UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia de Alimentos

ANA PAULA DA SILVA BERNARDO

EFFECTS OF FREEZING AND THAWING, AND PRESENCE OF BONE AND SUBCUTANEOUS FAT ON DRY-AGED BEEF

EFEITOS DO CONGELAMENTO E DESCONGELAMENTO, E PRESENÇA DE OSSO E GORDURA SUBCUTÂNEA NO PROCESSO DE MATURAÇÃO A SECO DE CARNE BOVINA

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Orientador: Sérgio Bertelli Pflanzer Júnior
Coorientadora: Maristela da Silva do Nascimento

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RESUMO

A maturação é um dos principais métodos *post-mortem* utilizado para melhorar a maciez da carne bovina, e pode ser realizado tanto a vácuo ("wet aging"), em embalagens com baixa permeabilidade de vapor de água e gases, quanto à seco ("dry aging"), onde a carne é disposta, sem embalagem, em câmaras de refrigeração sob condições controladas de umidade, temperatura e velocidade do ar. Uma das grandes vantagens da maturação a seco é o desenvolvimento e intensificação de sabor e aroma mais desejáveis na carne. No entanto, este processo apresenta um alto custo, devido principalmente às perdas de peso que ocorrem por meio de evaporação e remoção das superfícies secas. Sendo assim, tanto o osso, quanto a gordura subcutânea podem reduzir a perda de peso durante a maturação a seco e aumentar o rendimento do processo, pois ambos atuam como uma barreira à evaporação. E ainda, considerando que a matéria prima para produzir carne maturada a seco deve ser de alta qualidade, que por sua vez é um produto de alto custo e menor disponibilidade no Brasil, o armazenamento congelado pode ser útil para aumentar a validade e expandir o mercado deste tipo de produto. Portanto, o presente estudo avaliou a viabilidade do congelamento, antes e após a maturação a seco, e seus efeitos na qualidade microbiológica e físico-química da carne, e ainda o impacto da presença de osso e gordura subcutânea no rendimento e características físico-químicas da carne maturada a seco. Para a avaliação dos efeitos do congelamento, antes e após a maturação a seco, foram coletados 12 pares de contrafilé, que por sua vez foram divididos ao meio e distribuídos de forma balanceada em 4 tratamentos: maturação por 28 dias (Dry); maturação por 28 dias, seguido de processamento e congelamento dos bifes (Dry+ST); congelamento por 28 dias, seguido de descongelamento rápido (FT - “Fast thawing”; 20 ºC/15 h) e maturação por 28 dias (FT+Dry); congelamento por 28 dias, seguido de descongelamento lento (ST - “Slow thawing”; 4 ºC/48 h) e maturação por 28 dias (ST+Dry). Foram avaliadas as perdas de peso do processo, as características físico-químicas (pH, aw, substâncias reativas ao ácido tiobarbitúrico (TBARS), força de cisalhamento e cor instrumental), a contagem microbiológica, e o perfil de compostos voláteis. O congelamento, antes ou após maturação, alterou o perfil de compostos voláteis da carne, quando comparado à carne não congelada. O congelamento prévio a maturação não afetou a qualidade microbiológica ($P > 0,05$), no entanto este processo aumentou significativamente a perda de peso ($P < 0,05$) em comparação à carne submetida apenas ao procedimento de maturação, sendo considerado um procedimento inviável devido ao menor rendimento. Já o congelamento após a maturação a seco

não teve impacto negativo na qualidade físico-química, sendo considerado uma alternativa para armazenamento de carne maturada a seco. Em relação a presença de osso e gordura subcutânea, foram coletados 8 pares de contrafilé, que também foram divididos ao meio e distribuídos de forma balanceada entre 4 tratamentos: com osso e com gordura subcutânea; com osso e sem gordura subcutânea; sem osso e com gordura subcutânea; e sem osso e sem gordura subcutânea. Foram avaliadas as perdas de peso e rendimento total do processo, e as características físico-químicas das amostras (pH, aw, substâncias reativas ao ácido tiobarbitúrico – TBARS, força de cisalhamento, suculência e cor instrumental). Os resultados indicaram que os tratamentos não afetaram os valores de pH, TBARS, força de cisalhamento e suculência instrumental ($P > 0,05$). Além disso, ambos, osso e gordura subcutânea, apresentaram efeito protetor semelhante, reduzindo as perdas de peso e aumentando o rendimento do processo ($P < 0,05$), sendo considerados fatores primordiais para a maturação a seco onde se objetiva elevar o aproveitamento da matéria prima.

Palavras-chaves: maturação a seco, congelamento, osso, gordura subcutânea, rendimento.

ABSTRACT

Aging is one of the most common post-mortem methods used to increase beef tenderness. There are two types of aging: wet aging, in a vacuum package with low water vapor and gas permeability; and dry aging, exposing the beef without package, in a highly controlled aging chamber. This process of aging contributes to the development of more desired flavors on the beef. However, dry aging is a high-costly process, due to the weight loss caused by evaporation and trimming. Thus, the presence of bone and subcutaneous fat on the dry-aged beef cuts could reduce the weight loss and increase the process yield, as both act as a barrier to the evaporation. Furthermore, considering that the raw material used to produce dry-aged beef is from high-quality beef, which is a high-cost product with less availability in Brazil, the freezing storage could be a feasible alternative to increase the shelf-life and expand the market of dry-aged beef. Therefore, this study evaluated the feasibility of freezing, prior to and after dry aging, and its effects on the microbiological and physical-chemical quality, and yet the impact of bone and subcutaneous fat on the yield and physical-chemical traits of dry-aged beef. To evaluate the effects of freezing, prior to and after dry aging, strip loins (n = 24) from 12 carcasses were collected, cut in a half, and assigned to four treatments: non-frozen dry aging (Dry); dry aging, steak fabrication, freezing and slow thawing (Dry+ST); freezing, fast thawing (FT; 20 ºC/15 h) and dry aging (FT+Dry); freezing, slow thawing (ST; 4 ºC/48 h) and dry aging (ST+Dry). Weight loss, physical-chemical traits (pH, aw, thiobarbituric acid-reactive substances (TBARS), shear force and color display), microbial counts, and volatile compounds profile were analyzed. The results indicated that freezing, prior to and after dry aging, changed the volatile compounds profile compared to non-frozen samples. Freezing prior to dry aging did not affect the microbiological quality ($P > 0.05$), however, this process highly increased the weight loss ($P < 0.05$) compared to treatments dry-aged only, turning the process unfeasible. Freezing after dry aging had no negative impact on the physical-chemical quality of the samples, and it was considered a feasible alternative to storage dry-aged beef. Regarding the presence of bone and subcutaneous fat, bone-in loins from eight carcasses (n = 16) were collected, cut in a half, and evenly assigned to four treatments: bone-in with subcutaneous fat, bone-in without subcutaneous fat, boneless with subcutaneous fat, and boneless without subcutaneous fat. Weight loss, total yield, and physical-chemical traits (pH, aw, thiobarbituric acid-reactive substances (TBARS), shear force, pressed juice percentage and color display) were analyzed. The results indicated that

the treatments did not affect shear force, pressed juice percentage, TBARS and pH ($P > 0.05$). Moreover, both bone and subcutaneous fat, had a similar protective effect on the lean beef, reducing the weight loss and increasing the process yield ($P < 0.05$), being considered important factors to dry aging process yield.

Keywords: dry aging, freezing, bone, subcutaneous fat, yield.

LISTA DE ILUSTRAÇÕES

FIGURAS DO ARTIGO 1

FIGURAS DO ARTIGO 2

LISTA DE TABELAS

TABELAS DO ARTIGO 1

TABELAS DO ARTIGO 2

SUMÁRIO

1. INTRODUÇÃO GERAL

O Brasil ocupa um lugar de destaque na pecuária bovina mundial. O país possui o segundo maior rebanho do mundo, com cerca de 238 milhões de cabeças, além de ser o maior produtor e exportador desta fonte proteica, produzindo aproximadamente 10,2 milhões de toneladas de carne e destinando 2,2 milhões de toneladas ao mercado de exportação (USDA, 2019a).

Do rebanho brasileiro, em torno de 80% possui influência de gado zebu (*Bos indicus*) (Ferraz & Felício, 2010), majoritariamente alimentados a pasto. Animais zebuínos são conhecidos por apresentar menores taxas de maturação, e, além disso a dieta a pasto dificulta a deposição de gordura nas carcaças, o que pode comprometer sua qualidade sensorial (Carvalho et al., 2014; Miller et al., 2001; Koohmaraie, 1994; Shorthose & Harris, 1990).

Uma das maneiras mais comuns utilizadas para melhorar os atributos sensoriais da carne, como maciez e sabor, é a maturação (Sitz et al., 2006; Campbell et al., 2001). A maturação pode ser realizada de duas formas: a vácuo, em embalagens com baixa permeabilidade a gases e vapor de água; ou a seco, onde a carne é exposta, sem embalagem, a condições controladas de temperatura, umidade e velocidade do ar da câmara de maturação.

Até os anos 60 o único método conhecido e utilizado para maturação de carne era a seco. A partir dos anos 70, com o desenvolvimento de embalagens para acondicionamento a vácuo, a maturação a seco foi internacionalmente substituída pela maturação úmida (Savell, 2008). A maturação em embalagens a vácuo apresenta vantagens como menores: perdas de peso; encolhimento; e perdas econômicas (Savell, 2008). Dessa forma, a maturação úmida ganhou popularidade, devido à sua maior conveniência, rendimento e extensão da vida de prateleira, enquanto o produto maturado a seco se tornou um item para mercados especiais ou "gourmet" (Warren & Kastner, 1992).

Portanto, a maturação a seco pode ser definida como a maturação de carnes de alta qualidade, sob condições rigidamente controladas de temperatura, umidade relativa e velocidade do ar (Kim et al., 2016). Estes parâmetros precisam ser cuidadosamente controlados e monitorados para minimizar a perda de peso, inibir o crescimento microbiano e resultar em melhorias dos atributos sensoriais como sabor e maciez (Kim et al., 2016; Savell, 2008).

No entanto, devido ao rígido controle do processo, demanda por maiores espaços em câmaras (Smith et al., 2014), e maiores perdas de peso comparado a maturação a vácuo (Oreskovich et al., 1988; Parrish et al., 1991; Warren & Kastner, 1992; Dikeman et al., 2013), a produção de carne maturada a seco se torna um procedimento de alto custo (Miller et al., 1985; Smith et al., 2008, DeGeer et al., 2009). Com isso, reduzir a perda de peso durante o processo de maturação a seco, e aumentar o rendimento do produto final é de grande importância para redução dos custos da produção de carne maturada a seco. Sendo assim, a presença de osso e gordura subcutânea nos cortes cárneos utilizados para maturação a seco podem auxiliar na redução das perdas por evaporação, já que ambos funcionam como uma barreira que previne a perda de líquido por evaporação. Alguns estudos sobre maturação a seco demonstraram que cortes cárneos com osso apresentam menores perdas de peso quando comparados a carnes desossadas (Lepper-Blilie et al., 2016; DeGeer et al., 2009; Laster et al., 2008). Além disso, é de amplo conhecimento que o tecido muscular apresenta maiores perdas por evaporação do que o tecido adiposo (Johnson et al., 1988). Estudos já demonstraram que a gordura subcutânea possui a capacidade de reduzir o encolhimento de carcaças, por atuar como uma barreira à perda de umidade, prevenindo a evaporação de conteúdo líquido do tecido muscular (Savell et al., 2005; Smith & Carpenter, 1973). Dessa forma, é de grande relevância entender o impacto de ambos, osso e gordura subcutânea, no rendimento do processo de maturação a seco.

