color and cooked a* (r = 0.82), overall texture and cooked instrumental resistance (r = 0.96), cooked springiness and cooked instrumental cohesiveness (r = 0.98), product uniformity and raw instrumental resistance (r = 0.99), sensory cohesiveness and saltiness (r = 0.88), bitter flavor and cooking yield (r = 0.96), and sensory cooked color and cooked b* (r = 0.81). On the other hand, several other parameters demonstrated negative correlation, including cooking yield and sensory raw color (r = – 0.95), instrumental cooked hardness and sensory hardness (r = – 0.95), and sensory cohesiveness and sensory hardness (r = – 0.82).

The compounds responsible for taste in meat products are, in general, non-volatile, water-soluble, and low molecular weight molecules (Lawless & Heymann, 1998). The treatments exhibiting increased cooking yield (T-2 and T-4) possibly might have retained hydrophilic molecules responsible for bitterness. Furthermore, the treatment demonstrating the greatest (P < 0.05) bitter flavor (T-2) contained the highest concentration of potassium chloride, which is known to impart bitterness (Desmond, 2006).

#### 3.1.3.7 Consumer sensory testing

##### 3.1.3.7.1 *Hedonic scale testing*

The data on consumer hedonic testing on cooked caiman steaks are presented in table 6. T-1 steak demonstrated lower (P < 0.05) overall acceptance than T-2 and lower (P < 0.05) texture than the low-sodium treatments. All other treatments were similar (P > 0.05) with respect to overall acceptance and texture. The results on T-1 reflected the data from instrumental texture (Table 4) and QDA (Table 5); low tenderness and high cohesiveness scores could have contributed to the low overall acceptance of T-1. In general, appearance, flavor, and odor attributes were not different (P > 0.05) for the treatments indicating that untrained panelists could not differentiate between the control, MTG-treated, and low-sodium restructured caiman steaks based on these attributes. Noticeably, no palatability attribute led to rejection of low-sodium

caiman steaks suggesting their positive consumer acceptance and economic potential. In agreement to our results, Horita et al. (2011) observed no differences in the appearance, odor, and texture between mortadellas prepared with blends of CaCl2, $MgCl_2$, and KCl as partial replacers for NaCl and the control mortadellas. On the other hand, in the same study they observed low flavor for the formulation containing 0.5% NaCl, 1% KCl, and 0.5% $MgCl_2$, a finding that we did not observe. One of the major bottlenecks of sodium reduction is consumer acceptance (Desmond, 2006). KCl and $MgCl_2$ usually have a bitter/metallic flavor and reduced saltiness, both of which lead to consumer rejection (Verma & Banerjee, 2012) of low-salt products. However, this was not observed in the present study in consumer sensory testing, possibly due to the presence of MTG in the low-sodium caiman steaks.

The purchase intention, expressed as the percentage of total consumers willing to purchase the product, was different for the treatments. The greatest purchase intention was for T-3 steaks (70.49%), whereas T-1 was the least preferred one for purchase (50.82%). The purchase intention for control was 60.66%, while that of T-2 and T-4 was 68.85% each. The restructured steaks with lowest purchase intention were the ones containing 1.5% NaCl (control and T-1). The increased purchase intention for T2, T-3, and T-4 indicated the market potential for low-salt restructured caiman steaks.

#### 3.1.3.7.2 *Just-About-Right (JAR) Profile*

The data from JAR profile of cooked restructured caiman steaks are presented in table 7. Saltiness was close to ideal for all the treatments in the nine-point scale. Furthermore, the treatments did not differ for saltiness ($P > 0.05$). This finding indicated that the consumers are unable to differentiate the low-sodium restructured steaks and regular salt ones based on saltiness. Firmness was close to ideal for control, T-2, T-3, and T-4, and suggested the similarity in texture for these products. On the other hand, T-1 demonstrated more firmness than ideal ($P < 0.05$) possibly due to the presence of both NaCl and MTG. The observed similarities between control and low-sodium caiman (T2, T-3, and T-4) restructured caiman steaks insinuated that replacement of NaCl with

KCl and $MgCl_2$ (in combination with MTG) can be employed as a suitable salt-reduction strategy in processed meats without compromising sensory quality.

3.1.3.7.3 *Partial Least Squares Regression (PLSR)*

PLSR technique examined the meat quality data from instrumental and sensory evaluations. The PLSR model (Figure 2) explained 99.8% of consumer acceptance and 75% of the trained panelist sensory scores and instrumental parameters showing an accumulated Q2 of 0.887. The QDA and instrumental attributes were considered relevant when their respective Variable Important to the Projection was greater than 0.8 (Wold, Sjostrom, & Eriksson, 2001). Those relevant parameters were retained as determinants for product acceptance (Figure 3). Succulence and cooking yield positively influenced the product acceptance, while cooked color and cohesiveness exerted a negative effect on restructured caiman steaks. Noticeably, bitter flavor did not contribute to rejection of T-2, T-3, and T-4, indicating that consumers are unable to distinguish NaCl substitution by KCl and/or $MgCl_2$ at 50% ratio.

3.1.3.7.4 *Penalty Analysis*

Penalty Analysis was used in sensory data analysis to identify potential directions for product improvement on the basis of consumer data (Table 8). Combining the data from JAR profile and consumer hedonic testing makes it possible to identify the parameters (for each treatment) that can be improved to increase consumer acceptance. Major detrimental attributes were the ones with more than 0.5 penalty score and 20% occurrence. Table 8 summarizes the attributes that decreased the acceptance for the restructured caiman steaks, and the major detrimental attribute on acceptance were different for the treatments. Firmness and saltiness did not penalize overall acceptance of control caiman steaks. However, based on too much saltiness T-1 and T-2 were penalized by 36% and 34% of panelists, respectively. Furthermore, the consumers concluded that T-1 and T-2 steaks were slightly more salty than ideal. On

the other hand, T-3 and T-4 were not penalized for saltiness, indicating the potential for these formulations. Apparently, MTG contributed to an enhanced salt perception in T-2 steaks, and this finding suggested that MTG can be exploited as a logical strategy to enhance salt perception and consumer acceptance of low-salt meats. Increased firmness was considered by 47%, 54%, and 49% of consumers as a source of penalty for T-2, T-3, and T-4, respectively. The outcomes of penalty analysis indicated that, in general, the consumers found the JAR parameters (saltiness and/or firmness) were greater than ideal for the MTG-treated restructured caiman steaks. This result indirectly suggested that lowering NaCl and MTG concentrations may improve the product acceptance.

### 3.1.4 Conclusions

The results suggest that the product formulations of T-3 and T-4 restructured caiman steaks can be employed as a suitable salt reduction strategy. While MTG improved the texture (instrumental and sensory), salt replacers (KCl and $MgCl_2$) improved cooking yield, succulence, and consumer acceptance. The meat industry could exploit the synergistic effect of restructuring, MTG, and salt replacers (KCl and $MgCl_2$) for development of low-sodium value-added products from caiman trimmings.

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Table 1: Formulations of restructured caiman steaks.

| | Ingredients (% w/w) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **NaCl** | **MTG [a]** | **KCl** | **$MgCl_2$** | **Sodium tripolyphosphate** | **Chilled water** | **Caiman meat** | **Onion powder** | **Garlic powder** |
| CON [b] | 1.5 | 0 | 0 | 0 | 0.4 | 1.0 | 95.1 | 1.0 | 1.0 |
| T-1 | 1.5 | 1.0 | 0 | 0 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-2 | 0.75 | 1.0 | 0.75 | 0 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-3 | 0.75 | 1.0 | 0 | 0.75 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-4 | 0.75 | 1.0 | 0.375 | 0.375 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |

[a] MTG = Microbial transglutaminase
[b] CON = Control

Table 2: Description and references of sensory attributes used in Quantitative Descriptive Analysis of restructured caiman steaks.

| Sample | Attribute | Definition | References |
|---|---|---|---|
| Raw | Color | Light gray | Light: Cooked root of *Colocasia esculenta*<br>Dark: Raw tilapia skin |
| Cooked | Color | Very light gray | Light: Cooked root of *Colocasia esculenta*<br>Dark: Raw shrimp |
| Cooked | Product uniformity | Pattern of product's particles clustering | Slight: Brazilian meat ball (Kibe)<br>A lot: Restructured chicken breast |
| Cooked | Spicy odor | Garlic and onion odor | Slight: Chicken meat ball<br>A lot: Brazilian rice spice mix (onion, garlic, and salt) |
| Cooked | Spicy flavor | Garlic and onion flavor | Slight: Brazilian meat ball (Kibe)<br>A lot: Brazilian rice spice mix (onion, garlic, and salt) |
| Cooked | Salt flavor | Taste characterized by sodium chloride | Slight: Brazilian meat ball (Kibe)<br>A lot: 3% of NaCl in water |
| Cooked | Bitter flavor | Astringent taste | Slight: Brazilian meat ball (Kibe)<br>A lot: 3% of Lite salt [a] in water |
| Cooked | Tenderness | Strength needed to shear at the first bite | Slight: Chicken sausage<br>A lot: Salami |
| Cooked | Succulence | Amount of juice expelled on chewing | Slight: Brazilian meat ball (Kibe)<br>A lot: Chicken sausage |
| Cooked | Cohesiveness | Tendency of meat particles to stick together | Slight: Brazilian meat ball (Kibe)<br>A lot: Salami |
| Cooked | Overall texture | Visual and textural similarity to restructured steak | Slight: Chicken patties<br>A lot: Restructured chicken breast |

[a] Lite salt = 50% blend of sodium chloride and potassium chloride.

Table 3: pH and proximate composition of raw restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **CON** | **T-1** | **T-2** | **T-3** | **T-4** | **SEM** |
| pH | 5.72[b] | 5.73[b] | 5.76[a] | 5.77[a] | 5.78[a] | 0.002 |
| Moisture (%) | 76.15[a] | 76.23[a] | 76.98[a] | 76.75[a] | 76.93[a] | 0.660 |
| Protein (%) | 18.80[a] | 17.96[b] | 17.23[b] | 17.38[b] | 17.80[b] | 0.128 |
| Fat (%) | 0.94[a] | 0.85[a] | 0.84[a] | 0.91[a] | 0.83[a] | 0.018 |
| Ash (%) | 2.84[a] | 3.05[a] | 3.09[a] | 2.73[a] | 2.94[a] | 0.069 |

[a–b] Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

Table 4: Cooking yield, instrumental color, and instrumental textural attributes of restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **CON** | **T-1** | **T-2** | **T-3** | **T-4** | **SEM** |
| Cooking yield | 71.39$^{d}$ | 75.47$^{c}$ | 87.22$^{a}$ | 78.70$^{b}$ | 84.64$^{a}$ | 0.749 |
| Raw attribute | | | | | | |
| *L** | 48.82$^{a}$ | 49.17$^{a}$ | 50.10$^{a}$ | 49.49$^{a}$ | 48.57$^{a}$ | 0.479 |
| *a** | 4.65$^{c}$ | 5.21$^{bc}$ | 5.68$^{ab}$ | 5.84$^{a}$ | 4.81$^{c}$ | 0.082 |
| *b** | 7.75$^{b}$ | 6.80$^{cd}$ | 7.48$^{bc}$ | 8.65$^{a}$ | 6.49$^{d}$ | 0.113 |
| Hardness | 30.44$^{b}$ | 52.22$^{a}$ | 65.25$^{a}$ | 35.67$^{b}$ | 55.51$^{a}$ | 2.605 |
| Springiness | 0.576$^{c}$ | 0.649$^{b}$ | 0.722$^{a}$ | 0.596$^{c}$ | 0.647$^{b}$ | 0.008 |
| Cohesiveness | 0.369$^{b}$ | 0.349$^{b}$ | 0.532$^{a}$ | 0.383$^{b}$ | 0.390$^{b}$ | 0.010 |
| Resistance | 0.064$^{c}$ | 0.136$^{b}$ | 0.183$^{a}$ | 0.117$^{b}$ | 0.133$^{b}$ | 0.004 |
| Cooked attribute | | | | | | |
| *L** | 63.69$^{a}$ | 62.53$^{a}$ | 62.30$^{a}$ | 60.58$^{a}$ | 61.83$^{a}$ | 0.648 |
| *a** | 3.73$^{a}$ | 3.70$^{a}$ | 3.49$^{a}$ | 4.20$^{a}$ | 4.06$^{a}$ | 0.113 |
| *b** | 7.93$^{a}$ | 7.80$^{a}$ | 7.73$^{a}$ | 8.39$^{a}$ | 8.50$^{a}$ | 0.243 |
| Hardness | 149.5$^{d}$ | 236.6$^{a}$ | 204.0$^{b}$ | 216.9$^{ab}$ | 173.8$^{c}$ | 4.029 |
| Springiness | 0.826$^{b}$ | 0.933$^{a}$ | 0.917$^{a}$ | 0.912$^{a}$ | 0.879$^{b}$ | 0.006 |
| Cohesiveness | 0.484$^{d}$ | 0.613$^{a}$ | 0.592$^{b}$ | 0.604$^{ab}$ | 0.571$^{c}$ | 0.002 |
| Resistance | 0.166$^{c}$ | 0.258$^{a}$ | 0.232$^{b}$ | 0.224$^{b}$ | 0.234$^{b}$ | 0.003 |

$^{a–d}$ Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

Table 5: Quantitative descriptive analysis scores of restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Attribute** | **CON** | **T-1** | **T-2** | **T-3** | **T-4** | **SEM** |
| Raw color | 4.14[a] | 4.08[a] | 3.92[a] | 4.01[a] | 3.99[a] | 0.102 |
| Cooked color | 2.17[bc] | 2.44[ab] | 2.04[c] | 2.50[ab] | 2.65[a] | 0.128 |
| Spicy odor | 4.45[a] | 4.20[a] | 4.05[ab] | 4.22[a] | 3.54[b] | 0.223 |
| Spicy flavor | 3.79[a] | 4.00[a] | 3.77[a] | 3.46[a] | 3.42[a] | 0.216 |
| Salty flavor | 2.94[b] | 3.72[a] | 3.35[ab] | 2.77[b] | 3.12[b] | 0.217 |
| Bitter flavor | 0.28[c] | 0.63[b] | 1.17[a] | 0.69[b] | 0.84[b] | 0.097 |
| Product uniformity | 6.58[a] | 6.74[a] | 6.85[a] | 6.71[a] | 6.71[a] | 0.138 |
| Tenderness | 5.58[a] | 3.73[d] | 4.80[bc] | 4.62[c] | 5.42[ab] | 0.235 |
| Succulence | 5.32[b] | 5.04[b] | 5.94[a] | 4.37[c] | 6.04[a] | 0.215 |
| Cohesiveness | 5.57[b] | 6.42[a] | 5.68[b] | 5.59[b] | 5.71[b] | 0.161 |
| Overall texture | 7.27[a] | 7.56[a] | 7.42[a] | 7.44[a] | 7.51[a] | 0.133 |

[a–d] Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

Table 6: Consumer sensory scores of restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Attribute** | **CON** | **T-1** | **T-2** | **T-3** | **T-4** | **SEM** |
| Overall acceptance | 6.54[ab] | 6.30[b] | 7.00[a] | 6.69[ab] | 6.71[ab] | 0.212 |
| Texture | 6.31[ab] | 5.82[b] | 6.64[a] | 6.51[a] | 6.61[a] | 0.234 |
| Flavor | 6.69[a] | 6.51[a] | 6.95[a] | 6.71[a] | 6.72[a] | 0.236 |
| Odor | 6.44[a] | 6.47[a] | 6.66[a] | 6.36[a] | 6.56[a] | 0.238 |
| Appearance | 5.98[a] | 5.98[a] | 6.36[a] | 5.03[b] | 6.23[a] | 0.254 |

[a–b] Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

Table 7: Just-About-Right profile scores of restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Attributes** | **CON** | **T-1** | **T-2** | **T-3** | **T-4** | **SEM** |
| Saltiness | 5.23[a] | 5.33[a] | 5.25[a] | 5.07[a] | 5.25[a] | 0.141 |
| Firmness | 5.82[b] | 6.38[a] | 5.64[b] | 5.64[b] | 5.54[b] | 0.171 |

[a–b] Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

Table 8: Penalty analysis of Just-About-Right (JAR) attributes of restructured caiman steaks.

| | Saltiness | | Firmness | |
|---|---|---|---|---|
| **Treatments** | **Not salty enough** | **Too Salty** | **Not firm enough** | **Too firm** |
| CON | – | – | – | – |
| T-1 | – | 36.07 [#] (– 0.53)* | – | – |
| T-2 | – | 34.43 (– 0.61) | – | 47.54 (– 0.61) |
| T-3 | – | – | – | 54.10 (– 0.97) |
| T-4 | – | – | – | 49.18 (– 0.71) |

[#] The percentage of consumers who found each treatment to be too salty or too firm for JAR saltiness and firmness.

* The number in parentheses is the change in mean compared to the consumer response score to overall acceptance.

– Indicates less than 20% of consumer selected the corresponding JAR category.

Figure 1: Instrumental and sensory data of restructured caiman steaks in the plane defined by two principal components.

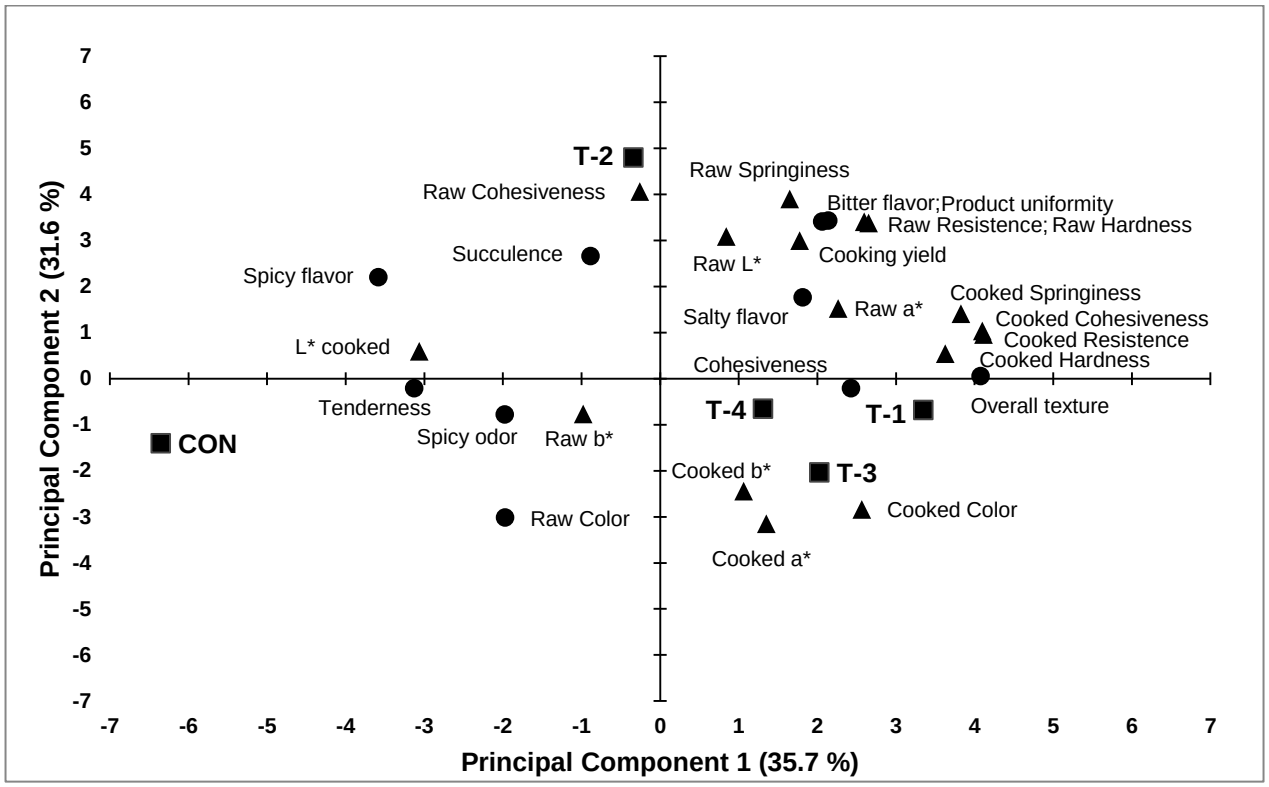


Figure 2: Partial Least Square Regression model for sensory and instrumental attributes of restructured caiman steaks.