Além disso, como mencionado anteriormente a maturação a seco é normalmente realizada a partir de carne de alta qualidade, com alto nível mármore e espessa camada de gordura subcutânea. No entanto, no Brasil, a produção de carne de alta qualidade pode variar durante o ano, se tornando um produto com menor disponibilidade e de alto custo. Portanto, o congelamento de carne de alta qualidade pode ser considerado uma alternativa para estocagem deste produto, proporcionando melhores negociações de preços, e consequentemente aumentando a disponibilidade de carnes maturadas a seco no mercado nacional.

O congelamento é um dos métodos mais comuns utilizados para comercialização de carne, sendo uma das formas mais eficientes e efetivas de preservação dos alimentos (Kim et al., 2017). Além do mais, o congelamento pode trazer vantagens como melhoria da maciez da carne bovina, fato já demonstrado em alguns estudos sobre congelamento prévio à maturação a vácuo (Grayson et al., 2014; Lagerstedt et al., 2008; Shanks et al., 2002; Crouse & Koohmaraie, 1990), e após a maturação a seco (Kim et al., 2017). Como contraponto, o congelamento e

descongelamento da carne podem resultar em aumento da perda de peso por exsudato (Kim et al., 2017), e também está ligado à redução da qualidade sensorial devido ao aumento da oxidação de lipídeos e proteínas causado, principalmente, pelos danos às células musculares, que resultam na liberação de enzimas e pró-oxidantes (Leygonie et al., 2012).

Deste modo, devido à grande disponibilidade de carne de gado zebuíno no mercado Brasileiro, é de grande relevância realizar um estudo que avalie os efeitos tanto do congelamento, quanto da presença de osso e gordura subcutânea sobre o rendimento e características físico-químicas de carnes de animais zebuínos maturadas a seco.

2. OBJETIVO

O objetivo deste trabalho foi avaliar a viabilidade do congelamento, antes e após a maturação a seco, e os efeitos da presença de osso e gordura subcutânea no rendimento e características físico-químicas de carne de bovinos típicos brasileiros (zebuínos).

3. REVISÃO BIBLIOGRÁFICA

3.1. Maturação

Maturação é um processo de amaciamento da carne pela ação de enzimas musculares endógenas que apresentam função proteolítica no período post-mortem (Devine, 2004). A maturação é realizada através da estocagem da carne, sob condições de refrigeração, promovendo o amaciamento e desenvolvimento do sabor característico de carne maturada (Warren & Kastner, 1992).

Durante o período post-mortem diversas alterações bioquímicas ocorrem no músculo esquelético. Algumas destas alterações se dão devido à proteólise gerada pelo sistema de enzimas endógenas da carne (Toldrá et al.,1995). As principais enzimas endógenas que atuam no processo de maturação são proteases cálcio dependentes, conhecidas como calpaínas (Huff-Lonergan et al., 2010; Huff-Lonergan & Lonergan, 1999; Huff-Lonergan et al., 1996; Koohmaraie, 1992; Koohmaraie, 1988). E as duas isoformas melhor caracterizadas são as μ-calpaínas e m-calpaínas (Huff-Lonergan et al., 2010).

Durante o processo de proteólise causado pelas calpaínas, há um desarranjo das subunidades musculares, devido à fragmentação da linha-Z e da degradação de proteínas estruturais como titina, nebulina, desmina e troponina-T (Koohmaraie et al., 1988), o que leva a um aumento da maciez da carne. As calpaínas já foram amplamente estudadas por vários autores que determinaram sua participação no processo de proteólise e consequente aumento da maciez da carne durante a maturação (Goll et al., 1986).

Para que a proteólise post-mortem ocorra, são necessários quatro componentes chaves: íons cálcio, m-calpaína, μ-calpaína e calpastatina. Além disso, o pH e a temperatura influenciam fortemente o processo proteolítico (Koohmaraie, 1992). Sendo que a proteólise é mais intensa em temperaturas mais altas, e a redução do pH durante o período post-mortem favorece a atividade da enzima μ-calpaína sobre a m-calpaína (Dransfield, 1993).

No primeiros dois dias post-mortem ocorre uma grande liberação de íons cálcio, que juntamente com a atividade da m-calpaína leva a uma proteólise intensa. Porém após 24 horas post-mortem a m-calpaína perde aproximadamente 60% da sua atividade enzimática (Dransfield,

1993; Koohmaraie et al., 1987). Ainda assim, o processo de proteólise continua, agora pela atividade em conjunto das enzimas m e μ-calpaínas. Após 6 dias de maturação, a m-calpaína não apresenta mais atividade enzimática e apenas μ-calpaína atua sobre as proteínas estruturais da carne (Dransfield, 1993; Koohmaraie et al., 1987).

As m e μ-calpaínas estão presentes no músculo "in vivo", porém não expressam atividade proteolítica dado ao pH elevado e a atividade da enzima calpastatina. A calpastatina atua como um inibidor de calpaínas. Contudo, a queda do pH durante o período post-mortem favorece a atividade proteolítica das calpaínas e diminui a capacidade inibitória da calpastatina (Cottin et al., 1981), garantindo que o processo proteolítico continue durante todo o período de maturação da carne (Devine, 2004).

A última etapa da proteólise ocorrida na maturação é a geração de aminoácidos livres (Toldrá et al., 1995). De fato, a maturação deixa os alimentos mais saborosos (Yamaguchi & Ninomiya, 1999). O estudo de Nishimura et al. (1988) relacionou o aumento de aminoácidos livres em carne maturada com a melhora no sabor. A pesquisa desenvolvida por Polak et al. (2007) encontrou um aumento na concentração de aminoácidos livres em carne bovina com períodos maiores de maturação incluindo um aumento no teor de glutamato.

O glutamato está presente em diversos alimentos como pescados, queijos e carnes nos quais ele melhora o gosto e a palatabilidade (Bagnasco et al., 2014). Segundo Yamaguchi and Ninomiya (1999), o gosto umami, relacionado com uma descrição de saboroso, cárneo, "similar a caldo de carne", é transmitido, entre outros compostos, pelo glutamato. Dessa forma, a melhora do sabor na carne maturada que se associa ao aumento do teor de aminoácidos livres pode estar relacionada ao aumento de compostos geradores de gosto umami como o glutamato.

3.2. Maturação a seco (″Dry aging″)

Fundamentalmente, existem dois tipos de maturação: a maturação úmida ("Wet-aging"), que consiste em acondicionar a carne em embalagem a vácuo com baixa permeabilidade a gases e vapor da água, sob refrigeração; e a maturação a seco ("Dry aging"), que implica na estocagem da carne, sem embalagem, sob condições controladas de temperatura, umidade e fluxo de ar (Ahnström et al. 2006, Campbell et al. 2001). Independente do processo de maturação utilizado, a maciez sensorial ou instrumental parece não ser afetada (Vilella et al., 2019)

A maturação a seco é o método mais antigo de maturação. Até os anos 60 a única forma de se maturar carne era através da maturação a seco (Savell, 2008). Porém, a partir da década de 70, com o surgimento da tecnologia de embalagem a vácuo, a maturação a seca foi amplamente substituída pela maturação a vácuo, devido principalmente à facilidade de armazenamento e transporte, menores perdas de peso, e consequentemente menores perdas econômicas (Savell, 2008).

Atualmente, maturação a seco pode ser definida como o método de maturação de carnes de alta qualidade sob condições controladas de umidade, temperatura e velocidade do ar (Kim et al., 2016). Estes parâmetros precisam ser rigidamente controlados e monitorados a fim de reduzir as perdas de peso e ao mesmo tempo inibir o crescimento microbiano, resultando em aumento da maciez e melhoria do sabor (Kim et al., 2016; Savell, 2008).

O grande inconveniente de se realizar a maturação a seco é o alto custo do processo devido à necessidade de maiores espaços em câmaras de refrigeração, encolhimento das peças, e perdas de processo por evaporação e remoção das superfícies secas (Stenström et al., 2014; DeGeer et al., 2009; Smith et al., 2008; Parrish et al., 1991; Miller et al., 1985). Com isso, o produto maturado a seco se tornou um item para mercados especiais ou "gourmet" (Warren & Kastner, 1992).

O principal motivo para realização da maturação a seco é o desenvolvimento de sabor característico do produto (Stenström et al., 2014; Li et al., 2014; Degeer et al., 2009; Savell, 2008; Ahnström et al., 2006; Warren & Kastner, 1992), como sabor de carne assada (Campbell et al. 2001; Warren & Kastner, 1992) e gosto umami (Li et al., 2014). Porém esse desenvolvimento de sabor é controverso, já que outros estudos não encontraram diferença quando comparado carnes de maturação a seco e a vácuo (Laster et al., 2008; Parrish et al., 1991).

O desenvolvimento do sabor durante o processo de maturação está relacionado ao aumento da concentração de aminoácidos livres (Polak et al., 2007; Nishimura et al., 1988), incluindo o glutamato, um dos compostos responsáveis pelo gosto umami (Yamaguchi & Ninomiya, 1999).

3.3. Efeitos do congelamento

Embora as tecnologias de congelamento e refrigeração tenham se desenvolvido no último século, o congelamento da carne com intuito de aumentar seu "shelf life" já é praticado há milhares de anos (Leygonie et al., 2012). Além disso, o congelamento desempenha um papel essencial na indústria da carne, garantindo que os produtos sejam distribuídos de forma segura pelo mundo todo (Leygonie et al., 2012). No entanto, o processo de congelamento e descongelamento da carne resulta em um aumento da perda de peso por exsudação (Kim et al., 2017), e pode ainda influenciar diretamente a qualidade sensorial devido à oxidação lipídica e proteica causado pela liberação de enzimas pró-oxidantes devido à ruptura das células musculares, que por sua vez é ocasionada pela formação de cristais de gelo entre essas células (Leygonie et al., 2012).

Durante o congelamento ocorre formação de cristais de gelo, que resultam no rompimento das fibras musculares e estrutura celulares (Kim et al., 2017; Vieira et al., 2009; Petrović et al., 1993). Devido à esse rompimento das fibras, o congelamento pode também aumentar a maciez da carne, fato já demonstrado em vários estudos (Kim et al., 2017; Grayson et al., 2014; Lagerstedt et al., 2008; Shanks et al., 2002; Wheeler et al., 1992; Crouse & Koohmaraie, 1990). Alguns estudos indicam que o congelamento prévio à maturação a vácuo aumenta a maciez da carne quando comparada com carnes não congeladas e maturadas pelo mesmo período (Grayson et al., 2014; Lagerstedt et al., 2008; Shanks et al., 2002; Crouse & Koohmaraie, 1990). E em relação à maturação a seco, Kim et al. (2017) demonstraram que o congelamento (“blast chiller” ou congelamento criogênico) após a maturação a seco também resultou no aumento da maciez da carne.

Outro efeito importante do congelamento e descongelamento é a alteração no conteúdo e distribuição da água nos tecidos da carne, que pode ser avaliada de diversas maneiras, como perdas por gotejamento, descongelamento, teor de umidade, capacidade de retenção de água e cocção (Leygonie et al., 2012). De acordo com Lagerstedt et al. (2008) carnes congeladas apresentam maiores perdas de água (gotejamento) quando comparadas à carnes mantidas resfriadas pelo mesmo período. Nos estudos científicos há um consenso de que o congelamento contribui para a redução da capacidade de retenção de água da carne (Leygonie et al., 2012; Vieira et al., 2009; Ngapo et al., 1999; Añón & Cavelo, 1980).

Em relação ao descongelamento, estudos indicaram que a redução do tempo de descongelamento pode resultar em uma menor quantidade de exsudato (Haugland, 2002; Ambrosiadis et al., 1994; Gonzalez-Sanguinetti et al., 1985). Este fato pode estar relacionado à reabsorção da água pelas fibras, devido ao aumento da atividade de água pelo derretimento do gelo extracelular (Gonzalez-Sanguinetti et al., 1985).

3.4. Efeitos da presença de osso e gordura subcutânea no rendimento

A maturação a seco consiste em dispor o corte cárneo, sem embalagem, na câmara de refrigeração sob condições controladas (Ahnström et al., 2006). Porém, é um procedimento de alto custo devido às grandes perdas por evaporação e remoção das superfícies secas (Dikeman et al., 2013; Warren & Kastner, 1992; Parrish et al., 1991; Oreskovich et al., 1988). Neste contexto, duas características que podem afetar a perda de peso durante o processo de maturação a seco é a presença de osso e a espessura da gordura subcutânea, pois ambos apresentam a capacidade de atuar como uma barreira protetora, reduzindo a perda de peso por evaporação.

Em relação à presença de osso, estudos feitos sobre maturação a seco de carne indicaram menor perda de peso, e consequentemente maior rendimento, em carnes maturadas com osso (DeGeer et al., 2009; Laster et al., 2008). Ainda, DeGeer et al. (2009) demonstraram aumento de 10-12% no rendimento quando a maturação a seco é realizada com osso.

Quanto à gordura subcutânea, já foi comprovado que o tecido muscular (magro) apresenta maior capacidade de perda de umidade comparado ao tecido adiposo (Johnson et al., 1988). Pascoal et al. (2011) avaliaram perda de peso em carcaças com gordura subcutânea uniforme, suficiente e insuficiente e verificaram que quanto maior a espessura de gordura subcutânea, menores são as perdas por encolhimento. No entanto, como 80% do gado brasileiro é de origem zebuína (Ferraz & Felício, 2010), que em geral apresenta pouca deposição de gordura subcutânea, quando comparado à animais de raças britânicas (Restle et al., 1999; Whipple et al., 1990; Wheeler et al., 1990; Crouse et al., 1989), é de grande importância avaliar como a espessura de gordura pode afetar a maturação a seco de carne proveniente de animais zebuínos. Já que essa característica de menor gordura subcutânea das carcaças pode afetar o rendimento do processo de maturação a seco, pois o tecido adiposo atua como uma barreira à perda de peso por evaporação (Savell et al., 2005; Smith & Carpenter, 1973).

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5. ARTIGO 1

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Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef

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ABSTRACT

This study evaluated the effects of freezing, prior to and after dry aging, on the microbiological and physical-chemical quality of beef. Strip loins (n = 24) from 12 carcasses were assigned to four treatments: non-frozen dry aging (**Dry**); dry aging, steak fabrication, freezing and slow thawing (**Dry+ST**); freezing, fast thawing (FT; 20 ºC/15 h) and dry aging (**FT+Dry**); freezing, slow thawing (ST; 4 ºC/48 h) and dry aging (**ST+Dry**). Freezing conditions were -20 ºC/28 days and dry aging conditions were 2 ºC/70% relative humidity, for 28 days. Freezing prior to dry aging did not affect the microbial counts compared to Dry. However, FT+Dry and ST+Dry increased (16%) total process loss ($P < 0.05$) compared to Dry and Dry+ST. Moreover, freezing changed volatile compounds profile. Thus, freezing prior to dry aging was not a feasible process due to increased process loss, while freezing after dry aging was considered a viable alternative to preserve the steaks without compromising beef physical-chemical traits.

Keywords: dry-aged beef; freezing; thawing; microbiological beef quality; volatile compounds.

5.1. Introduction

Dry-aged beef is the product of aging unpackaged beef under controlled conditions of temperature, relative humidity and air velocity. This aging process improves tenderness and contributes to "dry-aged beef" flavor (Savell, 2008). However, dry aging is a costly process (Miller, Davis, & Ramsey, 1985; Smith et al., 2008, DeGeer et al., 2009) as it requires strictly controlled conditions, larger spaces in chambers (Smith et al., 2014), and causes higher weight loss compared to wet-aging (Oreskovich et al., 1988; Parrish et al., 1991; Warren & Kastner, 1992; Dikeman et al., 2013).

Additionally, dry-aged beef is usually produced with high-quality beef from British cattle, which presents high marbling and thick subcutaneous fat. However, in Brazil, about 80% of the cattle has influence of zebu breeds (Ferraz & Felício, 2010) raised on grass-fed system, and known for their low marbling and thin subcutaneous fat. Thus, the Brazilian production of high-quality beef is limited and can vary during the year, making dry-aged beef a seasonal and costly product. Therefore, freezing could be an alternative to store high-quality beef, avoiding the seasonality in supply, allowing negotiations for best prices, and increasing the availability of dry-aged beef in the market.

Freezing is one of the most common and efficient methods to preserve and market beef (Kim et al., 2017). Several studies have reported that freezing may increase tenderness when applied prior to aging in a vacuum bag (Grayson et al., 2014; Lagerstedt et al., 2008; Shanks, Wulf, & Maddock, 2002; Crouse & Koohmaraie, 1990) and after the dry aging process (Kim et al., 2017). However, freezing and thawing can increase the weight loss by purging (Kim et al., 2017), and may reduce the sensory quality due to fat and protein oxidation, caused by damage in the muscle cells that release pro-oxidative enzymes (Leygonie, Britz, & Hoffman, 2012).

Although several studies have reported the effects of freezing and thawing on wet-aged beef (Kim et al., 2015; Grayson et al., 2014; Lagerstedt et al., 2008; Shanks, Wulf, & Maddock, 2002; Crouse & Koohmaraie, 1990), few studies have investigated freezing and thawing prior to or after dry aging and how this procedure impacts dry-aged beef quality attributes. Therefore, this study assessed the viability to produce dry-aged beef with previously frozen loins and analyzed the effects of thawing at two different rates (temperature x time) on the yield, physical-chemical traits, volatile compounds, and microbial characteristics. In addition, it was evaluated the impact of freezing steaks after dry aging on physical-chemical quality attributes.

5.2. Material and methods

5.2.1. Raw material preparation and aging conditions

Twelve pairs of strip loins (left and right-side) from grass-fed zebu steers (around 3 years old; 288 kg ± 35 kg average carcass weight; and 3.3 ± 0.3 mm of subcutaneous fat measured between the 12th and 13th ribs) were collected at 3 days postmortem from a commercial beef plant.

The strip loins were vacuum packaged in bags (50 µm thick; O_2 permeability of 20 cm^3 O_2/m^2/24 h at 23 °C, atmospheric pressure and 0% relative humidity; maximum CO_2 permeability of 100 cm^3 CO_2/m^2/24 h at 23 °C, atmospheric pressure and 0% relative humidity; Cryovac® BB 2620, Cryovac Brasil Ltda.), placed on ice, and transported to the Meat Laboratory at the University of Campinas, São Paulo, Brazil.

At the laboratory, one steak was cut out from the middle of each strip loin for sample characterization analyses (pH, moisture and fat content, Warner-Bratzler shear force, and volatile compounds), providing other two loin sections per strip loin (four per animal). Each section position (anterior and posterior) was balanced allocated into one of the four treatments, avoiding any position effect among the treatments: dry aging for 28 days, steak fabrication and analyses on non-frozen steaks (Dry); dry aging for 28 days, followed by steak fabrication, freezing of steaks at -20 ºC for 30 days, slow thawing (ST) at 4 ºC/24 h and analyses (Dry+ST); freezing of loins at -20 ºC for 30 days, fast thawing (FT) at 20 ºC/15 h (controlled room temperature), dry aging for 28 days, followed by steak fabrication and analyses (FT+Dry); freezing of loins at -20 ºC for 30 days, slow thawing (ST) at 4 ºC/48 h, dry aging for 28 days, followed by steak fabrication and analyses (ST+Dry).

The sections assigned to FT+Dry and ST+Dry were vacuum packaged, and evenly distributed in a commercial freezer (BVR28 model, Brastemp - Whirlpool, Brazil) used exclusively for this experiment and set to -20 ºC. The remaining sections were placed in an adapted aging chamber (VN50R model, Metalfrio 2010 ©, Brazil) at 2 ºC, 70% relative humidity and 2.5 m/s of air velocity.

5.2.2. Weight loss and steak preparation

The strip loins sections were weighed prior to and after aging and the percentage of evaporation loss was calculated as: (initial weight – post-aging weight)/initial weight x 100. After aging, the dried surface was trimmed (approximately 5mm-thick) and weighed. The percentage of trimming loss was calculated as: (weight of trimmings/initial weight) x 100. The percentage of thawing loss was also determined by weighing the strip loin sections before and after the freezing/thawing process: (initial weight – post-thawing weight/initial weight) x 100.

After the trimming process, steaks (2.54 cm-thick) were cut out from the loin sections, using a ruler to measure the thickness of each steak. The steaks were assigned to moisture content and pH, thiobarbituric acid-reactive substances, shear force, color display, and volatile compounds. Samples for microbiological analyses were collected from the dehydrated lean surface, avoiding subcutaneous fat.

5.2.3. pH and water activity

The pH was measured in duplicate by inserting a calibrated puncture pH electrode (MP125 portable pH meter, Mettler Toledo, Brazil) directly into the steaks assigned to this analysis, cut from both fresh (non-frozen and non-aged) and aged samples. The water activity was measured in the dried trimmed crust (2 mm-thick), using a water activity analyzer (Aqualab 4TE, Decagon, São Paulo, Brazil).