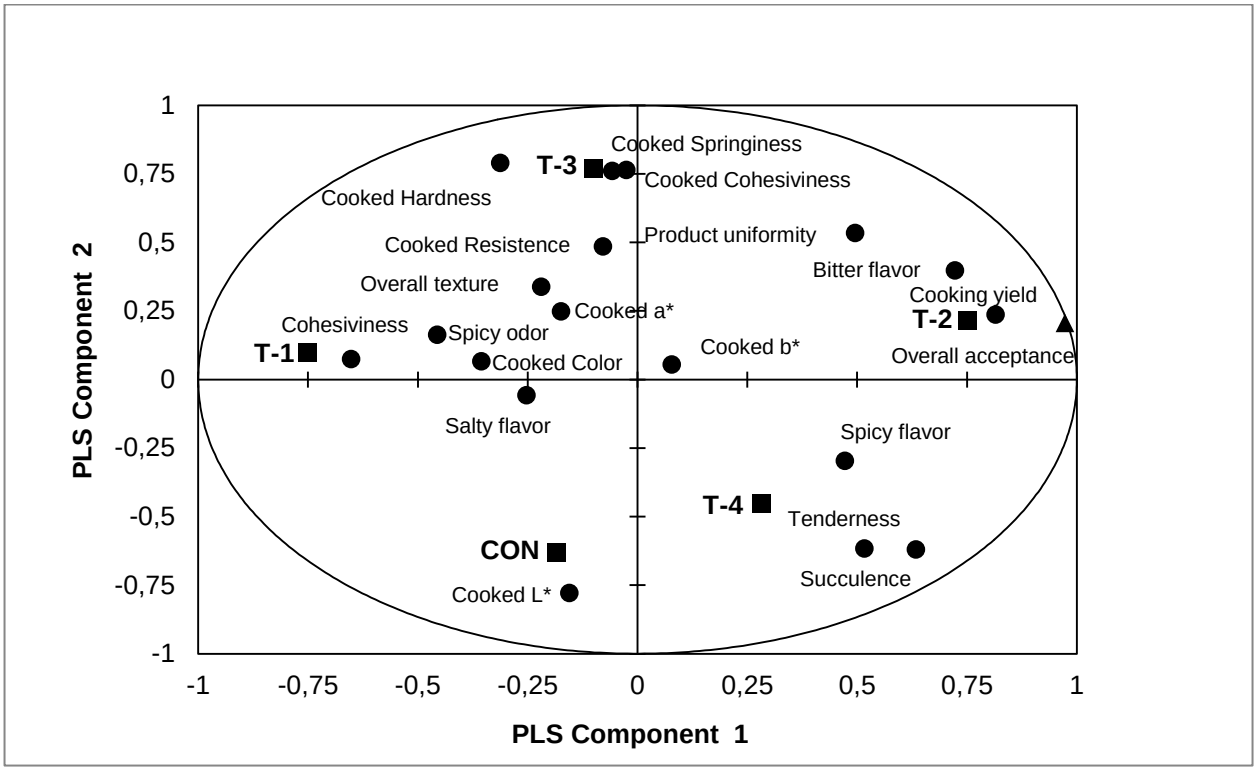
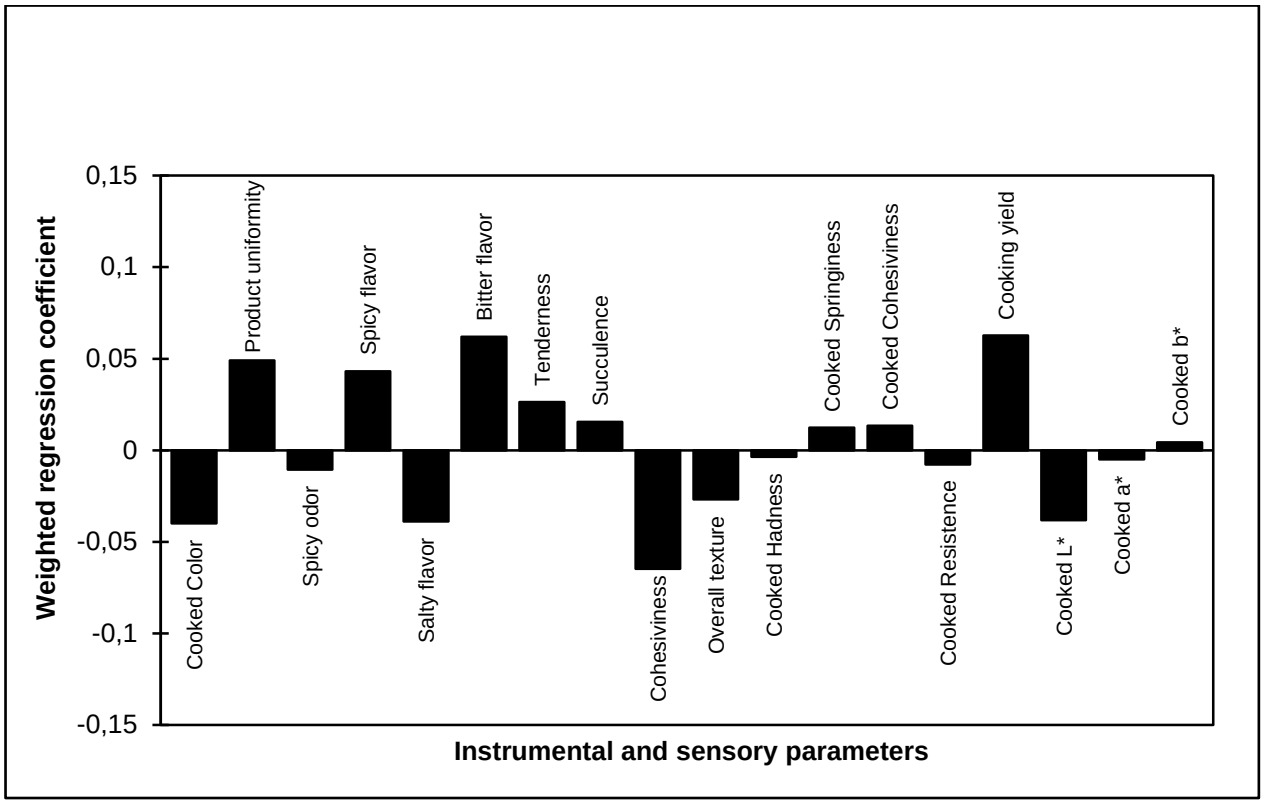


Figure 3: Weighted regression coefficients of instrumental and sensory parameters detrimental to consumer acceptance by partial least squares regression.



## 3.2 CHAPTER 2: BACTERIOLOGICAL QUALITY AND FATTY ACID PROFILE OF CAIMAN MEAT SUBJECTED TO HIGH HYDROSTATIC PRESSURE

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## ABSTRACT

Our objective was to examine the effects of high hydrostatic pressure (HHP) on physico-chemical properties and bacteriological quality of caiman (*Caiman crocodilus yacare*) meat. Caiman tail meat was cut into 25 g chunks, individually vacuum packaged, and allocated to four treatments: non-treated (NT; not subjected to HHP), and samples subjected to HHP at 200 MPa (P2), 300 MPa (P3), and 400 MPa (P4). Physico-chemical properties, fatty acid profile, and bacteriological quality were evaluated. All HHP treatments (P2, P3, and P4) demonstrated lower ($P < 0.05$) bacteriological loads than NT. HHP decreased ($P < 0.05$) n–3 polyunsaturated fatty acid content. In addition, HHP increased ($P < 0.05$) n–6/n–3 ratio as well as atherogenic index, which are critical indices for risk of cardiac diseases. The results suggest that while HHP improves bacteriological safety, it compromises the lipid profile of caiman meat, which is rich in polyunsaturated fatty acids.


**Keywords:** High hydrostatic pressure; *Caiman crocodilus yacare*; Fatty acid profile; Bacteriological quality

### 3.2.1 Introduction

The popularity of exotic meats is increasing due to their lower lipid content and greater concentration of polyunsaturated fatty acids than the meats from conventional livestock (Hoffman, 2008; Vicente-Neto et al., 2010; Hoffman & Cawthorn, 2013). An emerging exotic meat in Brazil is caiman (*Caiman crocodilus yacare*) meat, which is a valuable source of animal proteins of high biological value (Romanelli, Caseri, & Lopes Filho, 2002). Additionally, caiman meat is low in saturated fatty acids (SFA), high in polyunsaturated fatty acids (PUFA) and n–3 fatty acids, and has a desirable PUFA/SFA ratio (Vicente-Neto et al., 2010). Caiman meat has been well accepted in sensory studies and has potential for further processing (Romanelli et al., 2002; Paulino et al., 2012; Canto et al., 2012).

The growing consumer demand for high quality meat products has stimulated the industry to develop novel preservation technologies (Aubourg, Tabilo-Munizaga, Reyes, Rodriguez, & Perez-Won, 2010). High hydrostatic pressure (HHP) is a non-thermal preservation technology, wherein foods are subjected to pressure at or above 100 MPa using liquids as pressure transmitter (Clariana, Guerrero, Sarraga, & Garcia-Regueiro, 2012). The HHP treatment improves food safety by inactivating microorganisms and extending the shelf life (Vaudagna, Gonzalez, Guignon, Aparicio, Otero, & Sanz, 2012). An additional advantage of HHP is that it can be applied to pre-packaged foods, and thus can decrease the risk of recontamination (Toepfl, Mathys, Heinz, & Knorr, 2006).
Depending on the level of pressure and duration of exposure, HHP can cause physico-chemical changes in food matrix, such as proteolysis, lipolysis, and alteration of fatty acid composition (Canto et al., 2012; He et al., 2012; Clariana et al., 2012; McArdle, Marcos, Kerry, & Mullen, 2011; Wang et al., 2013). These HHP-induced physico-chemical changes can influence nutritive value (Yagiz, Kristinsson, Balaban, Welt, Ralat, & Marshall, 2009; McArdle, Marcos, Kerry, & Mullen, 2010) and sensory attributes (Kruk, Yun, Rutley, Lee, Kim, & Jo, 2011) of muscle foods. Previous studies reported that HHP improved microbial quality and induced changes in the physico-chemical properties of fresh beef (McArdle et al., 2010; 2011) and chicken (Bolumar, Andersen, & Orlien,

2011). However, the impacts of HHP on bacteriological quality and physico-chemical properties of PUFA-rich fresh caiman meat have not been examined. Therefore, the aim of the present study was to evaluate the effects of HHP on physico-chemical properties and bacteriological quality of caiman meat.

## 3.2.2 **Materials and methods**

### 3.2.2.1 Caiman meat

Using the tail meat from six caiman (*Caiman crocodilus yacare*) carcasses (30-month old, farm-raised) in each trial, the experiment was repeated four times to provide four replicates (n = 4). For each trial, the tail cuts of caiman carcasses were procured from a federally-inspected slaughterhouse at Caceres, Mato Grosso, Brazil. The cuts were deboned, and tail meat (500 g) from each carcass was individually vacuum-packaged and frozen. The frozen samples were transported in dry-ice to the meat laboratory of the Universidade Federal Fluminense, where they were thawed at 4°C overnight. Thawed meat was cut into 25 g chunks. The chunks were individually vacuum packaged, and the packages were randomly allocated to four pressure treatments. The vacuum packaged samples were maintained at 4°C (approximately for 2 hours) until high pressure processing.

### 3.2.2.2 High hydrostatic pressure treatment

High pressure processing was accomplished at the Embrapa Centro Nacional de Pesquisa de Tecnologia Agroindustrial de Alimentos (Rio de Janeiro, Brazil). There were four treatments: non-treated control (NT; vacuum-packaged but not subjected to HHP), and samples subjected to 200 MPa (P2), 300 MPa (P3), and 400 MPa (P4) HHP for 10 minutes at 20°C. Previous research (Kruk et al., 2011) reported that HHP above 400 MPa compromises quality of meat, and therefore levels above 400 MPa were not employed in this study. The equipment used for HHP treatment was a Stansted Fluid

Power pressurizer (model S-FL-850-9-W, Essex, United Kingdom). The meat packages were inserted into the stainless steel perforated cylinder (7 x 20 cm) of the equipment. The perforated cylinder containing the meat packages was placed inside the equipment chamber. A solution of 70% ethanol in water was used as the pressure-transmitting liquid. The chamber was hermetically closed, and two pneumatic pumps were sequentially activated to elevate the pressure. Vacuum-packaged meat was subjected to HHP for 10 minutes at 20°C. At the end of the cycle, the chamber was depressurized and opened, and the samples were removed from the perforated cylinder. Meat pH, proximate composition, and fatty acid profile were evaluated on samples immediately after HHP treatment, whereas microbial quality was examined at specific time points during refrigerated storage of HHP-treated vacuum packaged meat.

#### 3.2.2.3 Meat pH and proximate composition

The pH values were measured using a digital pH meter (Digimed, Sao Paulo, Brazil) after homogenizing 10 g of sample in 90 mL of distilled water (Conte-Junior, Fernandez, & Mano, 2008). The moisture, protein, ash, and lipid contents were determined according to AOAC (2005). Moisture was determined by drying the sample at 100–105°C until constant weight, protein content was estimated by Kjeldahl technique, ash content was determined after incineration at 550 °C in muffle furnace, and lipid content was obtained by petroleum ether extraction using Soxhlet apparatus (AOAC, 2005).

#### 3.2.2.4 Fatty acid profile

The lipids in caiman tail meat were cold-extracted and hydrolyzed as described by Bligh and Dyer (1959) with modifications suggested by Conte-Junior, Soncin, Hierro, and Fernandez (2007), and fatty acid methyl esters (FAME) were analyzed by gas chromatography. Briefly, the meat was chopped, and 5 g of samples was used for fatty acids extraction. Fatty acids were extracted using a methanol: chloroform (2:1 v/v) mixture. The extracted fatty acids were methylated under acidic conditions by adding

10% HCl in methanol (Kishino, Ogawa, Ando, Omura, & Shimizu, 2002; Chin, Liu, Storkson, Ha, & Pariza, 1992). Clarification was accomplished by adding 6 mL hexane to the mixture. FAME (1 µl) were analyzed using a gas chromatograph equipped with flame ionization detector (Perkin Elmer, Waltham, MA, USA). The separation was done on Carbowax/BTR column (30 m length, 0.32 mm internal diameter, and 0.25 µm particle size), while fatty acids determination were accomplished using the TCNav software (Perkin Elmer, Waltham, MA, USA). The injection and detector port temperatures were set at 260°C and 280°C, respectively. The initial temperature of the oven was set at 40°C, and the temperature ramp was programmed as: increase from 40°C to 240°C at 40°C/min, increase from 240°C to 260°C at 2°C/min, and hold at 260°C for 5 min. Helium was used as the carrier gas at 1.8 mL/min. FAME were identified by comparing the retention time of commercial standard of 15 individual fatty acid methyl esters (Supelco Inc., Bellefonte, PA, USA). Individual fatty acids were expressed as percentage of total identified FAME, and were then categorized as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids.

#### 3.2.2.5 Bacteriological analysis

Vacuum packaged samples (NT and HHP-treated) were stored at 4°C for three months, and the bacteriological quality was evaluated on 0, 15, 30, 45, 60, 75, and 90 days. Packages were opened under aseptic conditions, and the contents (25 g) were transferred to sterile plastic bags containing 225 mL sterile peptone saline (1 g of peptone and 8.5 g of NaCl per liter) and homogenized for 2 minutes in a stomacher (Stomacher 80, Seward Ltd., London, United Kingdom). The samples were serially diluted at a ratio of 1:9, and 1 mL aliquots were inoculated in duplicate by pour plate method into Petri dishes containing culture media. All culture media used were obtained from HiMedia (Mumbai, India). Total aerobic mesophilic (TAMB), total aerobic psychrotrophic (TAPB), and lactic acid (LAB) bacterial counts were evaluated. TAMB and TAPB viable counts were enumerated by pour plating on plate count agar (PCA) followed by incubation at 35°C for 48 h and 7°C for ten days, respectively (Erkan &

Uretener, 2010). LAB counts were analyzed by pour plating with over layer plates on de Man, Rogosa, and Sharpe (MRS) agar. The plates were incubated at 30°C for 120 h under anaerobic conditions. Immediately after counting, five colonies developed on MRS media were isolated and streaked on test tubes containing PCA agar for confirmation of LAB. The identity of isolated LAB was further confirmed through microscopic morphology, Gram staining, and catalase production (Hall, Ledenbach, & Flowers, 2001). After incubation, the colonies were counted and the results were expressed in Log (cfu/g). The presence of Salmonella spp. was evaluated on day 0 of storage according to the rapid method (Pignato, Marino, Emanuele, Iannotta, Caracappa, & Giammanco 1995), and the results were expressed as presence or absence in 25 g of samples.

#### 3.2.2.6 Statistical analysis

The experiment was a completely randomized block design with four replicates (n = 4). Data were analyzed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). One-way ANOVA was performed to compare the effects of HHP (NT, P2, P3, and P4) on physico-chemical parameters and fatty acid profile. The experiment for bacteriological quality was a 4 x 7 factorial with four treatments (NT, P2, P3, and P4) and seven storage time points (0, 15, 30, 45, 60, 75, and 90 days). The data on bacteriological growth were evaluated by two-way ANOVA. Tukey multiple range test was used to determine differences among means at 5% level of significance (GraphPad Software, Inc., La Jolla, CA, USA).

### 3.2.3 **Results and discussion**

#### 3.2.3.1 Meat pH and proximate composition

The results of pH and proximate composition of caiman tail meat are presented in table 1. HHP-treated caiman meat demonstrated greater ($P < 0.05$) pH than NT

samples. Among the HHP-treated samples, P4 demonstrated the greatest pH ($P < 0.05$). Our results on pH are in agreement with those of previous researchers, who observed that pressure levels greater than 200 MPa increased the pH of minced pork (Cheah & Ledward, 1996) and fresh beef (McArdle et al., 2010, 2011). The observed increase in pH of HHP-treated caiman meat can be attributed to the decrease in available acidic groups in muscle food matrix as a result of conformational changes in proteins (Ma & Ledward, 2004). associated with denaturation (Poulter, Ledward, Godber, Hall, & Rowlands, 1985). Redistribution of ions as well as increased ionization in the food matrices on exposure to elevated pressure also can contribute to an increase in meat pH (Macfarlane, McKenzie, & Turner, 1980).

HHP influenced ($P < 0.05$) the moisture content; P2 samples exhibited greater ($P < 0.05$) moisture content than their NT counterparts, whereas NT was not different ($P > 0.05$) from P3 and P4. While HHP did not influence ($P > 0.05$) protein and ash contents, it affected ($P < 0.05$) the fat content. HHP-treated caiman meat exhibited lower ($P < 0.05$) lipid content than NT. Kruk et al. (2011) also observed an increase in the moisture content of chicken breast fillets subjected to HHP above 300 MPa. The increase in moisture content could be attributed to the changes in protein structure induced by high pressure (Ma & Ledward, 2004). HHP-induced denaturation disrupts intramolecular bonds in and destabilizes the three-dimensional structure of muscle proteins (Silva, Foguel, & Royer, 2001). These changes, in turn, could alter the interactions between protein and water molecules; water molecules strongly bound to proteins are shifted to weaker binding sites within the polypeptide chain, leading to increased extractability (Silva et al., 2001). Carballo, Fernandez, Carrascosa, Solas, and Jimenez-Colmenero (1997) explained that high-pressure treatment of beef patties leads to rupture of adipocytes, which facilitates interactions between lipid and protein molecules. HHP-induced conformational and physico-chemical changes in proteins (Aubourg et al., 2010) increase hydrophobicity and binding affinity to lipids (Liu, Powers, Swanson, Hill, & Clark, 2005). Together, these cellular and biochemical changes can effectively hinder extraction of lipids using a non-polar solvent, which basic only extracts neutral lipids (Sahasrabudhe & Smallbone, 1983), resulting in low values up on quantification. In partial agreement, Briones-Labarca, Perez-Won, Zamarca, Aguilera-Radic, and Tabilo-

Munizaga (2012) observed lower lipid and greater moisture contents in red abalone treated with 550 MPa HHP compared to the controls.

#### 3.2.3.2 Fatty acid profile

The fatty acid profiles of NT and HHP-treated caiman tail meat are presented in Table 2, and HHP influenced ($P < 0.05$) the fatty acid profile. The main SFAs identified were C14:0, C16:0, and C18:0. In general, HHP decreased ($P < 0.05$) C18:0 content, whereas it increased ($P < 0.05$) the C14:0 and C16:0 levels. No difference ($P > 0.05$) was observed on total SFA contents among the three HHP treatments. Overall, the total SFA content was greater ($P < 0.05$) in HHP treatments than in NT. Among the MUFAs, while C16:1n9, C18:1n7, C18:1n9, and C20:1n9, were present in both NT and HHP-treated caiman meat, C22:1n11 and C24:1n9 values were under detection in HHP-treated samples. NT samples exhibited greater ($P < 0.05$) level of C20:1n9 than HHP-treated caiman meat. The total content of MUFA from NT and HHP treated samples did not exhibited differences ($P > 0.05$).