5.2.4. Moisture, fat and thiobarbituric acid-reactive substances (TBARS)

The internal moisture content was determined for both non-aged and aged samples, in triplicate, by grinding lean beef and drying it in a forced air convection oven, according to AOAC (1990) procedures. The fat content was determined according to the Bligh and Dyer (1959) procedures, in non-aged samples. The thiobarbituric acid-reactive substances (TBARS) were determined, in quadruplicate, on the internal lean beef after aging, according to Bruna et al. (2001), modifying the procedure by adding 20 mL of 5% TCA instead of 15 mL of 0.38 M $HClO_4$.

5.2.5. Warner-Bratzler shear force (WBSF)

Steaks (2.54 cm-thick) assigned for the shear force analysis were cooked using some adaptations from the American Meat Science Association cookery guidelines (AMSA, 2015). The steaks were placed on a metal rack over and aluminum tray and cooked in an electric oven (Fritomaq, Brazil) at 170 ºC until the internal temperature reached 71 ºC measured by copper-constantan thermocouples (E5CWL Omron, CSW) inserted into the geometric center of the steaks. Each steak was weighed, prior to and after cooking, and the cooking loss was calculated according to the following equation: (raw weight – cooked weight/raw weight) x 100.

After cooking, the steaks were kept at room temperature to cool (about 30 minutes) and then overwrapped in polyvinyl chloride film and chilled overnight at 4 ºC according to AMSA (2015) procedures. Six round cores of 1.27 cm diameter were removed from each steak parallel to the muscle fibers using a handheld coring device. The shear force (N) was measured by shearing each core in the center using a Warner-Bratzler blade attached to a Texture Analyzer (TA-XT Plus, Texture Technologies Corp./ Stable Micro Systems, UK) with a crosshead speed of 250 mm/minute (AMSA, 2015).

5.2.6. Color

Each steak assigned for the measurement of instrumental color was placed on a polystyrene tray covered with a polyvinyl chloride film and placed in a cooler at 4 ºC, without light exposure, for 5 days. The instrumental color was measured every day in triplicate using a portable colorimeter (CM 508-d, Hunter MiniScan TMXE) with attached moisture protector accessory and calibrated using white and black tile standards. The color was determined by CIE L*, a* and b* values using the illuminant D65 source and the standard observer of 10°, according to AMSA (2012) protocol. Chroma and hue angle were calculated by using the CIE L*, a*, and b* values, according to the following formulas; Chroma = $(a^{*2}+b^{*2})^{1/2}$ and hue = $\tan^{-1}(b^*/a^*)$ (AMSA, 2012).

5.2.7. Microbiological analyses

Samples for microbiological analyses were collected prior to aging and freezing (fresh samples), after thawing (FT+Dry and ST+Dry), and after dry aging (Dry, FT+Dry and ST+Dry).

Ten grams of the beef surface, measuring ± 2mm-thick, were aseptically collected from three loin sections of each step and treatment mentioned above. Each sample was homogenized with 90 mL of 0.1% peptone water (Difco, MD, USA) in a stomacher (Stomacher 400 circulator, Seward, UK) for 2 min at 230 rpm. When necessary, decimal dilutions were performed in 0.1% peptone water (Difco). Samples were analyzed for total bacterial count (TBC), psychrotrophic microorganisms (PSY), *Enterobacteriaceae* (ENT), lactic acid bacteria (LAB) and yeasts and molds (YM).

The TBC and PSY counts were determined in Plate Count Agar (PCA, Acumedia, MI, USA) with incubation at 35 ºC for 48 hours and 7 ºC for 10 days, respectively (Ryser & Schuman, 2015; Vasavada & Critzer, 2015). The LAB was determined using Man, Rogosa and Sharpe agar (MRS, Difco) incubated under anaerobic conditions (Probac, Brazil) at 35 ºC for 72 hours (Njongmeta et al., 2015). To quantify the *Enterobacteriaceae* family, Violet Red Bile Glucose agar (VRBG, Acumedia) in pour plate with overlay and incubation at 35 ºC for 24 hours was used (Kornacki, Gurtler, & Stawick, 2015). The YM counts were determined in Dichloran Rose Bengal Chloramphenicol agar (DRBC, Acumedia) incubated at 25 ºC for 5 days (Ryu & Wolf-Hall, 2015). The molds isolated from dry aged samples were cultivated in Czapek Yeast Autolysate agar (CYA) at 25 ºC for 7 days and identified by morphological characteristics (Pitt & Hocking, 2009).

5.2.8. Volatile compounds qualitative profile

A pool of 12 steaks (2.5 cm-thick) was obtained for each treatment. The steaks were cooked, separately, in a Combi oven (Tedesco, Santa Maria, RS, Brazil), pre-heated at 180 °C, until the samples reached an internal temperature of 75 °C. After cooking, the 12 steaks of each treatment were ground all at once, obtaining a pooled sample. Ten grams of this pool were weighed in 60 mL flasks in triplicate. The solid-phase microextraction technique was used for volatile compounds extraction, using a CAR/PDMS (Carboxen/polydimethylsiloxane, Sigma, Bellefonte, PA, USA) fiber as stationary phase. The extraction was performed at 60 °C for 10 minutes in a water bath, and then the fiber was exposed in the headspace for 65 minutes. Gas Chromatography coupled to Mass Spectrometry (GC-MS) (QP-2010 model, Shimadzu® Kyoto, Japan) was used to separate and identify beef volatile compounds. Thermal desorption was performed at 300 °C in a splitless mode injector for 1 minute. The volatile compounds were

separated by a DB- 5 MS (5 % phenyl, 95 % dimethylpolysiloxane) of 60 m x 0.25 mm internal diameter and 1 μm width of the stationary phase (J&W Scientific®, Santa Clara, CA, USA). The column temperature started at 40 °C for 2 minutes, increasing the temperature at the rate of 4 °C/min until 180 °C, followed by a rate of 60 °C/min up to 300 °C, remaining at this temperature for 5 minutes. The rate of 60 °C/min was used to reach the final temperature faster and cleaning the column, as the volatile compounds of interest are detected up to 180 °C. Helium was the carrier gas, at a flow rate of 1 mL/min. A quadrupole mass detector was operated in the following conditions: ionization energy 70eV, interface temperature of 300 °C, ions source temperature 200 °C. The mass spectrum was set at the scanning mode, monitoring the range from 35 to 350 *m/z*. Compounds were identified by their mass spectra, compared to the library database of GC-MS (NIST, 2002). To confirm identification, a n-alcane (C_7-C_{30}) (Supelco, Bellefonte, PA, USA) solution was injected into the equipment under the same conditions as the samples to obtain the LTPRI (linear temperature programmed retention index of the volatile compounds). The experimental identification was performed by comparing the LTPRI and the mass spectra with the reports from the literature, with a similarity of a minimum of 85% and maximum variation of ±10.

Specific compounds of each volatile compound were selected, transformed to log10 and a principal component analysis (PCA) was applied to check any separation between groups within the effects studied based on the volatile compounds.

5.2.9. Data analyses

This experimental design allocated 24 loins from 12 beef carcasses into 4 balanced treatments considering four freezing/thawing methods: non-frozen; frozen steaks after dry aging; frozen before dry aging and thawed at 20 ºC/15 h; frozen prior to dry aging and thawed at 4 ºC/48 h. The data were analyzed using the software Statistica 10.0 (StatSoft, 2010) for ANOVA one-way. For color, the data were analyzed using the variance analysis for repeated measures ANOVA. The means of interest were analyzed by the Tukey test at 5% level of significance.

5.3. Results and discussion

5.3.1. Sample characterization

Samples were characterized prior to aging. On average, raw samples had 3.3 ± 0.3 mm thickness of subcutaneous fat, a pH of 5.43 ± 0.02, moisture and fat content of 75.1 ± 0.2% and 1.9 ± 0.1%, respectively, and Warner-Bratzler shear force of 52.87 ± 2.83 N.

5.3.2. Thawing, trimming, evaporation and total process losses

Thawing losses were lower for FT+Dry compared to ST+Dry ($P < 0.05$; Figure 5.1). According to Haugland (2002) fast thawing (lower thawing time) reduces exudate formation. In addition, Gonzalez-Sanguinetti, Añón and Cavelo (1985) indicated that the lower exudate formation is related to water flow into intracellular spaces and water reabsorption by the muscle fibers, caused by increased water activity due to the melting of ice crystal formed in the extracellular spaces during the freezing process. The Dry+ST steaks, that were frozen after dry aging, had on average 1.1 ± 0.1% of thawing loss. The trimming loss reached about 16% and no difference was observed between the treatments ($P = 0.258$; Figure 5.1).

The FT+Dry and ST+Dry had higher evaporation and total process losses compared to Dry and Dry+ST ($P < 0.05$; Figure 5.1). Evaporation loss was about 4 percentage points higher for FT+Dry and ST+Dry and, adding to thawing loss, these samples had up to 11 percentage points more of total process loss than Dry and Dry+ST did. This increase in evaporation loss, and consequently in total process loss, is related to muscle fiber disruption due to the formation of ice crystals that damages the fiber structure and concentrates solutes leading to protein denaturation (Leygonie et al., 2012) and to a decrease in the water-holding capacity of meat (Leygonie et al., 2012; Vieira et al., 2009; Ngapo et al., 1999; Añón & Cavelo, 1980). Therefore, in this study, the freezing of the loins prior to dry aging was not considered a viable procedure to store high-quality beef. Increasing total process loss could also result in higher production costs, making the process inviable.

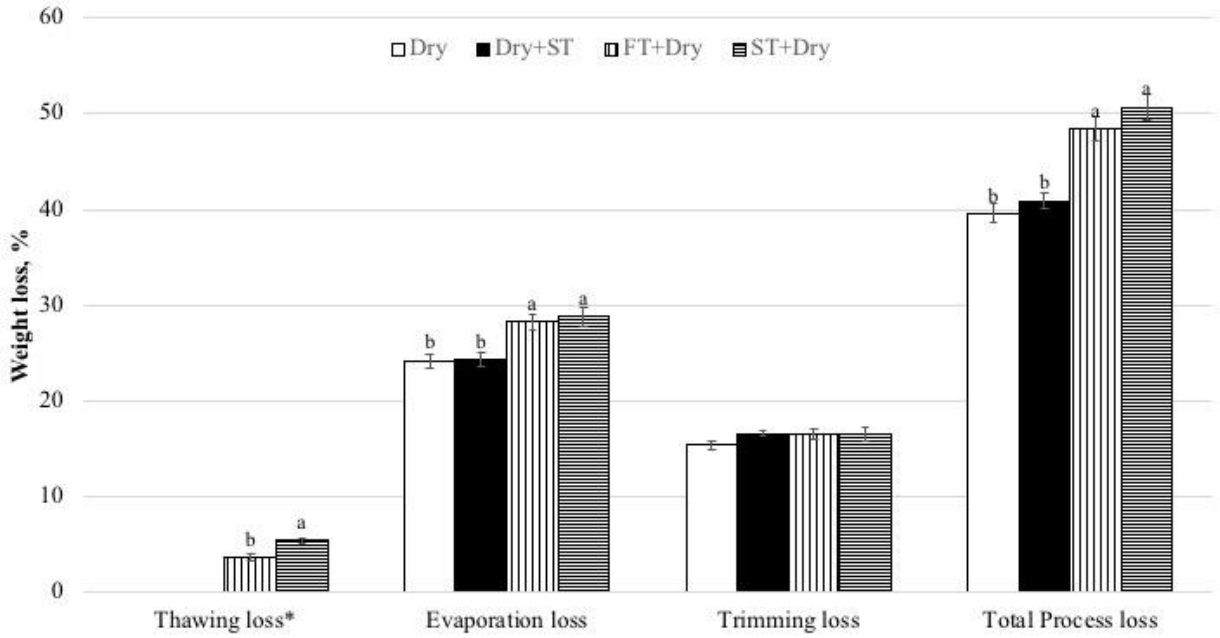

Fig. 5. 1. Effect (mean ± SEM) of treatments on thawing, evaporation, trimming and total process losses on beef samples (n=48).

a, b Different letters among treatments within each weight loss parameter (thawing, evaporation, trimming and total process loss) indicate significant differences ($P < 0.05$). * Thawing loss of Dry+ST was measured on thawed steaks (4 ºC/10 h) and had 1.1 ± 0.1 %. **Dry:** dry aging for 28 days; **Dry+ST:** dry aging for 28 days, steaks fabrication, freezing at -20 ºC and thawing at 4 ºC/24 h; **FT+Dry:** freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; **ST+Dry:** freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days.