The individual n–3 PUFAs identified were C18:4n3, C20:5n3, C22:5n3, and C22:6n3. All HHP-treated samples exhibited lower ($P < 0.05$) C18:4n3, C20:5n3, C22:5n3, and C22:6n3 contents than NT. Overall, total n–3 contents decreased ($P < 0.05$) with HHP treatment. Diets rich in n–3 PUFAs can reduce the incidence of cardiovascular diseases (Ulbricht & Southgate, 1991). The decrease in n–3 content indicated that HHP treatment can decrease the nutritive value of caiman meat. The observed decrease in n–3 fatty acid contents in HHP-treated caiman meat can be attributed to the structural and chemical changes induced in cells on exposure to high pressure. Application of HHP on muscle foods disrupts cell membranes and facilitates the interactions between unsaturated membrane lipids and cellular enzymes (Bolumar et al., 2011). This phenomenon makes the muscle lipid components susceptible to attack at their double bonds resulting in saturation and concomitant alteration in fatty acid composition (McArdle et al., 2010; Wang et al., 2013). Furthermore, Frankel (2005)

reported that n–3 fatty acids are highly susceptible to the modifications at the double bonds such as oxidation.

The main n–6 PUFAs detected were C18:2n6, and C20:4n6. HHP did not influence ($P > 0.05$) the levels of individual n–6 PUFAs in NT, P2, and P3. However, P4 exhibited lower values ($P < 0.05$) of 18:2n6 and greater values ($P < 0.05$) of C20:4n6 than in NT, P2, and P3. Furthermore, the concentration of total n–6 fatty acids was not affected ($P > 0.05$) by HHP, whereas was detected a decrease ($P < 0.05$) on P4 total PUFAs content when compared with NT samples. McArdle et al. (2011) reported that n–6 PUFAs are structural lipids resistant to physico-chemical changes induced by processing, and this could have contributed to the observed lack of changes in n–6 PUFA content in caiman meat when subjected to HHP.

The PUFA/SFA ratio was influenced ($P < 0.05$) by HHP, pressure treatment promotes the decrease on PUFA/SFA ratio, probably due to an increase ($P < 0.05$) on total content of SFA in HHP treated samples. The consumption of saturated fatty acids has been associated with the incidence of coronary heart diseases (Garcia-Segovia, Andres-Bello, & Martinez-Monzo, 2007). The relative proportions of SFA, PUFA, and MUFA are critical to the nutritive value of muscle foods (Wood, Richardson, Nute, Fisher, Campo, & Kasapidou, 2003; Wood, Enser, Fisher, Nute, Sheard, & Richardson, 2008). The PUFA/SFA ratio is used to estimate the nutritional quality of food lipids, and health guidelines have recommended this ratio should be above 0.4 (Wood et al., 2008). All the treatments in the present study demonstrated a PUFA/SFA ratio more than 0.4.

Another important index of cardiovascular health is the n–6/n–3 ratio, and nutritional guidelines recommends this ratio be less than 4.0 (Simopoulos, 2009). HHP increased ($P < 0.05$) the n–6/n–3 ratio in caiman meat. The n–6/n–3 ratio of NT was 2.15, whereas the value for caiman meat subjected to 200 MPa was above 4.0 reflecting an observed decrease n–3 fatty acids on exposure to HHP. No difference was detected ($P > 0.05$) among HHP-treated samples.

Atherogenic index, an indicator for cardiovascular disease risk, generally was greater ($P < 0.05$) in HHP-treated samples than in NT. Atherogenic index was proposed as an indicator for the nutritive value of lipids based on the effects of fatty acid composition on serum cholesterol (Ulbricht & Southgate, 1991). Greater values for

atherogenic index indicate an increased risk for cardiovascular diseases. The unsaturated fatty acids, regardless of the number and position of double bond or configuration, are effective in decreasing atherogenic index and thus improving heart health. Anti-atherogenic lipids such as PUFAs are known to improve cardiovascular health via reducing the formation of atherosclerotic plaques and decreasing the level of esterified fatty acids, cholesterol, and phospholipids (Dawczynski, Martin, Wagner, & Jahreis, 2010; Ramsden, Hibbeln, Majchrzak, & Davis, 2010). Our findings that HHP increased atherogenic index in caiman mean indicated that HHP-treatment can compromise the lipid composition of muscle foods rich in PUFAs.

In agreement with our results, Wang et al. (2013) studied the influence of HHP on yak (*Poephagus grunniens*) body fat and detected an increase on the total of SFA and decrease on PUFA when compared to samples treated with control samples. Morover, these authors demonstrated a decrease on PUFA/SFA ratio and increase on n–6/n–3 ratio. In addition, Yagiz et al. (2009) observed that while 150 MPa HHP did not change the n–6/n–3 ratio of Atlantic salmon, 300 MPa pressure increased the ratio in comparison with the controls. On the other hand, McArdle et al. (2010) observed no differences in the fatty acid compositions of control and fresh beef samples treated with HHP at 200, 300 and 400 MPa. Kang et al. (2013) observed no changes in the fatty acid profile of Korean native black goat meat on exposure to 100 MPa HHP. The variation on different studies results can be attributed to the differences in meat species, which is a major factor influencing fatty acid profile, (Wood et al., 2008), as well as the pressure/temperature/time used. Depending on the pressure level and duration of HHP processing, the meat becomes more susceptible to alteration on the fatty acid composition (Wang et al., 2013). Moreover, differences in individual fatty acid content among HHP treatments can be explained by phospholipids lipolysis, which increases free fatty acids content and can modify the final fatty acid profile (He et al., 2012).

#### 3.2.3.3 Bacteriological quality

The TAMB, TAPB, and LAB counts in HHP-treated and control caiman meat samples during 90 days storage at 4°C are presented in tables 3, 4, and 5, respectively.

An important criterion for quality and safety of fresh meat is the initial total bacterial count, and the acceptable limit for total bacterial count in fresh meat is 7 Log cfu/g (ICMSF, 1986). Irrespective of the treatment, all total bacterial counts increased ($P < 0.05$) during the storage (Tables 3, 4, and 5). While both TAMB and TAPB counts in NT reached the unacceptable limits by day 60, LAB count reached the threshold at day 75. In contrast, no HHP-treated samples reached 7 Log cfu/g limit for any bacterial counts during the storage.

HHP decreased ($P < 0.05$) the TAMB count in caiman meat in a dose-dependent manner. During refrigerated storage NT consistently demonstrated the highest ($P < 0.05$) TAMB loads at all the time points, except day 0 (Table 3). While P3 and P4 had lower ($P < 0.05$) TAMB counts than control samples, the greatest reduction in TAMB was observed in P4. On day 0, TAMB were not detected in P4, while the counts were lower ($P < 0.05$) in P3 than the two other treatments. On day 15, HHP-treated samples had lower TAMB counts ($P < 0.05$) than NT, while P4 had the lowest ($P < 0.05$) values on day 75 and 90.

In general, TAPB counts in caiman meat were decreased by HHP ($P < 0.05$) in a dose-dependent manner (Table 4). On day 0, TAPB were not detected in the HHP-treated samples. Furthermore, P3 and P4 also resulted in ND counts of TAPB on day 15. Until day 60, all HHP-treated samples demonstrated lower ($P < 0.05$) TAPB counts than the controls. However, on days 75 and 90 this trend was observable only in P4.

LAB counts were not detected on day 0 in any treatments, whereas they were present in all treatments through the rest of the storage (Table 5). HHP treatments consistently demonstrated lower ($P < 0.05$) counts than NT (Table 5). After 60 days storage, a dose-dependent reduction in LAB was observed in HHP treatments, with P4 exhibiting the lowest ($P < 0.05$) LAB counts. Moreover, Salmonella spp. was present only in NT samples indicating that HHP treatment successfully controlled this Gram-negative pathogen.

In agreement with our findings, several authors have previously reported the effectiveness of HHP in minimizing bacterial loads in fresh as well as processed muscle foods. McArdle et al. (2011) observed a decrease in TAMB and LAB counts in fresh beef subjected to 400 and 600 MPa HHP. Vacuum-packaged marinated beef loins subjected

to 600 MPa HHP exhibited a reduction in TAMB, TAPB, and LAB counts (Garriga, Grebola, Aymericha, Monforta, & Hugas, 2004). Furthermore, reductions in TAMB, TAPB and LAB were reported in ready-to-eat cured beef carpaccio treated with 400 MPa HHP (Vaudagna et al., 2012). Application of 200 MPa HHP increased the shelf life of sea bass and decreased the TAMB (Cheret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & Lamballerie, 2005). Furthermore, Yagiz, Kristinsson, Balaban, and Marshall (2007) observed that HHP above 450 MPa was sufficient to successfully prevent microbial proliferation in rainbow trout as well as mahi mahi. Additionally, exposing Atlantic salmon to 150 MPa HHP resulted in a reduction in TAMB counts up to 6 days of refrigerated storage, whereas 300 MPa HHP led to reduction in TAMB counts even after 6 days of storage (Yagiz et al., 2009).

The mechanisms through which HHP damages microorganisms involve physico-chemical changes in cellular structure and components, including denaturation or inactivation of proteins and enzymes, damaging cell membrane accompanied by the separation of membrane from the cell wall, release of intracellular constituents, compression of vacuoles, and condensation of nuclear materials (Carlez, Veciana-Nogues, & Cheftel, 1995; Smelt, 1998; Manas & Mackey, 2004; Patterson, 2005). It is well-documented (Hugas, Garriga, & Monfort, 2002; Garriga et al., 2004) that the effectiveness of HHP in controlling microbes depends on processing parameters (pressure level, temperature, and exposure time), chemistry of food (pH and composition), and bacterial biology (strain and growth stage). In general, Gram-negative bacteria, such as Salmonella, are more sensitive to HHP than Gram-positive ones (Carlez, Rosec, Richard & Cheftel, 1994; Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991), possibly due to the presence of double-layered phospholipids in the lipid membranes of Gram-negative bacteria. On exposure to HHP, the phospholipids undergo tight packing during the compression phase (promoting the transition towards a gel state), and during the decompression phase the double-layered arrangement is disrupted resulting in the formation of pores and leakage of cytoplasmic materials (Williams, 1994; Shimada, Andou, Naito, Yamada, Osumi, & Hayashi, 1993). In agreement with our results, Garriga et al. (2004) reported that Salmonella was not detected in beef subjected to 600 MPa HHP, while the presence of this pathogen was

observed in control samples. Furthermore, Marcos, Aymerich, and Garriga (2005) argued that exposing raw pork sausages to 300 MPa HHP was an additional procedure to control Salmonella.

### 3.2.4 Conclusions

The application of HHP improved the bacteriological quality of caiman tail meat, with samples exposed to 400 MPa demonstrating the greatest reduction in bacterial load. In addition, HHP eliminated Salmonella in caiman meat. Thus HHP represents a practical alternative to conventional processing methods to improve safety and extend the shelf-life of caiman meat. However, HHP increased n–6/n–3 ratio and atherogenic index, two major indices for the risk of cardiovascular diseases, in caiman meat indicating that this non-thermal processing strategy can compromise the lipid profile of muscle foods rich in PUFAs.

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Table 1: The pH and proximate composition on caiman tail meat subjected to high hydrostatic pressure (HHP)

| | Treatments * | | | |
|---|---|---|---|---|
| **Parameter** | **NT** | **P2** | **P3** | **P4** |
| pH | 5.83$^{c}$ | 5.99$^{b}$ | 6.10$^{b}$ | 6.23$^{a}$ |
| Moisture (%) | 73.13$^{b}$ | 75.85$^{a}$ | 75.13$^{ab}$ | 75.29$^{ab}$ |
| Protein (%) | 21.58$^{a}$ | 21.55$^{a}$ | 21.81$^{a}$ | 21.76$^{a}$ |
| Ash (%) | 0.75$^{a}$ | 0.75$^{a}$ | 0.73$^{a}$ | 0.74$^{a}$ |
| Lipid (%) | 3.54$^{a}$ | 1.13$^{b}$ | 1.56$^{b}$ | 1.58$^{b}$ |

$^{a–c}$ Means in a row without common superscripts are different ($P < 0.05$).
* NT = Non-treated; P2 = 200 MPa HHP; P3 = 300 MPa HHP; P4 = 400 MPa HHP.

Table 2. Fatty acid composition (% of total fatty acids) of all caiman tail meat treatments

| Variable | Treatments* | | | |
|---|---|---|---|---|
| | **NT** | **P2** | **P3** | **P4** |
| **Individual fatty acids** | | | | |
| C14:0 | 0.25$^{c}$ | 0.57$^{ab}$ | 0.40$^{bc}$ | 0.75$^{a}$ |
| C16:0 | 23.00$^{b}$ | 30.36$^{a}$ | 28.42$^{a}$ | 29.85$^{a}$ |
| C18:0 | 4.42$^{a}$ | 2.48$^{b}$ | 2.26$^{b}$ | 3.04$^{b}$ |
| C16:1 | 2.70$^{a}$ | 2.34$^{a}$ | 2.47$^{a}$ | 2.75$^{a}$ |
| C18:1n7 | 9.71$^{a}$ | 9.20$^{a}$ | 9.08$^{a}$ | 8.23$^{a}$ |
| C18:1n9 | 26.32$^{a}$ | 27.60$^{a}$ | 30.26$^{a}$ | 29.53$^{a}$ |
| C18:2n6 | 10.91$^{a}$ | 11.40$^{a}$ | 11.79$^{a}$ | 8.14$^{b}$ |
| C18:4n3 | 1.41$^{a}$ | 0.56$^{b}$ | 0.16$^{b}$ | 0.13$^{b}$ |
| C20:1n9 | 2.41$^{a}$ | 0.41$^{b}$ | 0.46$^{b}$ | 0.13$^{b}$ |
| C20:4n6 | 9.38$^{b}$ | 9.83$^{b}$ | 8.40$^{b}$ | 10.98$^{a}$ |
| C20:5n3 | 1.22$^{a}$ | 0.29$^{b}$ | 0.28$^{b}$ | 0.29$^{b}$ |
| C22:1n11 | 0.58 | ND | ND | ND |
| C22:5n3 | 5.53$^{a}$ | 3.85$^{b}$ | 4.17$^{b}$ | 4.20$^{b}$ |
| C22:6n3 | 1.25$^{a}$ | 0.43$^{b}$ | 0.55$^{b}$ | 0.40$^{b}$ |
| C24:1n9 | 2.12 | ND | ND | ND |
| **Sums of fatty acids** | | | | |
| SFA | 27.67$^{b}$ | 33.41$^{a}$ | 31.08$^{ab}$ | 33.64$^{a}$ |
| MUFA | 43.84$^{a}$ | 39.55$^{a}$ | 42.27$^{a}$ | 40.64$^{a}$ |
| PUFA | 29.70$^{a}$ | 26.36$^{ab}$ | 25.35$^{ab}$ | 24.14$^{b}$ |
| n – 3 | 9.41$^{a}$ | 5.13$^{b}$ | 5.16$^{b}$ | 5.05$^{b}$ |
| n – 6 | 20.29$^{a}$ | 21.23$^{a}$ | 20.19$^{a}$ | 19.12$^{a}$ |
| **Ratios** | | | | |
| PUFA/SFA | 1.07$^{a}$ | 0.79$^{b}$ | 0.82$^{b}$ | 0.72$^{b}$ |
| n – 6/ n – 3 | 2.15$^{b}$ | 4.13$^{a}$ | 3.91$^{ab}$ | 3.81$^{ab}$ |
| Atherogenic index | 0.41$^{b}$ | 0.55$^{a}$ | 0.53$^{ab}$ | 0.57$^{a}$ |

$^{a, b, c}$ Different letters within lines indicate significant differences among values ($p<0.05$).
* NT = Non-treated; P2 = 200 MPa HHP; P3 = 300 MPa HHP; P4 = 400 MPa HHP.

Table 3: Total aerobic mesophilic bacteria count (Log cfu/g) in caiman tail meat subjected to high hydrostatic pressure (HHP) during refrigerated storage under vacuum packaging

| | Treatments * | | | |
|---|---|---|---|---|
| **Days** | **NT** | **P2** | **P3** | **P4** |
| 0 | $3.3^{xe}$ | $2.8^{xb}$ | $1.6^{yc}$ | ND |
| 15 | $4.3^{xd}$ | $3.3^{yb}$ | $2.5^{yzc}$ | $1.6^{zc}$ |
| 30 | $5.3^{xc}$ | $3.6^{yb}$ | $2.2^{zc}$ | $2.0^{zc}$ |
| 45 | $6.5^{xb}$ | $4.0^{yb}$ | $3.4^{yb}$ | $3.5^{ya}$ |
| 60 | $7.6^{xa}$ | $3.2^{yb}$ | $3.1^{yb}$ | $2.7^{yb}$ |
| 75 | $7.3^{xa}$ | $5.3^{yza}$ | $5.6^{ya}$ | $4.0^{za}$ |
| 90 | $7.4^{xa}$ | $5.4^{yza}$ | $5.7^{ya}$ | $4.1^{za}$ |

$^{x–z}$ Means in a row without common superscripts are different ($P < 0.05$).
$^{a–e}$ Means in a column without common superscripts are different ($P < 0.05$).
ND = Not detected
* NT = Non-treated; P2 = 200 MPa HHP; P3 = 300 MPa HHP; P4 = 400 MPa HHP.

Table 4: Total aerobic psychrotrophic bacteria counts (Log cfu/g) in caiman tail meat subjected to high hydrostatic pressure (HHP) during refrigerated storage under vacuum packaging

| | Treatments * | | | |
|---|---|---|---|---|
| **Days** | **NT** | **P2** | **P3** | **P4** |
| 0 | $2.4^{d}$ | ND | ND | ND |
| 15 | $3.9^{xc}$ | $1.6^{yc}$ | ND | ND |
| 30 | $6.2^{xb}$ | $3.8^{yb}$ | $2.6^{yb}$ | $2.2^{yb}$ |
| 45 | $6.8^{xab}$ | $3.5^{yb}$ | $3.3^{yb}$ | $3.0^{yab}$ |
| 60 | $7.4^{xa}$ | $5.7^{ya}$ | $3.2^{zb}$ | $3.3^{za}$ |
| 75 | $7.0^{xa}$ | $5.9^{xa}$ | $6.0^{xa}$ | $3.8^{ya}$ |
| 90 | $7.1^{xa}$ | $6.0^{xa}$ | $6.1^{xa}$ | $3.9^{ya}$ |

$^{x–z}$ Means in a row without common superscripts are different ($P < 0.05$).
$^{a–d}$ Means in a column without common superscripts are different ($P < 0.05$).
ND = Not detected
* NT = Non-treated; P2 = 200 MPa HHP; P3 = 300 MPa HHP; P4 = 400 MPa HHP.

Table 5: Lactic acid bacteria total counts (Log cfu/g) in caiman tail meat subjected to high hydrostatic pressure (HHP) during refrigerated storage under vacuum packaging

| | Treatments * | | | |
|---|---|---|---|---|
| **Days** | **NT** | **P2** | **P3** | **P4** |
| 0 | ND | ND | ND | ND |
| 15 | $2.7^{xd}$ | $2.2^{yc}$ | $2.1^{yc}$ | $1.7^{yc}$ |
| 30 | $4.9^{xc}$ | $3.6^{yb}$ | $2.5^{yzc}$ | $1.6^{zc}$ |
| 45 | $6.6^{xb}$ | $3.3^{yb}$ | $3.6^{yb}$ | $2.7^{yb}$ |
| 60 | $6.8^{xab}$ | $2.9^{yzbc}$ | $3.3^{yb}$ | $2.1^{zbc}$ |
| 75 | $7.2^{xab}$ | $5.3^{ya}$ | $5.8^{ya}$ | $3.5^{za}$ |
| 90 | $7.4^{xa}$ | $5.5^{ya}$ | $6.0^{xya}$ | $3.7^{za}$ |

$^{x–z}$ Means in a row without common superscripts are different ($P < 0.05$).
$^{a–d}$ Means in a column without common superscripts are different ($P < 0.05$).
ND = Not detected
* NT = Non-treated; P2 = 200 MPa HHP; P3 = 300 MPa HHP; P4 = 400 MPa HHP.