5.3.3. pH, water activity, moisture and thiobarbituric acid-reactive substances (TBARS)

The final pH was not affected by the treatments (P = 0.274; Table 5.1). Previous studies have reported similar results, where the freezing process did not influence the pH either prior to or after aging (Kim et al., 2017; Kim et al., 2015; DeGeer et al., 2009).

Regarding the effects of treatments on the water content, the FT+Dry and ST+Dry showed lower values of surface water activity and moisture content compared to Dry and Dry+ST (P < 0.05; Table 5.1). These results were expected as freezing reduces the meat water-holding capacity due to muscle fiber disruption caused by ice crystals formation (Leygonie et al., 2012).

No differences were observed in TBARS values between the treatments (P = 0.971; Table 5.1). Moreover, the values obtained in the TBARS analysis were lower than the threshold for detection of rancid flavor, which is 2 mg MDA/kg (Vieira et al., 2009; Campo et al., 2006; Greene & Cumuze, 1981; Watts, 1962). These low TBARS could be related to the high levels of vitamin E found in beef from grass-fed cattle. Studies have demonstrated that beef from grass-fed cattle has higher vitamin E contents, which increases the beef stability against lipid oxidation (Warren et al., 2008; Nuernberg et al., 2005). Additionally, Nuernberg et al. (2005) indicated that beef from grass-fed cattle showed more oxidative stability than from cattle fed with concentrate diet, even for previously frozen beef. Although it was not determined, it seems that the beef from grass-fed cattle used in the current study had high Vitamin E contents, which provided the oxidative stability. Further investigation is necessary to evaluate the effects of Vitamin E and other potential antioxidants on freezing and dry aging processes.

Table 5. 1. Effect (mean ± SEM) of treatments on pH, surface aW, moisture, TBARS, Warner-Bratzler shear force (WBSF) and cooking loss (n=48) of beef samples.

	Dry	Dry+ST	FT+Dry	ST+Dry	P-value
pH	5.59 ± 0.02	5.53 ± 0.02	5.51 ± 0.02	5.45 ± 0.09	0.274
Surface aw	0.9419 ± 0.0017$_{a}$	0.9384 ± 0.0030$_{ab}$	0.9269 ± 0.0030$_{bc}$	0.9255 ± 0.0046$_{c}$	<0.05
Moisture, %	72.80 ± 0.29$_{a}$	72.91 ± 0.28$_{a}$	70.02 ± 0.27$_{b}$	69.61 ± 0.31$_{b}$	<0.0001
TBARS, mg MDA/kg	0.24 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.03	0.971
WBSF, N	34.43 ± 1.85	31.00 ± 2.03	31.69 ± 1.04	32.29 ± 1.07	0.449
Cooking loss, %	17.18 ± 0.57$_{a}$	17.03 ± 0.72$_{a}$	13.10 ± 0.51$_{b}$	12.62 ± 0.71$_{b}$	<0.0001

$_{a,b}$ Different letters in a row indicate significant differences ($P < 0.05$).

Dry: dry aging for 28 days; **Dry+ST:** dry aging for 28 days, steaks fabrication, freezing at -20 ºC and thawing at 4 ºC/24 h; **FT+Dry:** freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; **ST+Dry:** freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days.

5.3.4. Warner-Bratzler shear force (WBSF) and cooking loss

No differences were observed in shear force values for the different treatments ($P = 0.449$; Table 5.1). Aroeira (2014) also found no differences in shear force values between samples frozen prior to aging and non-frozen samples, both aged for the same period. Wheeler et al. (1990) reported no differences in shear force values of samples frozen after 14 days of aging compared to samples aged for 13 days and non-frozen samples. However, several studies indicate that freezing prior to aging increases beef tenderness (Kim et al., 2017; Lagerstedt et al., 2008; Shanks, Wulf, & Maddock, 2002; Crouse, & Koohmaraie, 1990) which can be associated to muscle fiber disruption through ice crystal formation (Leygonie et al., 2012; Petrović, Grujić, & Petrović, 1993) and/or by the release of proteolytic enzymes that increase the aging rate (Vieira et al., 2009). According to Setyabrata and Kim (2019), this divergence between studies could be related to the various intrinsic and extrinsic factors that affect shear force values; therefore, further investigations are necessary to evaluate the decrease in shear force due to freezing on sensory perception.

In this study, FT+Dry and ST+Dry samples had lower cooking loss compared to Dry and Dry+ST samples ($P < 0.05$; Table 5.1). This result was expected due to the lower moisture content found in the FT+Dry and ST+Dry after aging thus less water was available to be lost during the cooking procedure.

5.3.5. Color

No differences were observed in L* values (lightness) during 4 days of display ($P > 0.05$; Table 5.2). However, on the 5th day of display the FT+Dry had slightly lower L* values ($P < 0.05$; Table 5.2) compared to the other treatments, although this difference was less than three units, which was considered minimal. Even though several studies have described that freezing and thawing resulted in lower L* values (darker color) compared to non-frozen beef (Aroeira et al., 2017; Kim et al., 2017; Vieira et al., 2009), the results of this current study indicated that the beef lightness was not affected by freezing. This suggests that the dry aging process had more impact on the L* values than the freezing process did.

The Dry showed higher a* (redness), b* (yellowness), and chroma (color intensity) values ($P < 0.05$; Table 5.2) compared to the other treatments during the display period, except on the

2nd day, when Dry had a sharp decrease in a*, b*, and chroma values ($P < 0.05$; Table 5.2). These observations indicated that freezing, either prior to or after dry aging, reduced the beef color stability. Similar results were also observed in other studies, where frozen beef had lower a* values than non-frozen (Setyabrata & Kim, 2019; Kim et al., 2017; Kim et al., 2015; Vieira et al., 2009). The freezing of meat causes muscle fiber disruption and concentration of solutes, which results in protein denaturation, including myoglobin (Setyabrata & Kim, 2019; Leygonie et al., 2012). Thus, freezing increases the susceptibility of myoglobin oxidation (Setyabrata & Kim, 2019; Kim et al., 2015; MacDougall, 1982; Renerre, 1990) and consequently reduces color stability of the frozen/thawed meat compared to non-frozen one. However, in the current study the ST+Dry had the lowest hue angle values ($P < 0.05$; Table 5.2) during display compared to the other treatments, excepting on the 4th day where there was no difference between the treatments ($P = 0.141$; Table 5.2). For meat color analysis, the increase of hue values during display storage correspond to more discoloration (Kim et al., 2017; Vieira et al., 2009). Overall, hue angle showed similar values throughout the display period within each treatment.

Table 5. 2. Effect (mean ± SEM) of treatments on L*, a*, b*, hue angle, and chroma parameters of color stability during 5 days of display (n=48).

Treatment	Color parameter	Display Period (days)				
		1	2	3	4	5
Dry	L*	33.95 ± 0.68	29.19 ± 0.66	33.48 ± 1.04	32.71 ± 0.82	32.47 ± 0.90$_{ab}$
Dry+ST		32.65 ± 0.78	29.88 ± 0.66	31.22 ± 0.65	30.83 ± 0.62	31.30 ± 0.51$_{ab}$
FT+Dry		34.56 ± 0.61	28.93 ± 0.62	31.22 ± 0.82	31.68 ± 1.03	29.97 ± 0.75$_{b}$
ST+Dry		35.45 ± 0.87	31.19 ± 0.64	32.39 ± 0.71	33.55 ± 0.59	32.94 ± 0.61$_{a}$
P-value		0.070	0.084	0.168	0.102	<0.05
Dry	a*	23.84 ± 0.54$_{a}$	16.73 ± 0.83$_{ab}$	21.69 ± 0.95$_{a}$	20.82 ± 0.73$_{a}$	21.29 ± 0.79$_{a}$
Dry+ST		19.25 ± 0.66$_{b}$	19.29 ± 0.47$_{a}$	17.79 ± 0.43$_{b}$	16.61 ± 0.50$_{b}$	15.49 ± 0.40$_{b}$
FT+Dry		18.49 ± 0.81$_{b}$	18.76 ± 0.65$_{ab}$	15.48 ± 0.75$_{bc}$	15.48 ± 0.82$_{bc}$	14.46 ± 0.53$_{c}$
ST+Dry		16.65 ± 0.83$_{b}$	16.43 ± 0.80$_{b}$	14.59 ± 0.79$_{c}$	13.28 ± 0.54$_{c}$	12.12 ± 0.64$_{c}$
P-value		<0.0001	<0.05	<0.0001	<0.0001	<0.0001
Dry	b*	20.67 ± 0.42$_{a}$	9.56 ± 0.77$_{b}$	17.73 ± 0.94$_{a}$	17.36 ± 0.53$_{a}$	17.65 ± 0.64$_{a}$
Dry+ST		15.55 ± 0.57$_{b}$	16.32 ± 0.44$_{a}$	16.14 ± 0.34$_{ab}$	14.44 ± 0.49$_{b}$	12.94 ± 0.41$_{b}$
FT+Dry		13.63 ± 0.71$_{bc}$	15.81 ± 0.71$_{a}$	13.41 ± 0.75$_{bc}$	13.44 ± 0.82$_{b}$	12.14 ± 0.69$_{b}$
ST+Dry		11.60 ± 0.79$_{c}$	12.25 ± 0.93$_{b}$	11.77 ± 1.02$_{c}$	10.76 ± 0.65$_{c}$	8.94 ± 0.72$_{c}$
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dry	Hue angle	40.93 ± 0.25$_{a}$	29.49 ± 0.90$_{c}$	39.14 ± 0.30$_{bc}$	39.86 ± 0.41	39.69 ± 0.42$_{a}$
Dry+ST		38.93 ± 0.52$_{a}$	40.22 ± 0.51$_{a}$	42.24 ± 0.33$_{a}$	40.96 ± 0.47	39.83 ± 0.60$_{a}$
FT+Dry		36.30 ± 0.62$_{b}$	40.02 ± 0.52$_{a}$	40.80 ± 0.54$_{ab}$	40.83 ± 0.87	39.73 ± 0.72$_{a}$
ST+Dry		34.59 ± 0.78$_{b}$	36.25 ± 0.96$_{b}$	38.29 ± 1.03$_{c}$	38.73 ± 1.06	35.88 ± 1.20$_{b}$
P-value		<0.0001	<0.0001	<0.0001	0.141	<0.05
Dry	Chroma	31.56 ± 0.67$_{a}$	19.30 ± 1.07$_{b}$	28.02 ± 1.32$_{a}$	27.12 ± 0.89$_{a}$	27.66 ± 0.99$_{a}$
Dry+ST		24.75 ± 0.84$_{b}$	25.28 ± 0.60$_{a}$	24.02 ± 0.53$_{b}$	22.02 ± 0.67$_{b}$	20.19 ± 0.54$_{b}$
FT+Dry		22.98 ± 1.05$_{bc}$	24.55 ± 0.94$_{a}$	20.48 ± 1.05$_{bc}$	20.52 ± 1.12$_{b}$	18.91 ± 0.84$_{b}$
ST+Dry		20.31 ± 1.11$_{c}$	20.52 ± 1.18$_{b}$	18.78 ± 1.25$_{c}$	17.12 ± 0.78$_{c}$	15.09 ± 0.92$_{c}$
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$_{a,b}$ Different letters in a column, for each color parameter, indicate significant differences ($P < 0.05$).

Dry: dry aging for 28 days; Dry+ST: dry aging for 28 days, steaks fabrication, freezing at -20 ºC and thawing at 4 ºC/24 h; FT+Dry: freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; ST+Dry: freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days.

5.3.6. Microbiological analyses

The samples analyzed prior to aging and freezing (fresh samples) showed low microbial load, TBC ranged from 2.79 to 3.34 log CFU/g, PSY was around 2.55 log CFU/g and LAB from <1.16 to <1.42 log CFU/g. *Enterobacteriaceae* and yeasts and molds were below the limit of detection (1 log CFU/g for ENT; 2 log CFU/g for YM). These results are similar to others reported in the literature (Li et al., 2014; Li et al., 2013; DeGeer et al., 2009).

Favorable conditions for microbial growth may occur during meat thawing, due to cell disruption and destruction of muscle fibers caused by freezing (Choe, Stuart, & Kim, 2016). The temperature increase and exudate release create an ideal environment for microbial development (Gill, 2014). However, in the current study no statistical difference ($P > 0.05$) was observed among fresh samples and the samples analyzed immediately after thawing.

After 28 days of dry aging, for TBC and PSY, the highest counts obtained were 3.54 log CFU/g in FT+Dry samples and 5.05 log CFU/g in ST+Dry samples, respectively, corroborating with Ahnström et al. (2006), Gudjónsdóttir et al. (2015), and Li et al. (2013). For LAB, 2.56 log CFU/g was achieved in the ST+Dry samples, similar values have been reported in dry-aged beef (Hulánková et al., 2018). *Enterobacteriaceae* were < 2 log CFU/g and YM ≤ 3 log CFU/g. The PSY counts obtained in FT+Dry and ST+Dry were approximately 1 log CFU/g higher than Dry; nevertheless, the results were not statistically different ($P > 0.05$; Table 5.3). LAB counts were higher for ST+Dry compared to Dry ($P < 0.05$), but similar to FT+Dry counts. *Aspergillus sp*. was isolated in 75% of the FT+Dry samples. In addition, *Cladosporium sp*. was identified in 25% of the FT+Dry and ST+Dry samples. Although commonly found in refrigerated meats (Fung, 2014), there were no previous reports on the presence of these molds in dry-aged beef. Therefore, the data indicated that freezing prior to dry aging had no major impact on the microbial load. In addition, all the processes resulted in microbiological counts ≤ 5 log CFU/g, i.e., had an acceptable microbiological quality (Feiner, 2006; Hulánková et al., 2018).

Table 5. 3. Microbial counts (log CFU/g ± SEM) on beef samples.

Microbial groups	Dry		FT+Dry			ST+Dry		
	Fresh sample	After dry aging	Fresh sample	After thawing	After dry aging	Fresh sample	After thawing	After dry aging
TBC[1]	2.79 ± 0.24$_{a}$	3.27 ± 0.25$_{a}$	3.34 ± 0.37$_{a}$	3.36 ± 0.35$_{a}$	3.54 ± 0.16$_{a}$	2.88 ± 0.44$_{a}$	2.70 ± 0.32$_{a}$	3.18 ± 0.08$_{a}$
PSY[2]	2.58 ± 0.29$_{b}$	4.07 ± 0.34$_{ab}$	2.55 ± 0.28$_{b}$	3.04 ± 0.23$_{b}$	4.79 ± 0.59$_{a}$	2.58 ± 0.29$_{b}$	2.98 ± 0.06$_{b}$	5.05 ± 0.14$_{a}$
EB[1]	<1.00 ± 0.00$_{a}$	<1.00 ± 0.00$_{a}$	<1.00 ± 0.10$_{a}$	<1.10 ± 0.10$_{a}$	<1.83 ± 0.49$_{a}$	<1.00 ± 0.00$_{a}$	<1.00 ± 0.00$_{a}$	<1.16 ± 0.16$_{a}$
LAB[1]	<1.26 ± 0.14$_{b}$	<1.53 ± 0.53$_{b}$	<1.16 ± 0.16$_{b}$	<1.26 ± 0.14$_{b}$	<1.80 ± 0.41$_{ab}$	<1.42 ± 0.28$_{b}$	<1.10 ± 0.10$_{b}$	2.56 ± 0.24$_{a}$
YM[2]	<2.00 ± 0.00$_{a}$	3.02 ± 0.47$_{a}$	<2.00 ± 0.00$_{a}$	<2.10 ± 0.10$_{a}$	2.96 ± 0.32$_{a}$	<2.00 ± 0.00$_{a}$	<2.00 ± 0.00$_{a}$	<2.73 ± 0.37$_{a}$

[1] Limit of detection: 1 log CFU/g; [2] Limit of detection: 2 log CFU/g. $_{a,b}$ Different letters in a row indicate significant differences ($P < 0.05$).
Dry: dry aging for 28 days; **FT+Dry:** freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; **ST+Dry:** freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days. **TBC:** total bacterial count; **PSY:** psychrotrophic microorganisms; **EB:** *Enterobacteriaceae*; **LAB:** lactic acid bacteria; **YM:** yeasts and molds.

5.3.7. Volatile compounds qualitative profile

Altogether, 73 volatile compounds were identified and classified as alcohols (n = 19), aldehydes (n = 13), ketones (n = 13), hydrocarbons (n = 12), aromatic compounds (n = 5), carboxylic acids (n = 4), esters (n = 3), sulfur compounds (n = 2), ether (n = 1) or terpenoid (n = 1). Twenty-six of these compounds were found in all samples analyzed. Many of these compounds detected in all samples are commonly related to cooked beef, for example 1-Hexanol, 2-ethyl- (green); 1-Pentanol (mild, fruit); Octanal (fatty, green); Heptanal (fatty); Pentanal (almond, pungent, malt); Hexanal (grassy taste); 2-Heptanone (fruity); Benzaldehyde (burning aromatic taste) (Faridnia et al., 2015; Calkins & Hodgen, 2007; Insausti et al., 2002). Several volatile compounds found in cooked meat include products of lipid oxidation, for example, hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters (Mottram, 1998).

In this study, 10 volatile compounds were found only in non-aged samples (fresh) and nine compounds in aged samples, frozen or non-frozen (Table 5.4). Therefore, it can be suggested that the volatile compounds found in non-aged samples were transformed into other products, when submitted to aging, as they did not appear in the aged samples, frozen or non-frozen. Generally, hydrocarbons do not have odoriferous importance and many of them were isolated in non-aged samples (fresh): 2- octene (Z)-, 1- octene, tridecane, hexane, octane. While in dry-aged samples, only two hydrocarbons were found: ethane (methylthio)- and nonane 5-butyl.

Three volatile compounds were only detected in non-frozen dry-aged samples (Table 5.4). From these compounds, nonanoic acid is found in both dry and wet-aged beef (King et al., 1995), and 1,3 octadiene is found in beef (Calkins & Hodgen, 2007). Studies indicated that, in aged beef, volatile compounds from fatty acid oxidation increases and affect the beef flavor intensity (Khan, Jo, & Tariq, 2015; Gorraiz et al., 2002).

In relation to the frozen samples, thiazoles, a product formed from lipid oxidation products combined with Maillard reaction compounds were found. Additionally, other compounds found included ketones (2-heptanone 3 methyl and 2-heptanone, 4,6 dimethyl - both associated to "green" flavor); octanoic acid (fatty, soapy , rancid, cheesy); benzyl alcohol (bitter); 2,3 butanediol (buttery); 1-butanol, 2-methyl (+/-) (roasted, onion); 2-propanol, 1-methoxy (compound found in coffee).

Several studies have reported that the freezing/thawing process increases lipid oxidation compared to non-frozen aged meat (Setyabrata & Kim, 2019; Ali et al., 2016; Xia et al., 2009; Hansen et al., 2004). Lipid oxidation is the major form of deterioration in frozen meat and results in the accumulation of volatile carbonyls, alcohols, and acids, which are responsible for off-flavors (Utrera, Parra, & Estévez, 2014). Moreover, ketones are some of the main aroma compounds derived from lipid oxidation (Resconi, Escudero, & Campo, 2013). However, in this study, TBARS values were low, under the threshold for detection of rancid flavor, and no differences between treatments were observed (Table 5.1), although different compounds related to lipid oxidation were identified in the aged samples, frozen or non-frozen (Table 5.4). This suggested that, regardless of the treatment, volatile compounds were formed due to the lipid oxidation, possibly in low quantities. These results could be better explained by further investigations about the effects of vitamin E and other potential antioxidants on beef from grass-fed cattle submitted to freezing and dry aging processes, and by the quantification of volatile compounds identified in this study.

Table 5. 4. Volatile compounds identified in cooked beef samples regard aging and freezing treatments (pooled sample).