## 3.3 CHAPTER 3: DIFFERENTIAL ABUNDANCE OF SARCOPLASMIC PROTEOME EXPLAINS ANIMAL EFFECT ON BEEF LONGISSIMUS LUMBORUM COLOR STABILITY Ψ – SUBMITTED TO MEAT SCIENCE.

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**Abstract**

The sarcoplasmic proteome of beef *Longissimus lumborum* demonstrating animal-to-animal variation in color stability was examined to correlate proteome profile with color. *Longissimus lumborum* (36 h post-mortem) were obtained from 73 beef carcasses, aged for 13 days, and fabricated to 2.5-cm steaks. One steak was allotted to retail display, and another steak was immediately vacuum packaged and frozen at –80°C. Aerobically packaged steaks were stored under retail display, and color was evaluated on days 0 and 11. The steaks were ranked based on redness and color stability on d 11, and ten color-stable and ten color-labile carcasses were identified. The sarcoplasmic proteome of frozen steaks from the selected carcasses were analyzed. Nine proteins were differentially abundant in color-stable and color-labile steaks. Three glycolytic enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) were over-abundant in color-stable steaks and positively correlated ($P < 0.05$) to redness and color stability. These results indicated that animal variations in proteome contribute to differences in beef color.

**Keywords:** Beef color; Color stability; Glycolytic enzyme; *Longissimus lumborum*; Myoglobin; Sarcoplasmic proteome

### 3.3.1 Introduction

Color of fresh beef is the most important attribute influencing the purchase intention (Mancini & Hunt, 2005). Consumers prefer a bright cherry-red color for beef and associate it with freshness and wholesomeness. Color deterioration during retail display is perceived as undesirable because discolored meat is often considered unwholesome by consumers, leading to product rejection. In the United States, the beef industry loses more than $1 billion per year due to discoloration (Smith, Belk, Sofos, Tatum, & Williams, 2000). The pigment primarily responsible for meat color is myoglobin; the redox stability of myoglobin and color of fresh meat are governed by a multitude of endogenous as well as exogenous factors (Suman & Joseph, 2013).

Beef color stability is a highly muscle-specific trait (Joseph, Suman, Rentfrow, Li, & Beach, 2012; King, Shackelford, & Wheeler, 2011b; McKenna et al., 2005). With respect to the biochemistry of meat color, *Longissimus lumborum* (LL) is a major beef muscle that has been extensively studied. LL is a relatively color-stable muscle and demonstrates low oxygen consumption rate (O'Keeffe & Hood, 1982) and increased metmyoglobin reducing activity (Ledward, 1985; Seyfert et al., 2006), both of which favor formation of reduced redox ferrous forms of myoglobin. Previous research in the United States suggested that animal-to-animal variations influence the color stability and discoloration of beef LL steaks during retail display (King, Shackelford, Rodriguez, & Wheeler, 2011a; King et al., 2011b). Nonetheless, the molecular mechanisms of these variations are yet to be completely understood.

The advances in proteomic techniques, such as mass spectrometry, two-dimensional electrophoresis, and bioinformatics, have been applied successfully to explain fundamental bases of meat color phenomena (Suman, Rentfrow, Nair, & Joseph, 2014b). Previous research from our lab employed proteomic tools to interpret species-specific nature of meat color stability in livestock and poultry (Joseph, Suman, Li, Beach, & Claus, 2010; Nair, Suman, Li, Joseph, & Beach, 2014; Suman, Faustman, Stamer, & Liebler, 2007). Further investigations documented the contribution of sarcoplasmic proteome on muscle-specificity in beef color (Joseph et al., 2012; Suman et al., 2014a). Although a proteomic approach could elucidate the biochemical basis of

animal effect on beef color stability, investigations are yet to be undertaken in this direction. Therefore, the objectives of the present study were – (1) to characterize the sarcoplasmic proteome of LL steaks from beef carcasses demonstrating variations in retail color stability; and (2) to correlate the color stability attributes to differentially abundant proteome components.

### 3.3.2 **Materials and methods**

Animal care and use approval was not obtained for this experiment because beef carcasses were selected post-mortem from a USDA-inspected commercial facility.

#### 3.3.2.1 Sample collection

Seventy-three beef carcasses were selected from a commercial processing facility as they were presented for grading at approximately 36 h post-mortem. Carcass selection was conducted on 5 days approximately 2 weeks apart. After ribbing between the $12^{th}$ and $13^{th}$ ribs, carcass grade data were collected using an image analysis-based (VBG 2000) grading system (Shackelford, Wheeler, & Koohmaraie, 2003). All carcasses (USDA Select) demonstrated normal lean color and firmness, and had similar marbling scores (between $Slight^{00}$ and $Slight^{90}$). The carcasses were fabricated, and the strip loins (IMPS #180; NAMP, 2007) were obtained from the left sides. Subprimals were transported via refrigerated truck (0°C) to the U.S. Meat Animal Research Center abattoir and were aged until 13 days post-mortem. After aging, subprimals were cut, and the *Longissimus lumborum* (LL) muscle was separated. The most anterior third of LL was removed, and the remaining portion was cut into 2.54-cm steaks. One steak was allotted to simulated retail display, and another steak was immediately vacuum packaged and frozen at –80°C.

3.3.2.2 Simulated retail display and instrumental color evaluation

The steaks allotted to retail display were placed on polystyrene trays with soaker pads and over-wrapped with oxygen-permeable polyvinylchloride (PVC) film (stretchable meat film 55003815; Prime Source, St. Louis, MO, USA; oxygen transmission rate = 1.4 mL/cm$^2$/24 h at 23°C). Individually packaged steaks were placed under continuous fluorescent lighting (color temperature = 3,500 K; color rendering index = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35, GE, GE Lighting, Cleveland, OH, USA) for 11 days. Light intensity at the meat surface was approximately 2,000 lx. Retail display was conducted in a refrigerated room (1°C), and no temperature fluctuations associated with defrost cycles were encountered.

Instrumental color readings were taken at two random locations on each steak on days 0 and 11 of retail display. CIE *L** (lightness), *a** (redness), and *b** (yellowness) values were measured on the light-exposed steak surfaces with a HunterLab Miniscan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA, USA) using 2.54 cm diameter aperture, illuminant A, and 10° standard observer (AMSA, 2012). In addition, the ratio of reflectance at 630 nm and 580 nm (R630/580) was calculated as an indirect estimate of surface color stability; a greater ratio indicates a lesser amount of metmyoglobin/brown discoloration and thus greater color stability. Steaks allotted to day 0 were allowed to bloom at least 2 h (after aerobically packaged) in retail display before color evaluation.

Instrumental color data on steaks from the seventy-three carcasses were ranked based on the *a** value and R630/580 on day 11. From this ranking, the ten (n = 10) most color-stable and ten (n = 10) most color-labile steaks were identified to examine the molecular basis of animal-to-animal variation in color stability. The carcasses corresponding to these steaks were identified, and the vacuum-packaged frozen LL steaks from the selected twenty carcasses (collected during fabrication) were shipped in dry ice to the University of Kentucky for proteome analysis.

### 3.3.2.3 Myoglobin concentration

Myoglobin concentration was determined according to the method of Faustman and Phillips (2001). Duplicate 5 g frozen samples were homogenized in 45 mL ice cold 40 mM sodium phosphate buffer at pH 6.8. The homogenate was filtered using Whatman no. 1 filter paper, and the absorbance of the filtrate at 525 nm (A525) was recorded using UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA) with sodium phosphate buffer as blank. Myoglobin concentration was calculated using the following equation.

Myoglobin (mg/g) = [A525/(7.6 $mM^{-1}$ $cm^{-1}$ x 1 cm)] x [17,000/1000] x 10

where, 7.6 $mM^{-1}$ $cm^{-1}$ = millimolar extinction coefficient of myoglobin at 525 nm; 1 cm = path length of cuvette; 17,000 Da = average molecular mass of myoglobin; 10 = dilution factor.

### 3.3.2.4 Isolation of sarcoplasmic proteome

The sarcoplasmic proteome from beef LL steaks (color-stable and color-labile groups) was extracted as described by Joseph et al. (2012). Frozen samples (5 g) were cut and homogenized in 25 mL ice-cold extraction buffer (40 mM Tris, 2 mM EDTA, and pH 8.0). The homogenate was centrifuged at 10,000 x *g* for 15 min at 4 °C. The supernatant consisting of soluble sarcoplasmic proteome was filtered and utilized for analysis.

### 3.3.2.5 Two-dimensional electrophoresis (2–DE)

Bradford assay was used to determine the protein concentration of the sarcoplasm proteome extract (Bio-Rad, Hercules, CA, USA). The sarcoplasmic protein extract (1200 µg) was mixed with rehydration buffer optimized to 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% Bio-Lyte 5/8 ampholyte (Bio-Rad), and 0.001% bromophenol blue. The mix was applied onto immobilized pH gradient (IPG) strips (pH

5–8, 17 cm), and was subjected to passive rehydration for 16 h. After passive rehydration, the IPG strips were subjected to first-dimension isoelectric focusing (IEF) in a Protean IEF cell system (Bio-Rad) by applying a linear voltage increase initially, and a final rapid voltage ramping to reach a total of 80 kVh. Subsequently the IPG strips were equilibrated in SDS-containing buffers, first with equilibration buffer I (containing 6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT; Bio-Rad) followed by equilibration buffer II (containing 6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT, 2.5% iodoacetamide; Bio-Rad), each for 15 min. In the second dimension, 13.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 38.5:1 ratio of acrylamide to bis-acrylamide) was employed for protein separation, using Protean II XL system (Bio-Rad). The staining was performed for 48 h using colloidal Coomassie Blue, and the destaining was done for 48 h until the background was cleared. Color-stable and color-labile samples were run in parallel under the same conditions. Two gels per steak were produced; ten steaks were used for each group resulting in 40 gels.

#### 3.3.2.6 Gel image analysis

The gel images were digitalized using VersaDoc (Bio-Rad) and were analyzed by PDQuest software (Bio-Rad). Spots were detected and then matched with the aid of landmarks, which are well-resolved spots present in every gel image. Matched spots exhibiting a 1.5-fold or more intensity difference between color-stable and color-labile groups and associated with 5% statistical significance ($P < 0.05$) in the Student's t-test were considered differentially abundant (Joseph et al., 2012).

#### 3.3.2.7 Protein identification by mass spectrometry

Pipet tips were used to remove the selected spots from the gels. The spots were destained with 50 mM $NH_4HCO_3$/50% $CH_3CN$, homogenized using vortex for 10 min, and dried in a vacuum centrifuge. The proteins present in the gel pieces were first

reduced by addition of 10 mM DTT in 50 mM $NH_4HCO_3$, followed by alkylation with 50 mM iodoacetamide in 50 mM $NH_4HCO_3$, both for 30 min. The gel pieces were washed two times using 50 mM $NH_4HCO_3$ solution, once with $CH_3CN$, and then partially dried. Further, the gel pieces were rehydrated for 1 h (on ice) with 40 mM $NH_4HCO_3$/9% $CH_3CN$, containing proteomic grade trypsin (Sigma, St. Louis, MO, USA) at a concentration of 20 ng/µL. Additional volume of 40 mM $NH_4HCO_3$/9 % $CH_3CN$ was added, and the samples were incubated for 18 h at 37°C. Two extractions of the peptides from the gel pieces were done – first using 0.1 % trifluoroacetic acid, and second using a solution of 50% acetonitrile containing 0.1% trifluoroacetic acid; both extracts were then combined. The concentration and desalting of the peptide extracts were performed by solid phase extraction using a 0.1–10 µL pipet tip (Sarstedt, Newton, NC, USA) packed with 1 mm of Empore C-18 (3M, St. Paul, MN, USA). The peptides were eluted in 5 µL of 50 % $CH_3CN$/0.1% trifluoroacetic acid solution.

The concentrated and desalted peptide extracts (0.3 µL) were transferred onto an Opti-TOF 384 well insert (Applied Biosystems, Foster City, CA, USA) using 0.3 µL of 5 mg/mL α-cyano-4-hydroxycinnamic acid (Aldrich, St. Louis, MO, USA) in 50% $CH_3CN$/0.1% trifluoroacetic acid. A 4800 MALDI TOF-TOF Proteomics Analyzer (Applied Biosystems) was used to analyze the crystallized samples. From the initial MALDI MS spectrum for each spot, 15 peptide peaks with a signal-to-noise ratio of >20 were subjected to MS-MS for fragmentation and analysis by post-source decay. The MS-MS data were submitted for database similarity search using Protein Pilot 2.0 (Applied Biosystems) in the National Center for Biotechnology Information (NCBI) and UniProt databases to identify proteins.

#### 3.3.2.8 Statistical analysis

In this study, LL steaks from ten carcasses were used in each treatment (color-stable and color-labile) providing ten replicates (n = 10). The PROC MIXED procedure (SAS, 2011) with a repeated measure design was used to analyze the data on instrumental color parameters (*L**, *a**, *b**, and R630/580) at 0 and 11 days of retail display. The effects of treatment (color-stable vs. color-labile), retail display, and their

interaction were analyzed. Data for myoglobin concentration were analyzed for the effect of treatment. The differences between means were detected using Least Significant Difference (LSD) test at 5% significance ($P < 0.05$) level. In addition, PROC CORR procedure was used to determine the Pearson's correlation coefficients between the differentially abundant protein spots and the instrumental color parameters (SAS, 2011).

### 3.3.3 **Results and discussion**

#### 3.3.3.1 Myoglobin concentration

The concentration of myoglobin was not different ($P > 0.05$) between color-stable (4.05 ± 0.27 mg/g) and color-labile (4.56 ± 0.23 mg/g) steaks. In partial agreement with our results, Sammel et al. (2002) observed no difference in the myoglobin concentration of beef inside and outside *semimembranosus* muscles, which exhibited differences in color stability. In contrast to our findings, King et al. (2011a) reported greater myoglobin concentration in the color-labile beef loin steaks than in color-stable loin steaks. Several previous investigations attempted to characterize the relationship between myoglobin concentration and color stability in different beef muscles. McKenna et al. (2005) examined color biochemistry in 19 different beef muscles and reported that, in general, color-stable muscles exhibited lower myoglobin content than the color-labile ones. Furthermore, King et al. (2011b) reported that color-stable beef longissimus muscle exhibited lower myoglobin concentration than the color-labile *triceps brachii*. Jeong et al. (2009) also reported lower myoglobin concentration in color-stable beef Longissimus than in color-labile *Psoas major*.

#### 3.3.3.2 Instrumental color

The instrumental color data of color-stable and color-labile steaks on days 0 and 11 of retail display are presented in Table 1. All the color parameters demonstrated a

decrease ($P < 0.05$) from days 0 to 11. While several previous studies investigated intermuscular variation in beef color stability (Joseph et al., 2012; McKenna et al., 2005; Seyfert at al., 2006), limited work has been undertaken to examine color stability variations observed in a specific muscle from different carcasses. In the present study, on day 0, the color-labile steaks demonstrated greater ($P < 0.05$) *a** values (redness) than the color-stable ones; nonetheless *a** values of the two groups were numerically close. On the other hand, the *L** (lightness), *b** (yellowness), and R630/580 were similar ($P > 0.05$) for the two groups. In partial agreement with our data, King et al. (2011a) studied the color stability of the beef *Longissimus thoracis* and observed greater *a** values in the color-labile steaks than in the color-stable ones on the first day of display, whereas the *L** values and metmyoglobin content on meat surface were not different between the two groups.

After eleven days of refrigerated retail display, the *L** values were similar ($P > 0.05$) for both groups (Table 1). Color-stable steaks exhibited greater ($P < 0.05$) *a**, *b**, and R630/580 than the color-labile samples on day 11. Although both groups demonstrated a decrease ($P < 0.05$) in *a**, *b**, and R630/580, the color-labile steaks exhibited a greater decline between days 0 and 11 than their color-stable counterparts. In agreement with our results, King et al. (2011a) reported that longissimus steaks in color-stable group exhibited greater *a** and *b** values and lower metmyoglobin content on surface than the color-labile longissimus steaks on day 6 of retail display.

#### 3.3.3.3 Sarcoplasmic proteome profile

The image analyses of the Coomassie-stained gels identified twelve differentially abundant protein spots (Figure 1 and Table 2) in color-stable and color-labile longissimus muscles. Nine protein spots over-abundant ($P < 0.05$) in color-stable steaks (Table 3) were identified as phosphoglucomutase-1 (in 2 different spots), glyceraldehyde-3-phosphate dehydrogenase (in 3 different spots), pyruvate kinase M2, creatine kinase M-type, myosin regulatory light chain 2, and myosin light chain 1/3. The other three protein spots were over-abundant ($P < 0.05$) in color-labile samples (Table 3) and were identified as adenylate kinase isoenzyme 1, phosphatidylethanolamine-binding

protein 1, and myoglobin. The differentially abundant proteins were involved in glycolysis, ATP metabolism, muscle contraction, adenosine phosphates metabolism, signaling pathways, and oxygen transport (Table 3).

#### 3.3.3.4 Functional roles of differentially abundant proteins and their correlation with color traits

Six differentially abundant proteins were correlated ($P < 0.05$; Table 4) with instrumental color parameters. Five proteins (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2, myosin regulatory light chain 2, and myosin light chain 1/3) over-abundant in color-stable group exhibited a positive correlation ($P < 0.05$) with *a** value (r = 0.52–0.69). In addition, four proteins (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2, and myosin regulatory light chain 2) over-abundant in color-stable steaks were positively correlated (r = 0.54–0.65) with R630/580 ($P < 0.05$). On the other hand, phosphatidylethanolamine-binding protein 1, over-abundant in the color-labile group, demonstrated a negative correlation ($P < 0.05$) with *a** value (r = – 0.58) and R630/580 (r = – 0.59).

##### 3.3.3.4.1 *Glycolytic enzymes*

Three different enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2) involved in glycolytic metabolism were over-abundant ($P < 0.05$) in the color-stable group (Table 3). The presence of different isoforms of phosphoglucomutase-1 and glyceraldehyde-3-phosphate dehydrogenase exhibiting different isoelectric pH (Figure 1) could be attributed to possible post-translational modifications such as phosphorylation previously reported in these proteins (Anderson, Lonergan, & Huff-Lonergan, 2014; Bouley, Chambon, & Picard, 2004; Huang et al., 2011).

Phosphoglucomutase-1 catalyzes the reversible transfer of a phosphate group between positions 1 and 6 in a glucose molecule (Cori, Colowick, & Cori, 1938). Phosphorylation of threonine residue at position 466 increases the enzymatic activity of this protein (Gururaj, Barnes, Vadlamudi, & Kumar, 2004) accelerating the conversion of glucose-1-phosphate to glucose-6-phosphate (Anderson et al., 2014), which in turn favors the generation of substrates necessary for regeneration of NADH. Glyceraldehyde-3-phosphate dehydrogenase is an enzyme catalyzing the reversible conversion of glyceralehyde-3-phosphate and NAD+ to 1,3-bisphosphoglycerate and NADH (Kim & Dang, 2005). The active enzyme is composed of four identical subunits, each of which contains a reactive cysteine residue; the binding of NAD+ at the reactive cysteines activates the enzyme (Harris & Perham, 1965). The mammalian pyruvate kinase M2 is also a homo-tetrameric glycolytic enzyme (Wooll et al., 2001) catalyzing the dephosphorylation of phosphoenol pyruvate to pyruvate (Ainsworth & Macfarlane, 1973; Mazurek, 2011).

Phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2 were previously identified in proteome profile studies on bovine skeletal muscles (Bouley et al., 2004; Chaze, Bouley, Chambon, Barboiron, & Picard, 2006) and were related with fast-twitch fibers exhibiting high glycolytic activity (Okumura et al., 2005). The over-abundance of these enzymes can result in an increase in glycolytic metabolism and thus can stimulate the production of NADH and pyruvate, the latter of which is a mitochondrial substrate that promotes NADH regeneration (Ramanathan & Mancini, 2010). NADH is an important cofactor in enzymatic and non-enzymatic metmyoglobin reduction (Echevarne, Renerre, & Labas, 1990; Renerre & Labas, 1987). In support, Ramanathan and Mancini (2010) reported that the addition of pyruvate to beef mitochondria regenerated NADH (through tricarboxylic acid cycle) resulting in an electron transport-mediated metmyoglobin reduction. Several previous investigations documented the color-stabilization effect of NADH in beef and model systems. Kim et al. (2006) reported that the addition of NAD+, lactate, and LDH promoted non-enzymatic metmyoglobin reduction in model system possibly due to NADH regeneration. Moreover, in the same study beef LL steaks enhanced with 2.5% of potassium lactate exhibited increased NADH concentration and improved color stability

during retail display. Further studies (Kim, Keeton, Smith, Berghman, & Savell, 2009) investigated the differences in color stability among three beef muscles (LL, *Semimembranosus*, and *Psoas major*) and documented that the LL demonstrated greater *a** values and NADH concentration than *Psoas major* during seven days of retail display. Moreover, addition of pyruvate improved the color stability of beef LL steaks (Ramanathan, Mancini, & Dady, 2011) and muscle homogenates (Mohan, Hunt, Barstow, Houser, & Muthukrishnan, 2010). In addition, pyruvate decreased lipid oxidation in ground beef (Ramanathan, Mancini, Van Buiten, Suman, & Beach, 2012) and LL steaks (Ramanathan et al., 2011), and thus can minimize discoloration because lipid oxidation accelerates myoglobin oxidation (Faustman, Sun, Mancini, & Suman, 2010; O'Grady, Monahan, & Brunton, 2001).

The greater glycolytic metabolism in color-stable LL steaks indicates a possible low oxygen consumption, which minimizes myoglobin autoxidation resulting in lower metmyoglobin accumulation than in the color-labile steaks (O'Keefe & Hood, 1982; Renerre & Labas, 1987). Differences in glycolytic metabolism between LL steaks in the two color-stability categories could thus influence the inherent ability to reduce metmyoglobin and minimize discoloration. This in turn is explained by the observed positive correlation ($P < 0.05$) of the three glycolytic enzymes with *a** values and R630/580 (Table 4). In agreement, several studies reported a correlation between glycolytic enzymes and meat color traits. Joseph et al. (2012) investigated the differences in the sarcoplasmic proteome of color-stable (LL) and color-labile *Psoas major* beef muscles and reported that two glycolytic enzymes (β-enolase and triose phosphate isomerase) were over-abundant in LL and that β-enolase was positively correlated with *a** value. Previous investigations on pork quality reported correlation of phosphoglucomutase-1 and glyceraldehyde-3-phosphate dehydrogenase with instrumental color parameters. Zelechowska, Przybylski, Jaworska, and Sante-Lhoutellier (2012) studied the role of sarcoplasmic proteome in color attributes of pork longissimus and documented that phosphoglucomutase-1 was correlated positively to *L** value, whereas glyceraldehyde-3-phosphate dehydrogenase was positively correlated to *b** value. In contrast, Kwasiborski et al. (2008) reported a negative correlation of phosphoglucomutase-1 with *a** values in pork longissimus.

3.3.3.4.2 *Creatine kinase M-type*

Creatine kinase M-type was over-abundant ($P < 0.05$) in the color-stable LL steaks (Table 3). This sarcoplasmic kinase helps to maintain the ATP-ADP equilibrium in post-mortem skeletal muscles by catalyzing the interconversion of ADP and phosphocreatine to ATP and creatine (McLeish & Kenyon, 2005; Wallimann, Wyss, Brdiczka, Nicolay, & Eppenberger, 1992). The anoxia-induced depletion of ATP in post-mortem skeletal muscles leads to utilization of phosphocreatine by the creatine kinase M-type to generate creatine and ATP (Hamm, 1977). The LL muscle is mostly composed of type IIb fast-twitch fibers, which indicates a predominant glycolytic metabolism (Hamelin et al., 2007). Previous reports documented that fast-twitch muscles have greater creatine kinase content (Okumura et al., 2005) and phosphocreatine concentration (Kushmerick, Moerland & Wiseman, 1992) than the oxidative muscles. On their investigation using *in vitro* biological model of skeletal muscle, Lawler, Barnes, Wu, Song, and Demaree (2002) reported that creatine exhibited selective antioxidant property through its free radical scavenging ability. In addition, another study in living cells reported antioxidant functions of creatine through scavenging of reactive oxygen and nitrogen species (Sestili et al., 2006), which are associated with oxidative and nitrosative stress (Ryter et al., 2007). Free radicals promote protein oxidation in biological systems (Stadtman & Berlett, 1997) contributing to meat discoloration (Connolly, Brannan, & Decker, 2002; Faustman et al., 2010). The over-abundance of creatine kinase M-type in color-stable steaks can increase the creatine content, which can minimize myoglobin oxidation and improve color stability. In agreement with our results, Joseph et al. (2012) reported greater abundance of creatine kinase M-type in the color-stable beef LL steaks than in color-labile *Psoas major* steaks and documented a positive correlation between the enzyme and *a** values. Furthermore, in pork semimembranosus, Sayd et al. (2006) observed an over-abundance of creatine kinase in light muscles than in their dark counterparts. In addition, Kwasiborski et al. (2008) studied the sarcoplasmic proteome of pork longissimus and reported that creatine kinase was positively correlated to *a** value.

3.3.3.4.3 *Myofibrillar proteins*

Two myofibrillar proteins (myosin regulatory light chain 2 and myosin light chain 1/3) were over-abundant ($P < 0.05$) in color-stable LL steaks (Table 3). Previous research reported that LL muscle is composed predominantly of fast-twitch type IIb fibers (Hamelin et al., 2007; Hwang, Kim, Jeong, Hur, & Joo, 2010), which are associated with glycolytic metabolism (Peter et al., 1972). A myosin molecule consists of two heavy chains, two essential light chains, and two regulatory light chains (Schiaffino & Reggiani, 1996). The two types of essential light chains (myosin light chains 1 and 3) are transcribed from the same gene and thus exhibit significant similarities in their amino acid sequence (Barton, & Buckingham, 1985). In skeletal muscles, fast-twitch fibers are mainly composed of fast type myosin regulatory light chain 2 and myosin light chain 1/3 (Bicer & Reiser, 2004; Schiaffino & Reggiani, 1994). The appearance of myofibrillar proteins in sarcoplasmic fraction can be attributed to the 13 days aging prior to retail display. Previous studies (Lametsch, Roepstorff, & Bendixen, 2002; Lametsch et al., 2006) reported that the cleavage at myosin neck region during aging releases the myosin light chains from actomyosin complex resulting in their migration from myofibrillar proteome to the soluble sarcoplasmic proteome. The myofibrillar proteins over-abundant in color-stable steaks are fast-type indicating predominance of fast-twitch type IIb fibers in color-stable steaks than in color-labile ones. Fast-twitch type IIb fibers are strongly glycolytic (Peter et al., 1972), and the beef muscles demonstrating predominantly glycolytic metabolism are color-stable (O'Keefe & Hood, 1982).

Myosin regulatory light chain 2 and myosin light chain 1/3 were positively correlated ($P < 0.05$) to $a^*$ values (Table 4). Furthermore, myosin regulatory light chain 2 was positively correlated ($P < 0.05$) to R630/580 (Table 4). Our findings are in partial agreement with those of Oe et al. (2011), who investigated the proteome differences between *masseter* (slow-twitch) and *semitendinosus* (fast-twitch) muscles from Holstein cows. These authors observed greater abundance of the three proteins of our interest (myosin light chain 1 fast, myosin light chain 3 fast, and myosin regulatory light chain fast) in *semitendinosus* than in *masseter*. In addition, *semitendinosus* demonstrated

greater levels of glycolytic enzymes (enolase-3, aldolase-A, glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase) than *masseter* indicating that fast-twitch muscles are associated with glycolytic metabolism.

#### 3.3.3.4.4 *Adenylate kinase isoenzyme 1*

Adenylate kinase isoenzyme 1 catalyzes the reversible conversion of two molecules of ADP to ATP and AMP (Heil et al., 1974) and was over-abundant ($P < 0.05$) in the color-labile LL steaks (Table 3). While the exact mechanisms through which adenylate kinase influences color stability is not clear, our results are indirectly supported by muscle proteomic studies in Japanese Black cattle (Shibata et al., 2009). These authors investigated the differences in proteome profile of semitendinosus muscles from grass-fed and grain-fed cattle and reported that adenylate kinase 1 was over-abundant in the grass-fed animals. In addition, the muscles from grass-fed animals exhibited a greater content of slow-twitch myofibrillar proteins suggesting greater proportion of slow-twitch oxidative muscle fibers (Peter, Barnard, Edgerton, Gillespie, & Stempel, 1972) than their counterparts from grain-fed cattle. On the other hand, several glycolytic enzymes (β-enolase 3, fructose-1,6-bisphosphate aldolase A, and triosephosphate isomerase) were over-abundant in the grain-fed cattle indicating predominance of fast-twitch glycolytic muscle fibers. In general, muscles with increased oxidative metabolism demonstrate high oxygen consumption and are color-labile (O'Keeffe & Hood, 1982). Nonetheless, color attributes were not evaluated in the aforementioned study (Shibata et al., 2009) to assess the relationship between muscle proteome and color stability. In contrast, previous proteomic investigations in pork longissimus (Hwang, Park, Kim, Cho, & Lee, 2005; Kwasiborski et al., 2008) reported no correlation between adenylate kinase and color parameters.

### 3.3.3.4.5 *Phosphatidylethanolamine-binding protein 1*

Phosphatidylethanolamine-binding protein 1 was over-abundant ($P < 0.05$) in color-labile LL steaks (Table 3). This protein, also known as Raf kinase inhibitor protein (Yeung et al. 1999), belongs to phosphatidylethanolamine-binding protein family and are critically involved in cell signaling pathways (Keller, Fu, & Brennan, 2004). This is a cytosolic basic protein demonstrating affinity to organic anions and was named due to its capacity to bind with phosphatidylethanolamine (Bernier & Jolles, 1984; Bernier, Tresca, & Jolles, 1986). Further studies suggested that phosphatidylethanolamine-binding protein have a nucleotide-binding site (Schoentgen et al., 1992) and exhibits affinity to nucleotides such as ATP (Bucquoy, Jolles, & Schoentgen, 1994).

While phosphatidylethanolamine-binding protein 1 demonstrated a negative correlation ($P < 0.05$) with $a^*$ value and R630/580 (Table 4) in the present study, the exact mechanisms through which it influences color biochemistry are not clear. Nonetheless, findings from previous muscle proteomic studies were in agreement with our results. Kwasiborski et al. (2008) observed that this protein was negatively correlated to $a^*$ and $L^*$ values in pork longissimus. Moreover, Shibata et al. (2009) reported an over-abundance of phosphatidylethanolamine-binding protein in the semitendinosus muscles from grass-fed beef cattle compared with their counterparts from grain-fed animals; these authors also observed that the semitendinosus muscles from grass-fed animals exhibited predominance of slow-twitch fibers. Skeletal muscles consisting predominantly of slow-twitch fiber types are oxidative in metabolism (Peter et al., 1972) and thus are color-labile (O'Keefe & Hood, 1982). Results of the aforementioned studies indicated the necessity of further research on the role of phosphatidylethanolamine-binding protein 1 in meat color.

### 3.3.3.4.6 *Myoglobin*

Spot 12 (Figure 1) was over-abundant ($P < 0.05$) in color-labile steaks, and the protein in this spot was identified as myoglobin (Tables 2 and 3). However, myoglobin

concentration was similar ($P > 0.05$) in color-stable and the color-labile LL steaks. Therefore, three more protein spots exhibiting molecular weights similar to that of 12, but with different isoelectric points (Figure 2), were subjected to tryptic digestion and tandem mass spectrometry; all the spots were also identified as myoglobin (Table 5). Appearance of four spots identified as myoglobin suggested the possibility of post-translational modifications (Farley & Link, 2009). Post-translational modification of proteins via phosphorylation leads to an acidic shift in the isoelectric pH (Maurides, Akkaraju, & Jagus, 1989; Zhu, Zhao, Lubman, Miller, & Barder, 2005) as observed in figure 2. Nonetheless, we did not confirm phosphorylation of myoglobin. The spot over-abundant in color-labile steaks (spot 12) exhibited the most acidic isoelectric point (Figure 2) insinuating that myoglobin may be post-translationally modified at a greater degree in color-labile steaks than in color-stable ones and that this modification can compromise color stability. While previous studies have reported carbonylation of beef myoglobin (Alderton, Faustman, Liebler, & Hill, 2003; Suman et al., 2007), phosphorylation of myoglobin is yet to be reported in meat-producing livestock. Further research is necessary to examine the possibility of myoglobin phosphorylation and its implication in meat color stability.

Phosphoproteomics is an emerging area in life sciences (Mayya & Han, 2009), and recently an attempt was made to evaluate the relationship between protein phosphorylation and beef tenderness. Anderson et al. (2014) examined the role of phosphorylation in tenderness of beef Longissimus and reported that the least phosphorylated isoform of phosphoglucomutase enzyme was over-abundant in the less tender beef samples. These findings indicated the potential of protein post-translational modifications as biomarkers for meat quality.

### 3.3.4 **Conclusions**

The results of the present study indicate that the animal-to-animal variations observed in beef LL color stability during retail display could be attributed to the differences in sarcoplasmic proteome profile. The over-abundance of glycolytic enzymes

in the color-stable LL steaks contributes to improved color stability through NADH regeneration in post-mortem muscles. In addition, possible in situ post-translational modification of myoglobin in color-labile LL steaks appeared to compromise color stability. Further studies should examine the roles of post-translational modifications of myoglobin as well as the interactions between genome and muscle proteome in beef color stability so that biomarkers can be identified for this economically important quality attribute.

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Figure 1 – Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from beef *Longissimus lumborus* steak. Twelve protein spots differentially abundant in color- stable and color-labile steaks are numbered.

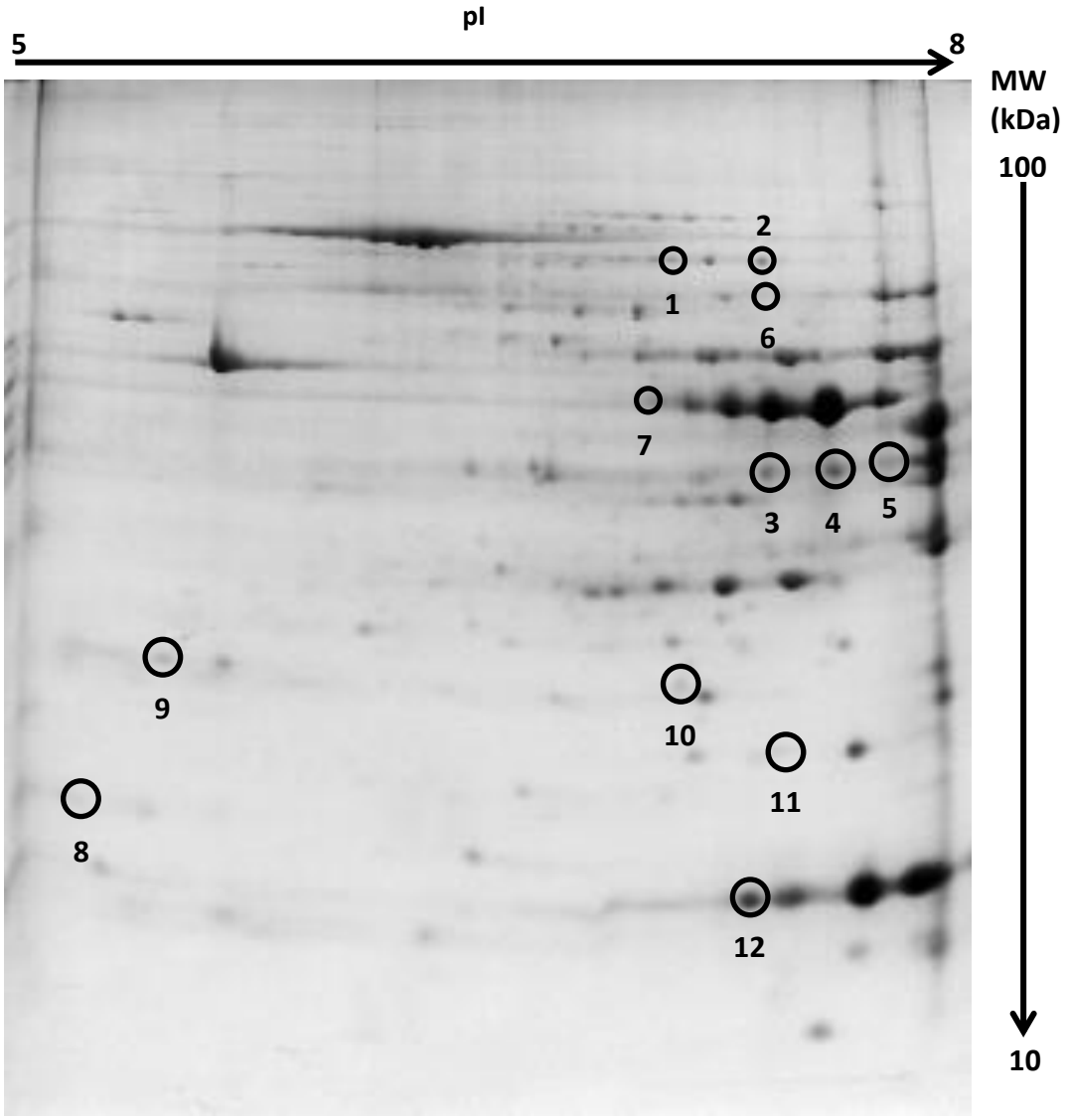

Figure 2 – Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from beef *Longissimus lumborum* steak. Four myoglobin spots detected in color-stable and color-labile steaks are numbered.

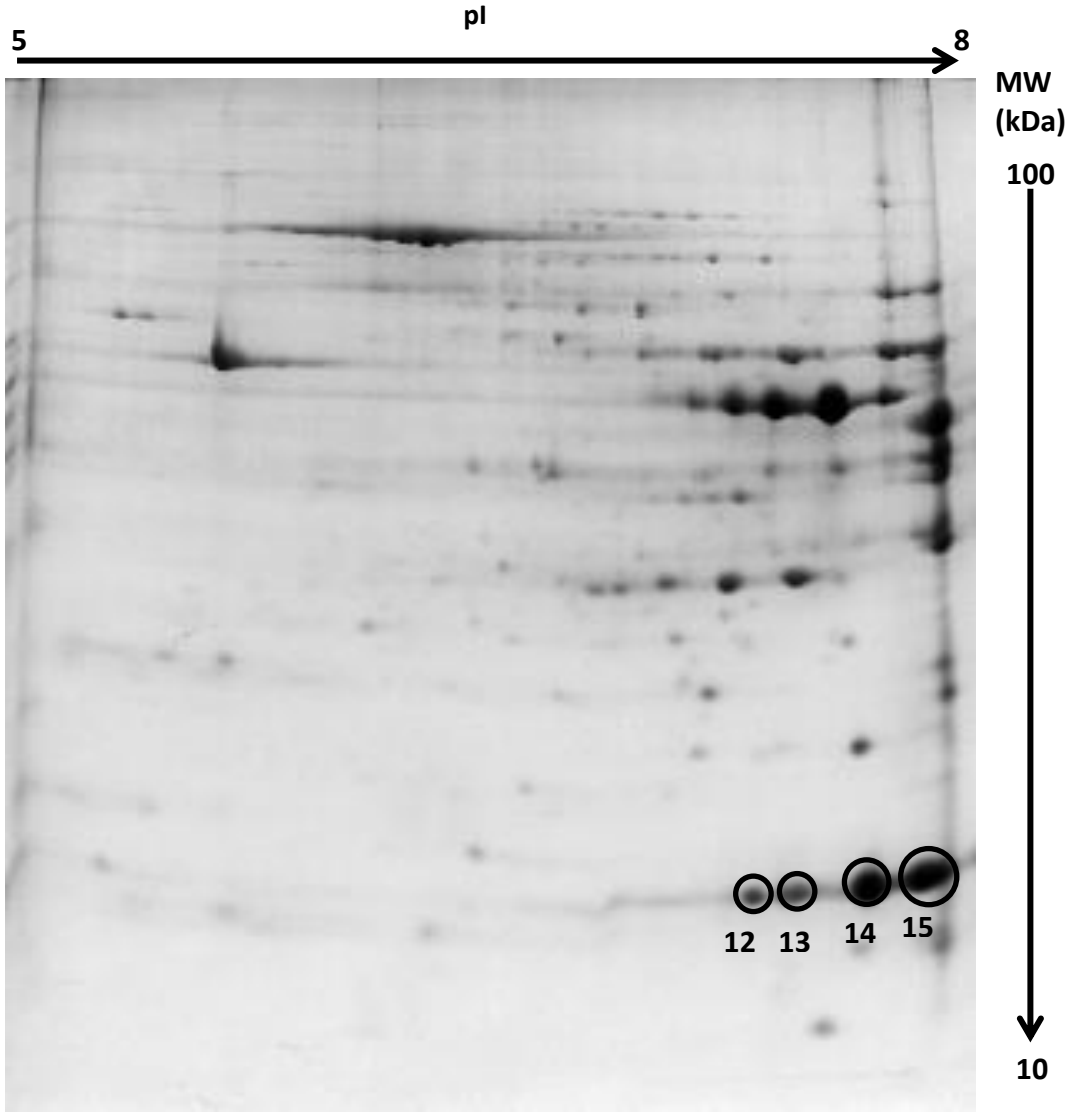

Table 1 – Instrumental color of color-stable and color-labile beef *Longissimus lumborum* steaks during refrigerated retail display.

| | | Display days | |
|---|---|---|---|
| **Parameter** | **Category** | **0** | **11** |
| *L** | Color-stable | 42.52 ± 0.83 $^{ax}$ | 40.48 ± 1.06 $^{bx}$ |
| | Color-labile | 43.32 ± 0.93 $^{ax}$ | 38.79 ± 1.18 $^{bx}$ |
| *a** | Color-stable | 34.65 ± 0.46 $^{ay}$ | 31.49 ± 0.90 $^{bx}$ |
| | Color-labile | 36.43 ± 0.40 $^{ax}$ | 12.22 ± 0.57 $^{by}$ |
| *b** | Color-stable | 27.12 ± 0.55 $^{ax}$ | 25.78 ± 0.76 $^{bx}$ |
| | Color-labile | 28.65 ± 0.47 $^{ax}$ | 17.42 ± 0.84 $^{by}$ |
| R630/580 | Color-stable | 8.71 ± 0.28 $^{ax}$ | 6.97 ± 0.47 $^{bx}$ |
| | Color-labile | 9.45 ± 0.36 $^{ax}$ | 1.17 ± 0.04 $^{by}$ |

Results (n = 10) are expressed as the mean ± standard error.
Means without common superscripts (a—b) in a row are different ($P < 0.05$).
Means without common superscripts (x—y) in a column within an attribute are different ($P < 0.05$).