Groups	Class	Compound	Fresh sample	Dry	Dry+ST	FT+Dry	ST+Dry
All Treatments	Alcohols	1-Penten-3-ol	x	x	x	x	x
	Alcohols	1-Hexanol	x	x	x	x	x
	Alcohols	1-Hexanol, 2-ethyl-	x	x	x	x	x
	Alcohols	1-Pentanol	x	x	x	x	x
	Alcohols	Ethanol, 2-butoxy-	x	x	x	x	x
	Aldehydes	Butanal, 2-methyl-	x	x	x	x	x
	Aldehydes	Butanal, 3-methyl-	x	x	x	x	x
	Aldehydes	Octanal	x	x	x	x	x
	Aldehydes	Heptanal	x	x	x	x	x
	Aldehydes	Nonanal	x	x	x	x	x
	Aldehydes	Pentanal	x	x	x	x	x
	Aldehydes	Hexanal	x	x	x	x	x
	Aldehydes	Decanal	x	x	x	x	x
	Aromatic C.	Ethylbenzene	x	x	x	x	x
	Aromatic C.	Furan, 2-pentyl-	x	x	x	x	x
	Aromatic C.	Toluene	x	x	x	x	x
	Aromatic C.	Benzaldehyde	x	x	x	x	x
	Carboxylic Ac.	Pentanoic acid	x	x	x	x	x
	Ketones	5-Hepten-2-one, 6-methyl-	x	x	x	x	x
	Ketones	2-Heptanone	x	x	x	x	x
	Ketones	2-Pentanone	x	x	x	x	x
	Ketones	2,3-Octanedione	x	x	x	x	x
	Ketones	2-Butanone, 3-hydroxy-	x	x	x	x	x
	Lactone	Butyrolactone	x	x	x	x	x
	Sulfur C.	Dimethyl trisulfide	x	x	x	x	x
	Sulfur C.	Disulfide, dimethyl	x	x	x	x	x
Fresh Sample (non-aged)	Alcohols	1-Undecanol	x				
	Alcohols	1-Octanol	x				
	Aldehydes	Undecanal	x				
	Esters	Formic acid, pentyl ester	x				
	Hydrocarbons	2-Octene, (Z)-	x				
	Hydrocarbons	1-Octene	x				
	Hydrocarbons	Tridecane	x				
	Hydrocarbons	Hexane	x				
	Hydrocarbons	Octane	x				
	Ketones	2-Heptanone, 6-methyl-	x				
Never Frozen	Alcohols	6-Hepten-1-ol		x			
	Carboxylic Ac.	Nonanoic acid		x			
	Hydrocarbons	1,3-Octadiene		x			

Table 5.4. cont. Volatile compounds identified in cooked beef samples regard aging and freezing treatments (pooled sample).

Groups	Class	Compound	Fresh sample	Dry	Dry+ST	FT+Dry	ST+Dry
Dry-aged	Alcohols	1-Butanol		x	x	x	x
	Alcohols	1-Butanol, 3-methyl-		x	x	x	x
	Alcohols	2,3-Butanediol		x	x	x	x
	Aldehydes	2-Butenal, 2-methyl-, (E)-		x	x	x	x
	Carboxylic acid	Hexanoic acid		x	x	x	x
	Esters	Ethyl Acetate		x	x	x	x
	Hydrocarbons	Ethane, (methylthio)-		x	x	x	x
	Hydrocarbons	Nonane, 5-butyl-		x	x	x	x
	Ketones	2,3-Butanedione		x	x	x	x
Frozen	Alcohols	2-Propanol, 1-methoxy-			x		x
	Alcohols	1-Butanol, 2-methyl-, (.+/-.)-				x	
	Alcohols	2,3-Butanediol, [R-(R@,R@)]-				x	x
	Alcohols	Benzyl Alcohol			x		
	Aromatic C.	Thiazole				x	
	Carboxylic Ac.	Octanoic Acid			x		
	Esters	Propanoic acid, 2-hydroxy-, ethyl ester			x		
	Ether	n-Butyl ether			x	x	x
	Ketones	2-Heptanone, 3-methyl-					x
	Ketones	2-Heptanone, 4,6-dimethyl-				x	x
Not grouped	Alcohols	3-Buten-1-ol, 3-methyl-	x	x	x	x	
	Alcohols	1-Heptanol	x	x	x		
	Alcohols	1-Octen-3-ol	x	x	x		
	Alcohols	1-Propanol, 2-methyl-		x	x	x	
	Aldehydes	Butanal		x			
	Aldehydes	2-Butenal, 3-methyl-		x		x	x
	Aldehydes	Methional/propanal, 3-methylthio		x		x	x
	Carboxylic Ac.	Butanoic acid, 3-methyl-		x		x	x
	Hydrocarbons	Nonane	x	x	x		x
	Hydrocarbons	1-Heptene	x	x		x	x
	Hydrocarbons	Decane	x	x			
	Hydrocarbons	Pentadecane		x		x	x
	Ketones	2,3-Pentanedione	x	x		x	x
	Ketones	2-Butanone	x		x		
	Terpenoids	.alpha.-Pinene	x		x	x	x

Dry: dry aging for 28 days; **Dry+ST:** dry aging for 28 days, steaks fabrication, freezing at -20 ºC and thawing at 4 ºC/24 h; **FT+Dry:** freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; **ST+Dry:** freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days.

A PCA (Figure 5.2) was created to illustrate the differences between each treatment based on individual volatile compounds. The first principal component (PC1) described 58.40% and the second principal component (PC2) described 26.22% of the total variation. The PC1 indicated a distinct difference between non-aged (fresh samples) and the other treatments, while the PC2 explained the differences between non-frozen and frozen treatments. The frozen samples, either prior to or after aging, were located in the lower right quadrant clearly separated from the dry-aged samples, located in the top right quadrant. These results indicated that both aging and freezing process modify the volatile compounds profile compared to non-aged beef. In addition, volatile compounds from lipid and protein degradation were identified in this study, which could affect the meat flavor. Therefore, further investigations are suggested to evaluate the effects of freezing and dry aging processes on the beef sensory attributes.

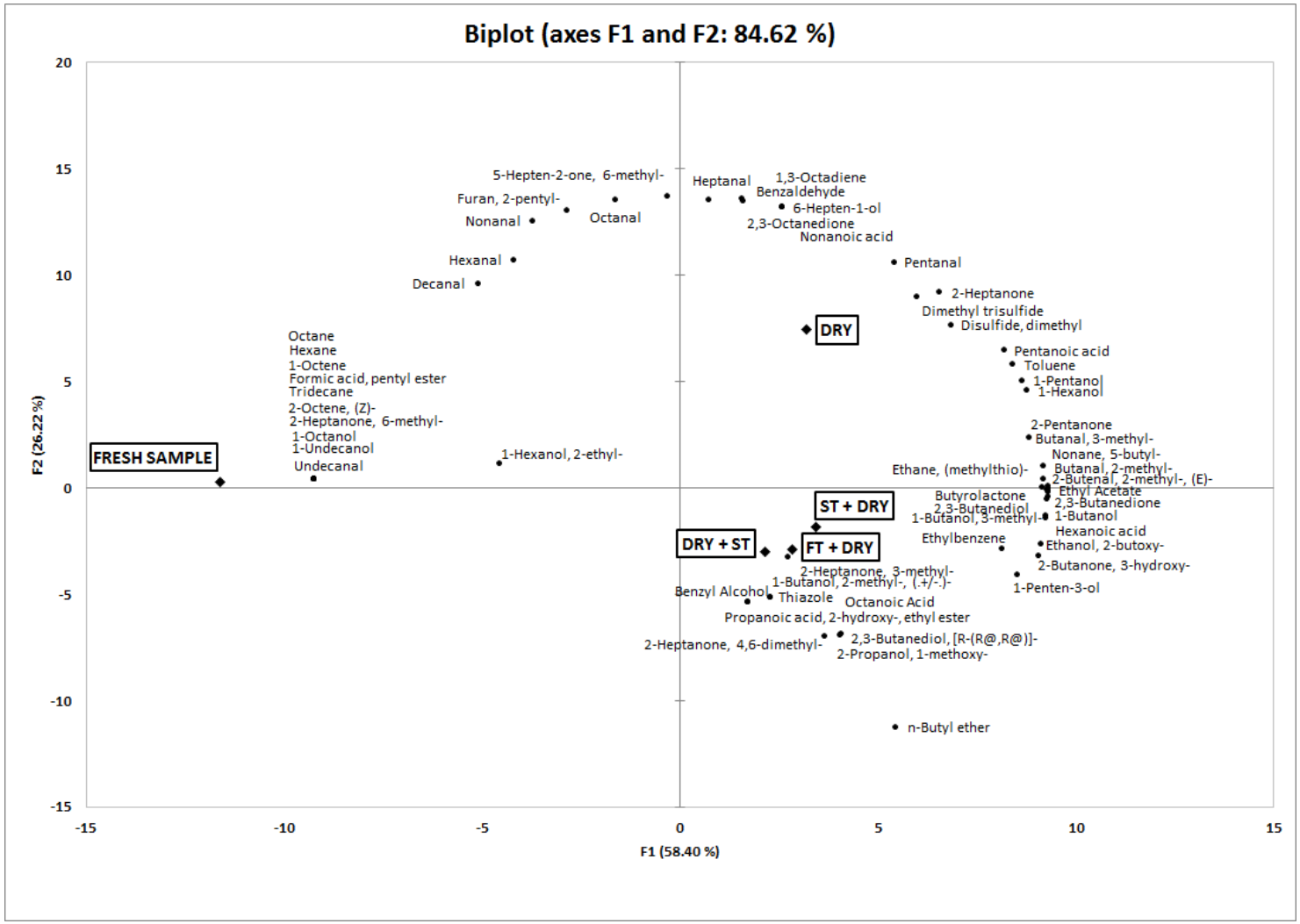

Figure 5. 2. Principal component analysis (PCA) score plot of cooked beef samples and volatile compounds.

Fresh Sample: non-aged beef; **Dry:** dry aging for 28 days; **Dry+ST:** dry aging for 28 days, steaks fabrication, freezing at -20 ºC and thawing at 4 ºC/24 h; **FT+Dry:** freezing at -20 ºC, fast thawing at 20 ºC/15 h and dry aging for 28 days; **ST+Dry:** freezing at -20 ºC, slow thawing at 4 ºC/48 h and dry aging for 28 days.

5.4. Conclusion

Freezing, prior to or after dry aging, changed the volatile compounds profile compared to non-frozen samples, although no differences were observed in lipid oxidation. Freezing prior to dry aging had no significant effect on the microbiological counts compared to non-frozen dry-aged samples. However, freezing prior to dry aging greatly increased the evaporation and total process loss, reducing moisture content, water activity, and cooking loss. Therefore, freezing the loins prior to dry aging was not considered a viable process. The freezing after dry aging did not have a major impact on the physical-chemical traits; however, further studies on sensory attributes are recommended to validate the consumer acceptance and the viability of freezing after dry aging, to preserve dry-aged beef for a longer period and, consequently, increase the opportunities for this high value market.

Conflict of interest

The authors declare no conflict of interest.