Table 2 – Differentially abundant sarcoplasmic proteins in color-stable and color-labile beef *Longissimus lumborum* steaks.

| Spot [a] | Accession number | Protein | Species | ProtScore/ matched peptides | Sequence coverage (%) |
|---|---|---|---|---|---|
| 1 | Q08DP0 | Phosphoglucomutase-1 | *Bos taurus* | 16.43/14 | 25.4 |
| 2 | Q08DP0 | Phosphoglucomutase-1 | *Bos taurus* | 22.00/13 | 30.1 |
| 3 | P10096 | Glyceraldehyde-3-phosphate dehydrogenase | *Bos taurus* | 8.40/6 | 21.9 |
| 4 | P10096 | Glyceraldehyde-3-phosphate dehydrogenase | *Bos taurus* | 5.65/4 | 10.8 |
| 5 | P10096 | Glyceraldehyde-3-phosphate dehydrogenase | *Bos taurus* | 7.36/6 | 17.1 |
| 6 | gi\|73587283 | Pyruvate kinase M2 | *Bos taurus* | 8.19/4 | 15.4 |
| 7 | Q9XSC6 | Creatine kinase M-type | *Bos taurus* | 11.82/9 | 20.2 |
| 8 | Q0P571 | Myosin regulatory light chain 2 | *Bos taurus* | 9.52/4 | 30 |
| 9 | A0JNJ5 | Myosin light chain 1/3 | *Bos taurus* | 12.00/11 | 42.7 |
| 10 | P00570 | Adenylate kinase isoenzyme 1 | *Bos taurus* | 13.03/11 | 40.2 |
| 11 | P13696 | Phosphatidylethanolamine-binding protein 1 | *Bos taurus* | 5.36/3 | 37.4 |
| 12 | P02192 | Myoglobin | *Bos taurus* | 12.00/11 | 48.1 |

[a] Spot number refers to the numbered spots in gel image (Figure 1)

For each spot, parameters related to protein identification are provided, including accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry.

Table 3 – Functional roles of the differentially abundant sarcoplasmic proteins in color-stable and color-labile beef *Longissimus lumborum* steaks.

| Spot [a] | Protein | Function | Over-abundant category | Spot ratio |
|---|---|---|---|---|
| 1 | Phosphoglucomutase-1 | Glycolysis related enzyme | Color-stable | 1.8 [b] |
| 2 | Phosphoglucomutase-1 | Glycolysis related enzyme | Color-stable | 2.1 [b] |
| 3 | Glyceraldehyde-3-phosphate dehydrogenase | Glycolysis related enzyme | Color-stable | 1.9 [b] |
| 4 | Glyceraldehyde-3-phosphate dehydrogenase | Glycolysis related enzyme | Color-stable | 2.0 [b] |
| 5 | Glyceraldehyde-3-phosphate dehydrogenase | Glycolysis related enzyme | Color-stable | 2.4 [b] |
| 6 | Pyruvate kinase M2 | Glycolysis related enzyme | Color-stable | 1.7 [b] |
| 7 | Creatine kinase M-type | ATP regeneration | Color-stable | 1.8 [b] |
| 8 | Myosin regulatory light chain 2 | Muscle contraction | Color-stable | 2.4 [b] |
| 9 | Myosin light chain 1/3 | Muscle contraction | Color-stable | 2.0 [b] |
| 10 | Adenylate kinase isoenzyme 1 | Adenosine phosphates metabolism | Color-labile | 1.6 [c] |
| 11 | Phosphatidylethanolamine-binding protein 1 | Signaling pathways | Color-labile | 1.7 [c] |
| 12 | Myoglobin | Oxygen transport | Color-labile | 2.3 [c] |

[a] Spot number refers to the numbered spots in gel image (Figure 1)
[b] Spot ratio of color-stable/color-labile
[c] Spot ratio of color-labile/color-stable

Table 4 – Pearson's correlation between instrumental color parameters on day 11 retail display and differentially abundant sarcoplasmic proteins in beef *Longissimus lumborum* steaks.

| Protein | Over-abundant category | Color parameter | Correlation coefficient |
|---|---|---|---|
| Phosphoglucomutase-1 | Color-stable | *a** value | + 0.57 |
| Glyceraldehyde-3-phosphate dehydrogenase | Color-stable | *a** value | + 0.61 |
| Pyruvate kinase M2 | Color-stable | *a** value | + 0.55 |
| Myosin regulatory light chain 2 | Color-stable | *a** value | + 0.69 |
| Myosin light chain 1/3 | Color-stable | *a** value | + 0.52 |
| Phosphatidylethanolamine-binding protein 1 | Color-labile | *a** value | – 0.58 |
| Phosphoglucomutase-1 | Color-stable | R630/580 | + 0.62 |
| Glyceraldehyde-3-phosphate dehydrogenase | Color-stable | R630/580 | + 0.65 |
| Pyruvate kinase M2 | Color-stable | R630/580 | + 0.54 |
| Myosin regulatory light chain 2 | Color-stable | R630/580 | + 0.62 |
| Phosphatidylethanolamine-binding protein 1 | Color-labile | R630/580 | – 0.59 |

Table 5 – Myoglobin spots identified in the sarcoplasmic proteome of color-stable and color-labile beef *Longissimus lumborum* steaks.

| Spot [a] | Accession no. | Protein | Species | ProtScore/matched peptides | Sequence coverage (%) |
|---|---|---|---|---|---|
| 12 | P02192 | Myoglobin | *Bos taurus* | 12.00/11 | 48.1 |
| 13 | P02192 | Myoglobin | *Bos taurus* | 9.49/7 | 48.1 |
| 14 | P02192 | Myoglobin | *Bos taurus* | 13.77/13 | 58.4 |
| 15 | P02192 | Myoglobin | *Bos taurus* | 14.00/13 | 58.4 |

[a] Spot number refers to the numbered spots in gel image (Figure 2)

For each spot, parameters related to protein identification are provided, including accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry.

# 4 OVERALL CONCLUSIONS

It is concluded that all the three different strategies applied to improve the meat utilization to promote the decrease on economic losses during meat chain processing, namely restructured caiman steak formulated with transglutaminase and NaCl substitutes addition, HHP application on caiman tail meat and molecular base investigation of beef color stability, were effective. Firstly, the synergy between microbial transglutaminase and NaCl substitutes (KCl and $MgCl_2$) represents an alternative to develop low-sodium value-added products from caiman trimmings. Moreover, the HHP technique efficient increased the caiman meat bacteriological safety during refrigerated storage. Nonetheless, this technology negatively influenced the lipid profile of a PUFA rich meat, evidencing the importance of studies to establish the best time/pressure association for each specific meat source. In addition, proteomic tool supported the elucidation of the molecular base influence on the beef color stability; animal-to-animal variation caused differences on the sarcoplasmic proteome between color-stable and color-labile group. An overabundance of proteins related to glycolytic metabolism improved color stability, possible due to a NADH regeneration in post mortem.

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# 6 APPENDIX

## 6.1 PAPER 1

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# Physico-chemical and sensory attributes of low-sodium restructured caiman steaks containing microbial transglutaminase and salt replacers

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ABSTRACT

Our objective was to examine the physico-chemical and sensory attributes of low-sodium restructured caiman steaks containing microbial transglutaminase (MTG) and salt replacers (KCl and $MgCl_2$). Trimmings from caiman carcasses were processed into restructured steaks with or without MTG and salt replacers; the five treatments were CON (1.5% NaCl), T-1 (1.5% NaCl + 1% MTG), T-2 (0.75% NaCl + 1% MTG + 0.75% KCl), T-3 (0.75% NaCl + 1% MTG + 0.75% $MgCl_2$), and T-4 (0.75% NaCl + 1% MTG + 0.375% KCl + 0.375% $MgCl_2$). T-4 demonstrated the greatest ($P < 0.05$) succulence and the lowest ($P < 0.05$) values for cooked hardness, springiness, and cohesiveness. The greatest ($P < 0.05$) purchase intention was for T-3. Furthermore, T-3 and T-4 were similar ($P > 0.05$) to controls in salty flavor. Our findings suggest that the combination of MTG, KCl, and $MgCl_2$ can be employed as a suitable salt reduction strategy in restructured caiman steaks without compromising sensory attributes and consumer acceptance.



## 1. Introduction

The increasing costs of meat production have prompted the industry to develop novel processing strategies to utilize the carcasses, and value-addition of low-value meat cuts generates additional revenue. Meat restructuring allows for efficient utilization of low-value cuts and carcass trimmings. Restructured meats exhibit consumer-desirable texture and appearance, and thus have an increased retail value (Marques, Marostica, & Pastore, 2010). Restructured meats are commonly manufactured using salt and mechanical processes to extract myofibrillar proteins, which form a protein matrix entrapping fat, water, and flavor compounds, resulting in a desirable texture and flavor (Pearson & Gillett, 1996).

The increase in consumer health consciousness has led to the development of healthy meat products through reformulation and using muscle foods with a high nutritive value (Trespalacios & Pla, 2007). Meat from caiman (*Caiman crocodilus yacare*) is low in fat and rich in polyunsaturated fatty acids (Paulino et al., 2011; Romanelli, Caseri, & Lopes Filho, 2002). Commercial caiman production (primarily for leather) is an emerging agricultural activity (Vicente Neto et al., 2007) in Brazil, where significant quantity of caiman meat is wasted due to the lack of suitable processing technology to utilize the carcass trimmings.

The role of sodium in hypertension and cardiovascular diseases (He & MacGregor, 2008) is a major concern in food industry because processed meats are a major source for sodium in human diet (Engstrom, Tobelmann, & Albertson, 1997). Therefore, sodium reduction is a priority in meat industry. Sodium reduction can lead to obstacles because salt (NaCl) is a reliable protein extractor, which enhances flavor and palatability traits. Potassium chloride (KCl) can be used as a salt replacer, but it imparts bitterness and decreases saltiness. Magnesium chloride ($MgCl_2$) has also been explored as a salt replacer (Hur et al., 2004; Ruusunen & Puolanne, 2005). In general, salt reduction strategies compromise sensory and textural attributes of meat products (Doyle & Glass, 2010; Ruusunen & Puolanne, 2005) and negatively influence consumer acceptance (Desmond, 2006). In this perspective, sodium reduction strategies in restructured meat products need further research (Cofrades, Lopez-Lopez, Ruiz-Capillas, Triki, & Jimenez-Colmenero, 2011; Lee & Chin, 2011).

Microbial transglutaminase (MTG) is an enzyme promoting protein aggregation in muscle foods through covalent cross-linking between glutamine and lysine residues (Lee & Lanier, 1995; Seguro, Kumazawa, Ohtsuka, Toiguchi, & Motoki, 1995). MTG is active at the pH range 5–8 and temperature range 2–60 °C. The efficiency of MTG is governed by the availability of the target amino acids, which depends on the species of meat (Ahhmed et al., 2007; Kawahara, Ahhmed, Ohta, Nakade, & Muguruma, 2007; Lennon, McDonald, Moon, Ward, & Kenny, 2010). In meat products, MTG is utilized to improve water-holding capacity

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and texture (Chin, Gob, & Xiong, 2009). Furthermore, MTG has been proposed as an ingredient to achieve protein gelation and matrix formation in low-salt meats (Colmenero, Ayo, & Carballo, 2005; Fulladosa, Serra, Gou, & Arnau, 2009).

Caiman carcass trimmings can be utilized for manufacturing heart-healthy, low-sodium processed meats through restructuring and salt reduction strategies. Nonetheless, investigations are yet to be undertaken to explore the use of MTG in combination with potassium chloride and magnesium chloride in low-sodium restructured caiman meat products. Therefore, the objective of the present study was to examine the sensory attributes, texture, and color of low-sodium restructured caiman meat steaks containing potassium chloride, magnesium chloride, and MTG.

## 2. Materials and methods

### 2.1. Caiman meat processing

Caiman carcass trimmings (tail, neck, legs, and back) were procured from a federally-inspected slaughterhouse at Caceres, Mato Grosso, Brazil. Trimmings from twenty caiman carcasses were obtained, and the meat (500 g) from each carcass was individually packaged and frozen immediately. Frozen trimmings were transported in dry-ice to the meat laboratory of the Universidade Federal Fluminense, where they were thawed at 4 °C overnight before processing. Thawed trimmings from five carcasses (2.5 kg) were pooled, mixed well, and ground through a 10 mm plate. The 2.5 kg meat was further divided into batches of 500 g representing five different treatments. The experiment was repeated four times providing four replicates (n = 4).

The formulations for the five treatments are presented in Table 1. The ingredients included caiman meat, chilled water, sodium tripolyphosphate, sodium chloride, potassium chloride, magnesium chloride, garlic powder, onion powder, and MTG (Active WM, Ajinomoto Co. Inc., Kawasaki, Japan). Active WM contained 99% (w/w) maltodextrin and 1% (w/w) MTG from *Streptoverticillium* sp. with an activity of 100 U/g.

The ingredients for 500 g batch were hand-mixed for 5 min, during which MTG was sprinkled. The meat mixture was bulk-packaged in a cylindrical shape (7 cm diameter and 20 cm length) with polyvinylchloride film. Several holes were punctured on the meat tubes to remove the trapped air, and the tubes were stored at 4 °C for 18 h for cold binding. Ten 2-cm thick steaks were cut from each tube, and the steaks were individually vacuum packaged and frozen at −18 °C for 24 h.

### 2.2. Meat pH and proximate composition

The pH of caiman trimmings and raw restructured steaks was measured using a digital pH meter (Digimed, Sao Paulo, Brazil) after homogenizing a 10 g sample in 90 mL distilled water (Conte-Junior, Fernandez, & Mano, 2008). Proximate composition (moisture, protein, fat, and ash) of caiman trimmings and raw restructured steaks was determined according to AOAC (2005).

### 2.3. Cooking yield

The raw frozen steaks were weighed and grilled in a pan until the internal temperature reached 35 °C. Then they were flipped to the other side and cooked to an internal temperature of 70 °C. Internal temperature was monitored using thermocouples inserted to the geometric center. Cooked steaks were cooled for 30 min at 25 °C and weighed. Cooking yield was calculated from the difference in the weight of raw and cooked steaks and expressed as the percentage of initial weight (Boles & Swan, 1996).

### 2.4. Instrumental color evaluation

CIE $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) values were measured at two random locations on each steak using a Konica Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan) with illuminant D65, 8 mm diameter aperture, and a 2° standard observer (AMSA, 2012). The colorimeter was calibrated with a standard white plate (Y = 94.2; x = 0.3160; y = 0.3326). The raw color measurements were taken on the surface immediately before grilling. The cooked samples were bisected parallel to the grilling surface and maintained at 25 °C for 30 min, at which time the internal color was determined.

### 2.5. Texture analysis

The raw and cooked steaks were subjected to Texture Profile Analysis (Bourne, 1978) using a TA.XT Plus texture analyzer (Stable Micro System, London, United Kingdom) equipped with a 75 mm diameter cylindrical metal probe. Steaks at 25 °C were cut into cubes (2 cm × 2 cm × 2 cm). Samples were compressed to 70% of their original height in two cycles at pre-test speed of 5 mm/s, test speed of 1 mm/s, and post-test speed of 5 mm/s. The time between the compressions was 2 s. The load cell used was 250 N. Eight repetitions were used per sample, and the readings were averaged before statistical analyses. The data obtained were processed by Texture Expert Software (Stable Micro System, London, United Kingdom) and expressed as hardness, springiness, cohesiveness, and resistance (Bourne, 1978). Hardness is defined as the force (N) necessary to attain 70% deformation and is determined as the peak force required for the first compression. Springiness is a dimensionless parameter, which is defined as the ratio of the height the sample returns after the first compression to the maximum deformation. Cohesiveness is defined as the strength of the internal bonds making up the body of the sample; it is also a dimensionless parameter calculated as the ratio of active work done under the second compression curve to the work done under the first compression curve. Resistance characterizes the ability of the product to regain its original shape after deformation and is also a dimensionless parameter. It is defined as

**Table 1**
Formulations of restructured caiman steaks.

| Treatment | Ingredients (% w/w) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | NaCl | MTG[a] | KCl | $MgCl_2$ | Sodium tripolyphosphate | Chilled water | Caiman meat | Onion powder | Garlic powder |
| CON[b] | 1.5 | 0 | 0 | 0 | 0.4 | 1.0 | 95.1 | 1.0 | 1.0 |
| T-1 | 1.5 | 1.0 | 0 | 0 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-2 | 0.75 | 1.0 | 0.75 | 0 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-3 | 0.75 | 1.0 | 0 | 0.75 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |
| T-4 | 0.75 | 1.0 | 0.375 | 0.375 | 0.4 | 1.0 | 94.1 | 1.0 | 1.0 |

[a] MTG = microbial transglutaminase.
[b] CON = control.

the ratio of work returned by the sample as compression force is removed to the work required for compression.

### 2.6. Quantitative Descriptive Analysis

Sensory profile of raw and cooked restructured caiman steaks was determined by eight experienced and trained panelists through Quantitative Descriptive Analysis (QDA) as previously described (Therkildsen, Stolzenbach, & Byrne, 2011). The panelists were regular consumers of meat products and were recruited from the graduate students of the Department of Food Technology at the Universidade Federal Fluminense. The panel consisted of eight members (three men and five women between 23 and 32 years of age). During training, the samples were offered to the panelists, and the attributes (appearance, aroma, flavor, and texture) were identified through an open discussion among the panel members moderated by a leader. After identifying the attributes, the panel further met for eight 2-hour sessions to establish, by consensus, the definitions and references to elaborate the scorecard. The panelists generated eleven clearly defined attributes with suitable references (Table 2) through an open discussion moderated by the leader. After identification of the attributes and definition of the references, the training with the descriptive terms was carried out using the perception intensity scale with anchor points of "light" or "dark" for color and "slight" or "a lot" for the other attributes. Before carrying out the QDA, the performance of the panel was evaluated to verify the ability to discriminate samples, repeatability, and agreement among the members (Damasio & Costell, 1991).

Visual color was evaluated in both raw and cooked samples. Steaks were presented to panelists on disposable white plastic plates under white light in individual booths constructed according to the specifications of the International Standards Organization (ISO, 1985) to evaluate the color attributes. Palatability attributes were evaluated only in cooked steaks. Cooked samples were cut into cubes (1 cm × 1 cm × 1 cm) and presented to the panelists as described above. Unsalted crackers and drinking water at room temperature were offered to clean the palate between samples. The panel completed the QDA under laboratory conditions and evaluated the samples based on previously determined references in four replicates for all attributes per panelist, using a scorecard with a non-structured 9 cm-long perception intensity scale. For color attributes 0 represented "light" and 9 represented "dark", while for the other attributes (appearance, aroma, flavor, and texture), 0 represented "slight" and 9 represented "a lot".

### 2.7. Consumer sensory testing

For cooked samples, consumer analysis was conducted with 60 panelists (age 18–63 years) recruited from the students, faculty, and staff of the Universidade Federal Fluminense. Panelists had no previous training in sensory analysis of meats. Acceptance test was used to evaluate the degree at which consumers like or dislike the products regarding the appearance, aroma, flavor, texture, overall acceptability, saltiness, firmness, and purchase intention. The samples were presented in randomized blocks in a sequential monadic way. Using a nine-point hedonic scale (1 = dislike completely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like completely), the consumers evaluated appearance, aroma, flavor, texture, and overall acceptability (Cruz et al., 2013). In addition, saltiness and firmness were evaluated using a nine-point Just-About-Right (JAR) scale (1 = extremely too little salty or firm than ideal; 2 = much less salty or firm than ideal; 3 = moderately less salty or firm than ideal; 4 = slightly less salty or firm than ideal 5 = just about right; 6 = slightly saltier or firmer than the ideal; 7 = moderately saltier or firmer than ideal; 8 = much saltier or firmer than ideal; and 9 = extremely too much salty or firm than ideal) according to Cervantes, Aoki, Almeida, Nepomuceno, and Pulzatto (2010).

The samples were evaluated at the sensory laboratory. The sensory evaluation was performed under individual booths, and necessary precautions were taken to ensure that the panelists made independent judgments. The samples were coded with random three-digit numbers, and the order of presentation was determined by random permutation. Samples of each treatment were provided to the panelists along with unsalted crackers and drinking water at room temperature to clean the palate between samples (Meilgaard, Civille, & Carr, 1999).

### 2.8. Statistical analysis

The experimental design was a randomized complete block design. Using the trimmings from five caiman carcasses in each trial, the experiment was repeated four times to provide four replicates (n = 4). One-way ANOVA was used to analyze cooking yield, instrumental data (texture and color), QDA, and consumer acceptance. Data were analyzed with XLSTAT (Addinsoft, Paris, France). Tukey's test was used to compare treatment means at 5% significance level ($P < 0.05$). Multivariate methods were also performed. The results of the QDA

**Table 2**
Description and references of sensory attributes used in Quantitative Descriptive Analysis of restructured caiman steaks.

| Sample | Attribute | Definition | References |
|---|---|---|---|
| Raw | Color | Light gray | light: Cooked root of *Colocasia esculenta*<br>Dark: Raw tilapia skin |
| Cooked | Color | Very light gray | light: Cooked root of *Colocasia esculenta*<br>Dark: Raw shrimp |
| Cooked | Product uniformity | Pattern of product's particles clustering | Slight: Brazilian meat ball (Kibe)<br>A lot: Restructured chicken breast |
| Cooked | Spicy odor | Garlic and onion odor | Slight: Chicken meat ball<br>A lot: Brazilian rice spice mix (onion, garlic, and salt) |
| Cooked | Spicy flavor | Garlic and onion flavor | Slight: Brazilian meat ball (Kibe)<br>A lot: Brazilian rice spice mix (onion, garlic, and salt) |
| Cooked | Salt flavor | Taste characterized by sodium chloride | Slight: Brazilian meat ball (Kibe)<br>A lot: 3% of NaCl in water |
| Cooked | Bitter flavor | Astringent taste | Slight: Brazilian meat ball (Kibe)<br>A lot: 3% of Lite salt[a] in water |
| Cooked | Tenderness | Strength needed to shear at the first bite | Slight: Chicken sausage<br>A lot: Salami |
| Cooked | Succulence | Amount of juice expelled on chewing | Slight: Brazilian meat ball (Kibe)<br>A lot: Chicken sausage |
| Cooked | Cohesiveness | Tendency of meat particles to stick together | Slight: Brazilian meat ball (Kibe)<br>A lot: Salami |
| Cooked | Overall texture | Visual and textural similarity to restructured steak | Slight: Chicken patties<br>A lot: Restructured chicken breast |

[a] Lite salt = 50% blend of sodium chloride and potassium chloride.

were also evaluated by principal component analysis in a correlation matrix with the data centered and scaled on the variable average. A matrix was elaborated with five rows and twenty-six columns, with the rows representing the treatments and the columns the instrumental and sensory descriptors. Descriptive information obtained from the trained panel was related to the consumer preference data using partial least squares regression (PLS). The PLS regression was used to model the acceptance test through instrumental color, textural analyses, and yield as well as the sensory data (van Schalkwyk, McMillin, Booyse, Witthuhn, & Hoffman, 2011). In addition, penalty analysis (PA) was used to analyze JAR data to identify possible alternatives for product improvement. This method is based on multiple comparisons to verify if the JAR scaling is significantly related to parameters in the acceptance test (Cervantes et al., 2010). In addition, the correlation between the instrumental and sensory data (for color and texture parameters) was determined using Pearson's correlation at 5% significance level ($P < 0.05$).

## 3. Results and discussion

### 3.1. Meat pH and proximate composition

The pH of caiman trimmings was 5.71, which was close to the values reported in fresh beef (De Marchi, Penasa, Cecchinato, & Bittante, 2013), pork (Holmer et al., 2009), and chicken (Basaran, Basaran-Akgul, & Rasco, 2010). The caiman trimmings contained 76.13% moisture, 18.94% protein, 1.02% fat, and 2.35% ash. The moisture and protein contents of caiman trimmings were comparable to chicken (Basaran et al., 2010; Trespalacios & Pla, 2007) and pork (Kim et al., 2008). On the other hand, the lipid content in caiman trimmings observed in the present study was lower than the values reported for beef (Nayak, Kenney, Slider, Head, & Killefer, 1998b), chicken (Trespalacios & Pla, 2007), and pork (Herrero, Cambero, Ordonez, de la Hoza, & Carmona, 2008). The observed ash content in caiman trimmings was greater than the values reported for chicken (Trespalacios & Pla, 2007) and beef (Nayak et al., 1998b).

The pH and proximate composition of raw restructured steaks are presented in Table 3. Control (CON) and T-1 samples demonstrated similar ($P > 0.05$) pH values, which were lower ($P < 0.05$) than those of the low-sodium products. The low-sodium products exhibited similar ($P > 0.05$) pH values. Our results indicated that MTG alone did not influence the pH of the product. In agreement with our findings, previous research documented that MTG alone did not influence the pH of restructured cooked pork shoulder (Dimitrakopoulou, Ambrosiadis, Zetou, & Bloukas, 2005), restructured ham (Lee & Chin, 2011), and restructured chicken meat (Basaran et al., 2010). In addition, the results of the present study are similar to those of Horita, Morgano, Celeghini, and Pollonio (2011), who observed that reduced-fat mortadella containing 1% of NaCl and 1% of KCl had greater pH than 2% of NaCl product. Protein extractability in meat depends on the ionic strength, pH, and type of the salt (Franks, 1993). The size of ions can also influence the gel strength (Tang, Tung, & Zeng, 1996). An increase in the concentration of chloride ions can increase the pH of food matrix, and this property is attributed to the negative charge provided by the chloride ions, which neutralizes the positive charges in matrix generated by an acidic pH (Rocha-Estrada, Cordova-Murueta, & Garcia-Carreno, 2010). On a molar basis, the chloride ion concentrations in the treatments were in the order: T-3 (0.39 M) > T-4 (0.35 M) = T-1 (0.35 M) = CON (0.35 M) > T-2 (0.32 M). The increased chloride ion concentration can partially explain the observed greater pH in T-3 than CON and T-1. The greater ($P < 0.05$) pH of T-2 and T-4 than CON and T-1 might be due to the differences in the size of the cations; hydrated sodium ion has a radius of 0.276 nm, while that of potassium ion is 0.232 nm (Helmke & Sparks, 1996). The smaller size of hydrated potassium ion can possibly increase diffusion into the muscle food matrix (Barat, Baigts, Alino, Fernandez, & Pérez-García, 2011; Sperelakis, 1995) increasing protein extractability and substrate availability for MTG. The action of MTG on protein substrates leads to the generation of ammonia (De Jong & Koppelman, 2002), which increases the pH of food matrix.

The raw restructured caiman steaks did not exhibit differences ($P > 0.05$) in moisture, fat, and ash contents (Table 3). However, protein content was greater ($P < 0.05$) in CON than in the other treatments, possibly due to its greater proportion of caiman meat used in the formulation (Table 1). Our data on moisture, fat, and ash contents are in agreement with those reported by Cofrades et al. (2011) on low-salt restructured poultry products containing only MTG. In addition, Horita et al. (2011) observed that reduced-fat mortadella prepared with sodium replacers had similar proximate composition as that of regular salt product. Furthermore, incorporation of MTG did not influence the proximate composition of restructured cooked pork shoulder (Dimitrakopoulou et al., 2005).

### 3.2. Cooking yield

The data on cooking yield are presented in Table 4. T-1 demonstrated greater ($P < 0.05$) cooking yield compared to CON, indicating that MTG alone increased processing yield. Furthermore, low-sodium restructured caiman steaks containing MTG and salt replacers (T-2, T-3, and T-4) had greater ($P < 0.05$) cooking yield than CON and T-1 suggesting the effectiveness of the combinations of MTG, KCl and/or $MgCl_2$ in improving product yield. The improved cooking yield in low-sodium treatments (T-2, T-3, and T-4) could be partially attributed to the increased pH, which was further away from the isoelectric point (pH 5.0–5.2) of major muscle proteins. As the pH moves away from the isoelectric point, muscle proteins become densely charged resulting

**Table 3**
pH and proximate composition of raw restructured caiman steaks.

| Parameter | Treatments | | | | | SEM |
|---|---|---|---|---|---|---|
| | CON | T-1 | T-2 | T-3 | T-4 | |
| pH | 5.72$^{b}$ | 5.73$^{b}$ | 5.76$^{a}$ | 5.77$^{a}$ | 5.78$^{a}$ | 0.002 |
| Moisture (%) | 76.15$^{a}$ | 76.23$^{a}$ | 76.98$^{a}$ | 76.75$^{a}$ | 76.93$^{a}$ | 0.660 |
| Protein (%) | 18.80$^{a}$ | 17.96$^{b}$ | 17.23$^{b}$ | 17.38$^{b}$ | 17.80$^{b}$ | 0.128 |
| Fat (%) | 0.94$^{a}$ | 0.85$^{a}$ | 0.84$^{a}$ | 0.91$^{a}$ | 0.83$^{a}$ | 0.018 |
| Ash (%) | 2.84$^{a}$ | 3.05$^{a}$ | 3.09$^{a}$ | 2.73$^{a}$ | 2.94$^{a}$ | 0.069 |

$^{a-b}$Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

**Table 4**
Cooking yield, instrumental color, and instrumental textural attributes of restructured caiman steaks.

| Parameter | Treatments | | | | | SEM |
|---|---|---|---|---|---|---|
| | CON | T-1 | T-2 | T-3 | T-4 | |
| Cooking yield | 71.39$^{d}$ | 75.47$^{c}$ | 87.22$^{a}$ | 78.70$^{b}$ | 84.64$^{a}$ | 0.749 |
| *Raw attribute* | | | | | | |
| $L^*$ | 48.82$^{a}$ | 49.17$^{a}$ | 50.10$^{a}$ | 49.49$^{a}$ | 48.57$^{a}$ | 0.479 |
| $a^*$ | 4.65$^{c}$ | 5.21$^{bc}$ | 5.68$^{ab}$ | 5.84$^{a}$ | 4.81$^{c}$ | 0.082 |
| $b^*$ | 7.75$^{b}$ | 6.80$^{cd}$ | 7.48$^{bc}$ | 8.65$^{a}$ | 6.49$^{d}$ | 0.113 |
| Hardness | 30.44$^{b}$ | 52.22$^{a}$ | 65.25$^{a}$ | 35.67$^{b}$ | 55.51$^{a}$ | 2.605 |
| Springiness | 0.576$^{c}$ | 0.649$^{b}$ | 0.722$^{a}$ | 0.596$^{c}$ | 0.647$^{b}$ | 0.008 |
| Cohesiveness | 0.369$^{b}$ | 0.349$^{b}$ | 0.532$^{a}$ | 0.383$^{b}$ | 0.390$^{b}$ | 0.010 |
| Resistance | 0.064$^{c}$ | 0.136$^{b}$ | 0.183$^{a}$ | 0.117$^{b}$ | 0.133$^{b}$ | 0.004 |
| *Cooked attribute* | | | | | | |
| $L^*$ | 63.69$^{a}$ | 62.53$^{a}$ | 62.30$^{a}$ | 60.58$^{a}$ | 61.83$^{a}$ | 0.648 |
| $a^*$ | 3.73$^{a}$ | 3.70$^{a}$ | 3.49$^{a}$ | 4.20$^{a}$ | 4.06$^{a}$ | 0.113 |
| $b^*$ | 7.93$^{a}$ | 7.80$^{a}$ | 7.73$^{a}$ | 8.39$^{a}$ | 8.50$^{a}$ | 0.243 |
| Hardness | 149.5$^{d}$ | 236.6$^{a}$ | 204.0$^{b}$ | 216.9$^{ab}$ | 173.8$^{c}$ | 4.029 |
| Springiness | 0.826$^{b}$ | 0.933$^{a}$ | 0.917$^{a}$ | 0.912$^{a}$ | 0.879$^{b}$ | 0.006 |
| Cohesiveness | 0.484$^{d}$ | 0.613$^{a}$ | 0.592$^{b}$ | 0.604$^{ab}$ | 0.571$^{c}$ | 0.002 |
| Resistance | 0.166$^{c}$ | 0.258$^{a}$ | 0.232$^{b}$ | 0.224$^{b}$ | 0.234$^{b}$ | 0.003 |

$^{a-d}$Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

in a concomitant increase in hydrophilicity and water retention (Puolanne & Halonen, 2010). Among low-salt products, the steaks containing KCl (T-2 and T-4) exhibited the greatest values ($P < 0.05$) indicating a possible synergistic effect of KCl and MTG. While all low-sodium products exhibited similar pH, the improved cooking yield in KCl-containing formulations (T-2 and T-4) could be partially due to an increase in protein extractability induced by the smaller-sized hydrated potassium ions in the matrix (Barat et al., 2011; Sperelakis, 1995).

Our results indicated that the incorporation of KCl and $MgCl_2$ in low-sodium restructured meats can increase the cooking yield. Divalent and monovalent cations promote changes in water-holding capacity through different mechanisms (Barat, Perez-Esteve, Aristoy, & Toldra, 2012). In agreement with our findings, previous reports documented that the replacement of sodium chloride by magnesium chloride or potassium chloride increased the extractability and solubility of myofibrillar proteins favoring gel formation in beef batter model systems (Nayak, Kenney, Slider, Head, & Killefer, 1998a; Pigott, Kenney, Slider, & Head, 2000). MTG catalyzes intra- and inter-protein cross-linking between glutamine and lysine, promoting network formation, and turning the muscle food matrix into a high molecular weight polymer entrapping water molecules, which improves processing yields. Moreover, MTG promotes glutamine deamination during which water acts as a nucleophile leading to the covalent attachment of the water molecule to the protein chain (Ohtsuka, Umezawa, Nio, & Kubota, 2001). All these mechanisms increase the water content in the meat products and effectively contribute to the increased cooking yield in MTG-containing restructured caiman steaks.

In agreement with our findings, Tseng, Liu, and Chen (2000) reported increased yield in low-salt chicken meat-balls containing MTG. Several researchers (Pietrasik, Jarmoluk, & Shand, 2007; Pietrasik & Li-Chan, 2002) observed that MTG addition decreased the cooking loss in pork batter gels. Moreover, Pietrasik (2003) documented that MTG positively influenced the parameters contributing to the water binding properties of beef gels. In contrast, other researchers observed no effect of MTG on cooking loss in porcine myofibrillar protein gel (Chin et al., 2009) and low-salt restructured poultry meat (Cofrades et al., 2011). The differences between our observations and these reports could be, in part, due to the variations in the concentration of MTG used.

### 3.3. Instrumental color

#### 3.3.1. Raw restructured caiman steak

Neither MTG nor salt replacers influenced ($P > 0.05$) $L^*$ values in raw restructured caiman steaks (Table 4). On the other hand, $a^*$ and $b^*$ values were different ($P < 0.05$) among the treatments (Table 4). T-1 and T-4 exhibited $a^*$ values similar ($P > 0.05$) to that of controls, whereas T-2 and T-3 demonstrated greater ($P < 0.05$) redness than the controls. The observed lack of difference in $a^*$ values of control, T-1, and T-4 caiman steaks indicated that the incorporation of MTG alone or in combination with KCl and $MgCl_2$ may not affect the surface redness during retail. Based on surface redness, which influences consumer purchase decisions (Mancini & Hunt, 2005), the low-sodium product T-4 can be retailed in a manner similar to the controls. The $b^*$ values of T-1 steaks were lower ($P < 0.05$) than those of the controls indicating that MTG decreased the yellowness. In addition, T-4 also exhibited lower ($P < 0.05$) $b^*$ values than CON. However, T-3 demonstrated greater ($P < 0.05$) $b^*$ values than CON.

The color of uncooked meat products is dictated by myoglobin concentration, fat and water contents, and non-meat ingredients (Pietrasik & Janz, 2009). When myoglobin content and redox state remain constant, the color of comminuted products is mostly governed by the parameters such as fat content, non-meat ingredients and added/lost water during the processing (Trespalacios & Pla, 2007). In agreement with our results, several previous studies reported that the $L^*$ values were unaffected by the addition of MTG in catfish patties (Min & Green, 2008) and pork (Nielsen, Petersen, & Moller, 1995). However, Nielsen et al. (1995) documented a decrease in $a^*$ values in MTG-treated pork. Min and Green (2008) observed that in catfish patties $a^*$ values did not change and $b^*$ values increased with the addition of MTG. These findings suggested a species-specific effect of MTG on meat color. The differences between our results and the previous reports could be attributed to the species-specific variations in the concentration and biochemistry of myoglobin in raw meat (Suman & Joseph, 2013).

#### 3.3.2. Cooked restructured caiman steak

The dull-brown color of cooked meat is due to the heat-induced denaturation of myoglobin (King & Whyte, 2006), and the process of cooking results in a decrease in redness and an increase in lightness and yellowness (Cofrades et al., 2011). The instrumental color parameters ($L^*$, $a^*$, and $b^*$ values) of cooked restructured caiman steaks were similar ($P > 0.05$; Table 4). These results indicated that cooked low-sodium and control restructured caiman steaks have similar color and may appear similar at the point-of-consumption. In support of the findings in the present study, Tseng et al. (2000) observed no differences among treatments in their study on low-salt chicken meat-balls. Furthermore, dicationic salts have been reported to have no effect on $L^*$ values in cooked beef batter (Pigott et al., 2000). Additionally, Horita et al. (2011) documented that salt reduction did not contribute to variations in $L^*$, $a^*$, and $b^*$ values of bologna. Furthermore, other researchers also reported that MTG levels had no effects on the cooked color of restructured pork shoulder (Dimitrakopoulou et al., 2005) and chicken kebab (Kilic, 2003).

### 3.4. Instrumental texture

#### 3.4.1. Raw restructured caiman steak

Hardness and springiness were greater ($P < 0.05$) for MTG-containing steaks than controls, except for T-3 which was comparable ($P > 0.05$) to control (Table 4). In addition, incorporation of MTG increased ($P < 0.05$) resistance in the raw restructured caiman steaks (Table 4). Among the MTG-treated samples, hardness was similar ($P > 0.05$) for T-1, T-2, and T-4 (Table 4). On the other hand, T-2 exhibited greater ($P < 0.05$) values for springiness, cohesiveness, and resistance than the other MTG-containing treatments as well as control (Table 4). The increase in hardness, springiness, and resistance observed in restructured caiman steaks containing MTG can be attributed to the enhanced protein cross-linking. The formation of large polymeric protein aggregates improves the gel structure between meat particles (De Jong & Koppelman, 2002). In agreement with our results, Herrero et al. (2008) concluded that hardness, springiness, and cohesiveness of pork increased with the incorporation of MTG. In addition, MTG has been suggested as an ingredient to improve functional and textural properties of food products (Yokoyama, Nio, & Kikuchi, 2004). Nayak et al. (1998a) reported that in low-fat beef batters $MgCl_2$ lowers actin solubility, which decreases the substrate availability for MTG; this phenomenon partially explains the lower value of hardness observed in T-3 than in other MTG-treatments. In contrast to our results, Nielsen et al. (1995) observed no effect of MTG addition on the textural attributes of pork. In the present study, the low values for textural parameters in meat systems without MTG indicated a pseudoplastic fluid behavior possibly due to low myofibrillar protein gelation.

#### 3.4.2. Cooked restructured caiman steak

After cooking, the MTG-treated steaks demonstrated greater ($P < 0.05$) values for instrumental texture parameters than CON, with the exception of springiness in T-4 (Table 4). Within the MTG treatments, T-4 had the lowest ($P < 0.05$) values for hardness, springiness, and cohesiveness, whereas T-1 had the greatest ($P < 0.05$) resistance. In general, replacing NaCl partially with KCl and/or $MgCl_2$ in the presence of MTG decreased ($P < 0.05$) resistance, cohesiveness, and hardness. The observed increase in texture parameters can be

attributed to the formation of glutamyl-lysyl bonds between myofibrillar proteins (Lee & Lanier, 1995). Protein–protein binding is enhanced by MTG, and a strong protein network among meat particles increases the breaking strength and results in a firm product (De Jong & Koppelman, 2002). Heat processing of MTG-incorporated restructured meat products promotes denaturation of protein molecules and leads to the exposure of buried reactive groups, which ultimately improves cohesiveness (Tellez-Luis, Uresti, Ramirez, & Vazquez, 2002). Several previous investigations supported the findings from the present study. Hammer (1998) reported that finely comminuted sausages containing 0.2% MTG were harder and firmer than the controls. Furthermore, Tseng et al. (2000) observed that the gel strength of low-salt chicken meat balls increased with the level of added MTG. Pietrasik (2003) reported that MTG increased hardness, cohesiveness, and springiness of beef gels.

### 3.5. Quantitative Descriptive Analysis

QDA differentiated cooked restructured caiman steaks based on cooked color, spicy odor, salty flavor, bitter flavor, tenderness, succulence, and cohesiveness (Table 5). Among the MTG-treatments, T-2 demonstrated the lowest ($P < 0.05$) values for cooked color, which was similar ($P > 0.05$) only to controls. T-4 exhibited lower ($P < 0.05$) spicy odor than CON, T-1, and T-3. However, spicy flavor was not different ($P > 0.05$) among the treatments. With respect to salty flavor, the data indicated that T-1 had the greatest scores ($P < 0.05$). T-3 and T-4 steaks were lower ($P < 0.05$) in salty flavor than T-1 possibly due to the partial replacement of NaCl with KCl and/or $MgCl_2$. However, T-3 and T-4 steaks were similar ($P > 0.05$) to controls in salty flavor. Overall, MTG-treated steaks had greater ($P < 0.05$) bitter flavor than controls. Among the low-sodium MTG-treatments, bitter flavor was greatest ($P < 0.05$) for T-2, which contained the highest level (0.75%) of potassium chloride. Potassium chloride, at a replacement level above 50%, is known to impart bitterness in low-salt foods (Desmond, 2006).

Control steaks demonstrated greater ($P < 0.05$) tenderness than T-1, T-2, and T-3. However, T-4 was rated similar ($P > 0.05$) to control with respect to tenderness. The least tender steaks were T-1 ($P < 0.05$), whereas T-3 and T-2 were intermediate. The most succulent steaks ($P < 0.05$) were T-2 and T-4, whereas the least ($P < 0.05$) succulent one was T-3. For cohesiveness, T-1 exhibited the greatest ($P < 0.05$) scores, whereas controls and low-sodium restructured steaks (T-2, T-3, and T-4) demonstrated similar values ($P > 0.05$). QDA did not identify any difference ($P > 0.05$) in raw color, product uniformity, spicy flavor, and overall texture. The results of QDA analysis indicated that the low-sodium T-4 steak has several sensory properties (salty flavor, tenderness, and cohesiveness) comparable to the controls, indicating the market potential for low-salt meats containing KCl and $MgCl_2$ as partial replacers for NaCl.

**Table 5**
Quantitative descriptive analysis scores of restructured caiman steaks.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Attribute | CON | T-1 | T-2 | T-3 | T-4 | SEM |
| Raw color | $4.14^{a}$ | $4.08^{a}$ | $3.92^{a}$ | $4.01^{a}$ | $3.99^{a}$ | 0.102 |
| Cooked color | $2.17^{bc}$ | $2.44^{ab}$ | $2.04^{c}$ | $2.50^{ab}$ | $2.65^{a}$ | 0.128 |
| Spicy odor | $4.45^{a}$ | $4.20^{a}$ | $4.05^{ab}$ | $4.22^{a}$ | $3.54^{b}$ | 0.223 |
| Spicy flavor | $3.79^{a}$ | $4.00^{a}$ | $3.77^{a}$ | $3.46^{a}$ | $3.42^{a}$ | 0.216 |
| Salty flavor | $2.94^{b}$ | $3.72^{a}$ | $3.35^{ab}$ | $2.77^{b}$ | $3.12^{b}$ | 0.217 |
| Bitter flavor | $0.28^{c}$ | $0.63^{b}$ | $1.17^{a}$ | $0.69^{b}$ | $0.84^{b}$ | 0.097 |
| Product uniformity | $6.58^{a}$ | $6.74^{a}$ | $6.85^{a}$ | $6.71^{a}$ | $6.71^{a}$ | 0.138 |
| Tenderness | $5.58^{a}$ | $3.73^{d}$ | $4.80^{b,c}$ | $4.62^{c}$ | $5.42^{ab}$ | 0.235 |
| Succulence | $5.32^{b}$ | $5.04^{b}$ | $5.94^{a}$ | $4.37^{c}$ | $6.04^{a}$ | 0.215 |
| Cohesiveness | $5.57^{b}$ | $6.42^{a}$ | $5.68^{b}$ | $5.59^{b}$ | $5.71^{b}$ | 0.161 |
| Overall texture | $7.27^{a}$ | $7.56^{a}$ | $7.42^{a}$ | $7.44^{a}$ | $7.51^{a}$ | 0.133 |

$^{a-d}$Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

The results of previous sensory studies on low-sodium meat products were in conflict with our findings in QDA. Matulis, McKeith, Sutherland, and Brewer (1995) examined the influence of salt on sensory characteristics of frankfurters and concluded that salt increased saltiness and decreased tenderness. Gimeno, Astiasaran, and Bello (1999) observed lower cohesiveness and greater tenderness for low-sodium dry-fermented sausages (containing KCl and $CaCl_2$) than the traditional ones. Furthermore, Dimitrakopoulou et al. (2005) reported no differences in juiciness for the control and MTG-containing cooked pork shoulders. The differences between our results and the previous reports could be attributed to the differences in the combinations of salt replacers used in the present study, which could have minimized the differences between control and the low-sodium products (T-2, T-3, and T-4).

### 3.6. Principal Component Analysis (PCA)

PCA explained 67.3% of total variance (Fig. 1). The principal component-1 contributed 35.7% in defining the treatments into 2 groups (CON and T-2; T-1, T-3, and T-4). For this division, cooked instrumental texture (hardness, springiness, cohesiveness, and resistance), overall texture, and spicy flavor were the relevant parameters. CON and T-2 demonstrated lower values for all these parameters, except spicy flavor, than the other treatments. The principal component-2 contributed 31.6% in characterizing the treatments. Despite having a low percentage, this component clearly divided the treatments based on raw instrumental texture (hardness, springiness, cohesiveness, and resistance), bitter flavor, and product uniformity. The high values for these factors separated T-2 from the other treatments. Additionally CON, T-1, T-3, and T-4 were rated high for cooked color. The combination of principal components 1 and 2 resulted in three groups – CON; T-2; and T-1, T-3 and T-4.

Pearson correlation analyses of product attributes (instrumental and sensory) indicated the existence of strong correlation between several attributes. Positive correlation ($P < 0.05$) was observed between sensory cooked color and cooked $a^*$ ($r = 0.82$), overall texture and cooked instrumental resistance ($r = 0.96$), cooked springiness and cooked instrumental cohesiveness ($r = 0.98$), product uniformity and raw instrumental resistance ($r = 0.99$), sensory cohesiveness and saltiness ($r = 0.88$), bitter flavor and cooking yield ($r = 0.96$), and sensory cooked color and cooked $b^*$ ($r = 0.81$). On the other hand, several other parameters demonstrated negative correlation, including cooking yield and sensory raw color ($r = -0.95$), instrumental cooked hardness and sensory hardness ($r = -0.95$), and sensory cohesiveness and sensory hardness ($r = -0.82$).

The compounds responsible for taste in meat products are, in general, non-volatile, water-soluble, and low molecular weight molecules (Lawless & Heymann, 1998). The treatments exhibiting increased cooking yield (T-2 and T-4) possibly might have retained hydrophilic molecules responsible for bitterness. Furthermore, the treatment demonstrating the greatest ($P < 0.05$) bitter flavor (T-2) contained the highest concentration of potassium chloride, which is known to impart bitterness (Desmond, 2006).

### 3.7. Consumer sensory testing

#### 3.7.1. Hedonic scale testing

The data on consumer hedonic testing on cooked caiman steaks are presented in Table 6. T-1 steak demonstrated lower ($P < 0.05$) overall acceptance than T-2 and lower ($P < 0.05$) texture than the low-sodium treatments. All other treatments were similar ($P > 0.05$) with respect to overall acceptance and texture. The results on T-1 reflected the data from instrumental texture (Table 4) and QDA (Table 5); low tenderness and high cohesiveness scores could have contributed to the low overall acceptance of T-1. In general, appearance, flavor, and odor attributes were not different ($P > 0.05$) for the

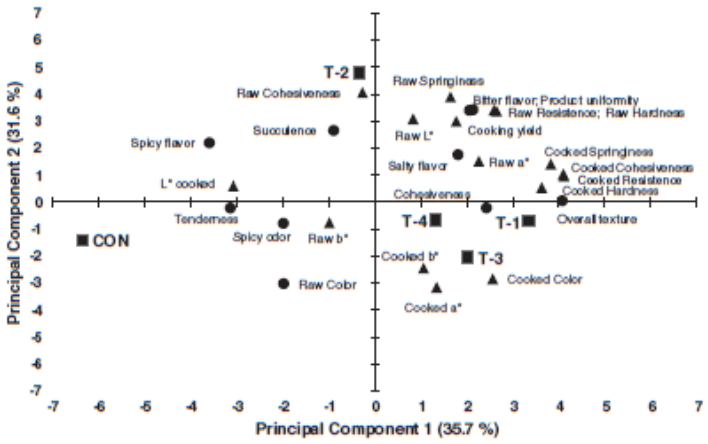


**Fig. 1.** Instrumental and sensory data of restructured caiman steaks in the plane defined by two principal components.

treatments indicating that untrained panelists could not differentiate between the control, MTG-treated, and low-sodium restructured caiman steaks based on these attributes. Noticeably, no palatability attribute led to the rejection of low-sodium caiman steaks suggesting their positive consumer acceptance and economic potential. In agreement to our results, Horita et al. (2011) observed no differences in the appearance, odor, and texture between mortadellas prepared with blends of $CaCl_2$, $MgCl_2$, and KCl as partial replacers for NaCl and the control mortadellas. On the other hand, in the same study they observed low flavor for the formulation containing 0.5% NaCl, 1% KCl, and 0.5% $MgCl_2$, a finding that we did not observe. One of the major bottlenecks of sodium reduction is consumer acceptance (Desmond, 2006). KCl and $MgCl_2$ usually have a bitter/metallic flavor and reduced saltiness, both of which lead to consumer rejection (Verma & Banerjee, 2012) of low-salt products. However, this was not observed in the present study in consumer sensory testing, possibly due to the presence of MTG in the low-sodium caiman steaks.

The purchase intention, expressed as the percentage of total consumers willing to purchase the product, was different for the treatments. The greatest purchase intention was for T-3 steaks (70.49%), whereas T-1 was the least preferred one for purchase (50.82%). The purchase intention for control was 60.66%, while that of T-2 and T-4 was 68.85% each. The restructured steaks with lowest purchase intention were the ones containing 1.5% NaCl (control and T-1). The increased purchase intention for T2, T-3, and T-4 indicated the market potential for low-salt restructured caiman steaks.

### 3.7.2. Just-About-Right (JAR) profile

The data from JAR profile of cooked restructured caiman steaks are presented in Table 7. Saltiness was close to ideal for all the treatments in the nine-point scale. Furthermore, the treatments did not differ for saltiness ($P > 0.05$). This finding indicated that the consumers are unable to differentiate the low-sodium restructured steaks and regular salt ones based on saltiness. Firmness was close to ideal for control, T-2, T-3, and T-4, and suggested the similarity in texture for these products. On the other hand, T-1 demonstrated more firmness than ideal ($P < 0.05$) possibly due to the presence of both NaCl and MTG. The observed similarities between control and low-sodium caiman (T2, T-3, and T-4) restructured caiman steaks insinuated that the replacement of NaCl with KCl and $MgCl_2$ (in combination with MTG) can be employed as a suitable salt-reduction strategy in processed meats without compromising sensory quality.

**Table 6**
Consumer sensory scores of restructured caiman steaks.

| Attribute | Treatments | | | | | SEM |
|---|---|---|---|---|---|---|
| | CON | T-1 | T-2 | T-3 | T-4 | |
| Overall acceptance | 6.54$^{ab}$ | 6.30$^{b}$ | 7.00$^{a}$ | 6.69$^{ab}$ | 6.71$^{ab}$ | 0.212 |
| Texture | 6.31$^{ab}$ | 5.82$^{b}$ | 6.64$^{a}$ | 6.51$^{a}$ | 6.61$^{a}$ | 0.234 |
| Flavor | 6.69$^{a}$ | 6.51$^{a}$ | 6.95$^{a}$ | 6.71$^{a}$ | 6.72$^{a}$ | 0.236 |
| Odor | 6.44$^{a}$ | 6.47$^{a}$ | 6.66$^{a}$ | 6.36$^{a}$ | 6.56$^{a}$ | 0.238 |
| Appearance | 5.98$^{a}$ | 5.98$^{a}$ | 6.36$^{a}$ | 5.03$^{b}$ | 6.23$^{a}$ | 0.254 |

$^{a-b}$Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

### 3.7.3. Partial Least Squares Regression (PLSR)

PLSR technique examined the meat quality data from instrumental and sensory evaluations. The PLSR model (Fig. 2) explained 99.8% of consumer acceptance and 75% of the trained panelist sensory scores and instrumental parameters showing an accumulated Q2 of 0.887. The QDA and instrumental attributes were considered relevant when their respective Variable Important to the Projection was greater than 0.8 (Wold, Sjostrom, & Eriksson, 2001). Those relevant parameters were retained as determinants for product acceptance (Fig. 3). Succulence and cooking yield positively influenced the product acceptance, while cooked color and cohesiveness exerted a negative effect on restructured caiman steaks. Noticeably, bitter flavor did not contribute to the rejection of T-2, T-3, and T-4, indicating that consumers are unable to distinguish NaCl substitution by KCl and/or $MgCl_2$ at 50% ratio.

### 3.7.4. Penalty analysis

Penalty analysis was used in sensory data analysis to identify potential directions for product improvement on the basis of consumer data

**Table 7**
Just-About-Right profile scores of restructured caiman steaks.

| Attributes | Treatments | | | | | SEM |
|---|---|---|---|---|---|---|
| | CON | T-1 | T-2 | T-3 | T-4 | |
| Saltiness | 5.23$^{a}$ | 5.33$^{a}$ | 5.25$^{a}$ | 5.07$^{a}$ | 5.25$^{a}$ | 0.141 |
| Firmness | 5.82$^{b}$ | 6.38$^{a}$ | 5.64$^{b}$ | 5.64$^{b}$ | 5.54$^{b}$ | 0.171 |

$^{a-b}$Means in a row without common superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.

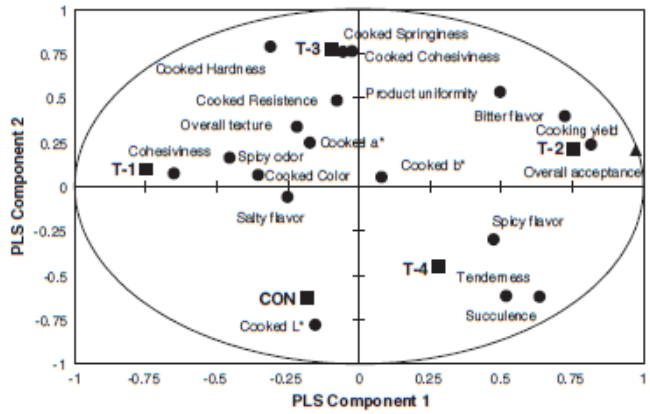


**Fig. 2.** Partial Least Square Regression model for sensory and instrumental attributes of restructured caiman steaks.

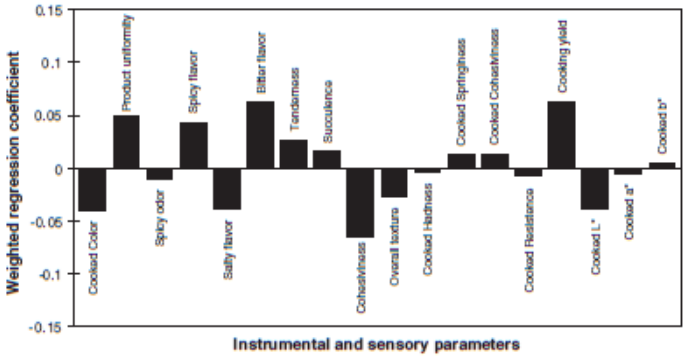


**Fig. 3.** Weighted regression coefficients of instrumental and sensory parameters detrimental to consumer acceptance by partial least squares regression.

(Table 8). Combining the data from JAR profile and consumer hedonic testing makes it possible to identify the parameters (for each treatment) that can be improved to increase consumer acceptance. Major detrimental attributes were the ones with more than 0.5 penalty score and 20% occurrence. Table 8 summarizes the attributes that decreased the acceptance for the restructured caiman steaks, and the major detrimental attribute on acceptance was different for the treatments. Firmness and saltiness did not penalize the overall acceptance of control caiman steaks. However, based on too much saltiness T-1 and T-2 were penalized by 36% and 34% of panelists, respectively. Furthermore, the consumers concluded that T-1 and T-2 steaks were slightly more salty than ideal. On the other hand, T-3 and T-4 were not penalized for saltiness, indicating the potential for these formulations. Apparently, MTG contributed to an enhanced salt perception in T-2 steaks, and this finding suggested that MTG can be exploited as a logical strategy to enhance salt perception and consumer acceptance of low-salt meats. Increased firmness was considered by 47%, 54%, and 49% of consumers as a source of penalty for T-2, T-3, and T-4, respectively. The outcomes of penalty analysis indicated that, in general, the consumers found the JAR parameters (saltiness and/or firmness) were greater than ideal for the MTG-treated restructured caiman steaks. This result indirectly suggested that lowering NaCl and MTG concentrations may improve the product acceptance.

**Table 8**
Penalty analysis of Just-About-Right (JAR) attributes of restructured caiman steaks.

| Treatments | Saltiness | | Firmness | |
|---|---|---|---|---|
| | Not salty enough | Too Salty | Not firm enough | Too firm |
| CON | – | – | – | – |
| T-1 | – | 36.07[a] (−0.53)[b] | – | – |
| T-2 | – | 34.43 (−0.61) | – | 47.54 (−0.61) |
| T-3 | – | – | – | 54.10 (−0.97) |
| T-4 | – | – | – | 49.18 (−0.71) |

A dash (–) indicates that less than 20% of consumer selected the corresponding JAR category.

[a] The percentage of consumers who found each treatment to be too salty or too firm for JAR saltiness and firmness.

[b] The number in parentheses is the change in mean compared to the consumer response score to overall acceptance.

## 4. Conclusions

The results suggest that the product formulations of T-3 and T-4 restructured caiman steaks can be employed as a suitable salt reduction strategy. While MTG improved the texture (instrumental and sensory), salt replacers (KCl and $MgCl_2$) improved cooking yield, succulence, and

consumer acceptance. The meat industry could exploit the synergistic effect of restructuring, MTG, and salt replacers (KCl and $MgCl_2$) for the development of low-sodium value-added products from caiman trimmings.

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