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6. ARTIGO 2

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Effects of bone and subcutaneous fat on dry-aged beef traits of zebu cattle (*Bos indicus*)

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ABSTRACT

This experiment evaluated the combined effects of bone (bone-in and boneless) and subcutaneous fat (with or without) on the yield and physical-chemical traits of dry-aged beef. Bone-in loins (from the 10th thoracic vertebra to 6th lumbar vertebra) from eight carcasses (n = 16) were collected at 2 days postmortem. The bone-in loins were cut in half and the sections were balanced assigned to four treatments: bone-in with subcutaneous fat; bone-in without subcutaneous fat; boneless with subcutaneous fat; and boneless without subcutaneous fat. All treatments were dry-aged for 21 days, at 2 ºC and 70% relative humidity. No interactions ($P > 0.05$) among bone and subcutaneous fat were found for total yield and physical-chemical traits. Both treatments bone-in and with subcutaneous fat had lower evaporation and trimming loss, and higher yield compared to boneless and samples without subcutaneous fat, respectively ($P < 0.05$). Bone-in showed higher moisture content and aw values than boneless ($P < 0.05$). Samples with subcutaneous fat had higher aw than samples without subcutaneous fat ($P < 0.05$), although no differences were observed on moisture content ($P > 0.05$). Shear force, pressed juice percentage, TBARS and pH were not affected by treatments ($P > 0.05$). Therefore, both bone and subcutaneous fat were considered important factors to prevent the evaporation loss during dry aging, increasing the total yield.

Keywords: dry-aged beef; bone-in; boneless; subcutaneous fat; total yield

6.1. Introduction

Brazil is the major exporter, and the second largest producer of beef in the world, with 2.2 million tons exported, and 10.2 million tons produced in 2019 (USDA, 2019a). The Brazilian herd has approximately 214 million head of cattle (MAPA, 2019), which about 80% has influence from zebu cattle (Ferraz & Felício, 2010). In addition, most Brazilian beef cattle can be considered as grass-fed based (Lobato et al., 2014), and only an estimated 10% of the beef produced are from cattle finished in feedlots (USDA, 2019b).

It is widely known that beef from grass-fed zebu cattle presents lower marbling and thinner subcutaneous fat than beef from grain-fed production. Moreover, beef from grass-fed cattle can be associated with negative terms of flavor, such as “grassy”, “gamey”, “bitter”, and “barny” (Maughan et al., 2012). However, the acceptability of beef is influenced by cultural aspects and eating habits (Lobato et al., 2019), and in Brazil beef from grass-fed grazing system seems to be well accepted by the consumers.

Additionally, the influence of zebu genes affects the beef tenderness due to its inherent characteristics as muscle structure, physiology, and enzymatic activity (Lobato et al., 2014; Lawrie, 2005). Tenderness is considered the most important sensorial attribute for beef consumption, and it can be improved by aging. There are two commonly methods of aging: wet aging, where the beef is package in a vacuum bag and stored; and dry aging, where the beef is exposed to the cooler conditions, without package. Savell (2008) defined dry aging as a process of storing unpackage carcasses, primals, and/or subprimals, at refrigeration temperatures for one to five weeks, allowing the enzymatic and biochemical processes to improve tenderness and develop the unique “dry-aged beef” flavor.

During dry aging process the beef loses weight due to moisture evaporation from the lean. Perhaps bone and subcutaneous fat can reduce the weight loss during dry aging, working as a barrier to prevent the liquid evaporation from the lean. A few studies comparing bone-in and boneless dry-aged beef showed lower weight loss for bone-in samples (Lepper-Blilie et al., 2016; DeGeer et al., 2009; Laster et al., 2008). Moreover, it is well known that the lean tissue presents higher evaporation loss capacity compared to fat tissue (Johnson et al., 1988). During carcass chilling, the subcutaneous fat reduces the carcass shrinkage and prevents evaporation from the lean meat (Savell et al., 2005; Smith & Carpenter, 1973). According to Pascoal et al. (2011) the higher the subcutaneous fat thickness, the lower the carcass weight loss during chilling.

Compared to wet aging, dry aging is considered a costly process (DeGeer et al., 2009; Smith et al., 2008; Miller et al., 1985). Producing dry-aged beef requires strict control of the cooler conditions and larger spaces in chambers (Smith et al., 2014), and results in higher weight loss (Kim et al., 2017). Furthermore, considering that the majority of the beef produced in Brazil is from grass-fed zebu cattle with thin subcutaneous fat, the understand of the impact of both subcutaneous fat and bone on the dry-aged beef is important to increase the yield of the process, achieving a consistent and less costly product. Therefore, the present study evaluated the combined effects of bone (bone-in and boneless) and subcutaneous fat (with or without fat) on the total yield and physical-chemical traits of dry-aged beef from grass-fed zebu cattle.

6.2. Material and methods

6.2.1. Raw material preparation and aging conditions

Eight paired Beef Loin, Strip Loin, Bone-in (similar to IMPS #175 described by USDA, 1996 and NAMP, 2003) from grass-fed zebu steers (around 30 months old; on average 290 kg of carcass weight; 6.0 ± 0.4 mm of subcutaneous fat thickness, measured between the 12th and 13th ribs; and normal pH of 5.60 ± 0.01) were collected in a commercial beef plant at 2 days postmortem. The loins were packed in plastic bags, placed in a portable cooler with ice, and transported by car (about 4 hours) to the Meat Laboratory at the University of Campinas.

At the Meat Laboratory, a steak was cut out from the middle of each bone-in loin and assigned to the characterization analyses (steaks of approximately 2.5 cm-thick for pH, moisture and fat content; and 2.5 cm-thick for Warner-Bratzler shear force). Then, each pair of bone-in loins provided four half-loin sections that were evenly assigned to four treatments: bone-in with subcutaneous fat, bone-in without subcutaneous fat, boneless with subcutaneous fat, and boneless without subcutaneous fat. The sections assigned to boneless treatments were deboned and sections for treatments without subcutaneous fat had the fat removed, following the natural connective tissue seam.

After the fabrication, the loin sections were aged for another 21 days in an adapted aging chamber (VN50R model, Metalfrio 2010 ©, Brazil) at 2 ºC, 70% relative humidity and 2.5 m/s of air velocity.

6.2.2. Weight loss and steak preparation

For evaporation and trimming loss, the pre- and post-aging weight were considered the initial and final weight, respectively, with bone for bone-in samples, and without bones for boneless samples. Moreover, for evaporation, trimming and total yield, neither pre- nor post-aging weight included the subcutaneous fat for the samples without subcutaneous fat.

The evaporation loss was calculated as: (pre-aging weight - post-aging weight)/pre-aging weight x 100. After weighing, the loin sections were deboned, when necessary, and the dried crust was trimmed. The trimming loss was calculated as: (weight of trimmings/pre-aging weight) x 100. For the total yield, for both bone-in and boneless samples, the pre aging weight was considered the initial weight with bones, according to the following equation: (post-aging deboned and trimmed loin section weight / pre-aging bone-in loin section weight) x 100.

After aging and trimming, the loin sections were weighed and cut into steaks. Each steak was assigned to one of the following analyses: pH and moisture content (approximately 2.0 cm-thick); thiobarbituric acid-reactive substances (approximately 1 cm-thick); Warner-Bratzler shear force and pressed juice percentage (approximately 2.5 cm-thick); instrumental color (approximately 1.5 cm-thick); and volatile compounds (approximately 1.5 cm-thick).

6.2.3. pH and water activity

The pH was measured, before and after aging, by inserting a calibrated pH probe (MP125 portable pH meter, Mettler Toledo, Brazil) directly into the beef, in duplicate. The surface water activity (aw) was measured in the dried trimmed crust (2 mm-thick), using a water activity analyzer (Aqualab 4TE, Decagon, São Paulo, Brazil).

6.2.4. Moisture, fat and thiobarbituric acid-reactive substances (TBARS)

The moisture content was determined in the non-aged and post-aged samples by grinding the internal lean beef, fat removed, and drying it in a forced air convection oven at 105 ºC for 20 hours, in triplicate, according to AOAC (1990) procedures. Fat content was determined only in the non-aged samples according to the Bligh & Dyer (1959) protocol. Thiobarbituric acid-reactive substances (TBARS) were determined on samples of lean after aging, in quadruplicate,

following Bruna et al. (2001) methodology modified by adding 20 mL of 5% TCA instead of 15 mL of 0.38 M $HClO_4$.

6.2.5. Cooking loss, Pressed juice percentage and Warner-Bratzler shear force (WBSF)

Pressed juice percentage and Warner-Bratzler shear force were both analyzed using the same steaks. Immediately after aging and fabrication, the steaks were prepared to cook. Steaks (2.5 cm-thick) were weighed, placed on a metal rack over an aluminum tray and cooked in an electric convection oven (Fritomaq, Brazil) at 170 ºC, until the internal temperature reached 71 ºC, measured by copper-constantan thermocouples (E5CWL Omron, CSW) inserted into the geometric center of each steak. After cooking, the steaks were reweighed to determine the cooking loss: (raw weight – cooked weight)/raw weight x 100.

Pressed juice percentage was determined according to Lucherk et al. (2017) methodology. Immediately after cooking, a slice (1.00 cm-thick) was cut from the center of each steak. Then three cubes (1.0 cm-width) were removed from each slice (1.00 cm-thick). Each one of the 3 cubes were weighed between two sheets of filter paper (Unifil Filter Paper 501.011, 80 g, 11 cm; Unifil) previously stored in a desiccator. Samples were compressed for 30 seconds at 78.45 N using a cylindrical compression probe (model P/36R, Texture Technologies Corp./ Stable Micro Systems, UK) coupled to a texture analyzer (model TA-XT Plus, Texture Technologies Corp./ Stable Micro Systems, UK). After compression, the sample was removed from the filter paper, which was reweighed. The results were expressed as the percentage of fluid loss during compression, according to the equation: (fluid released during compression/cube weight) x 100.

After cutting the samples for the pressed juice percentage method, the steaks were kept at room temperature for approximately 30 minutes until cooling, then they were overwrapped in polyvinyl chloride film and stored at 4 ºC overnight to proceed with the Warner-Bratzler shear force method, following the American Meat Science Association cookery guidelines (AMSA, 2015). Shear force was determined using six round cores (1.27 cm-diameter), cut parallel to the muscle fibers from each steak using a handheld coring device. Shear force was measured by shearing each core in the center using a Warner-Bratzler blade attached at a Texture Analyzer (TA-XT Plus, Texture Technologies Corp./ Stable Micro Systems, UK) with a crosshead speed of 250 mm/minute (AMSA, 2015).

6.2.6. Instrumental Color

Each steak assigned to instrumental color measurement was placed over a polystyrene tray covered with a polyvinyl chloride film and placed in a cooler at 4 ºC, with no lights, for 9 days. The instrumental color was measured every day in triplicate using a portable colorimeter (CM 508-d, Hunter MiniScan TMXE), attached to a moisture protector accessory and calibrated using white and black tile standards. The color was determined by CIE L* a* b* values using the illuminant D65 source and the standard observer of 10°, according to AMSA (2012) protocol.

6.2.7. Data analyses

Sixteen bone-in loins, from 8 beef carcasses, were used in this experiment. The bone-in loins were cut in half and then balanced assigned to the treatments. The experimental design was a 2x2 factorial with two bone effects (bone-in and boneless) and two subcutaneous fat effects (with and without fat). The data obtained were statistically analyzed using Statistica Version 10.0 (StatSoft, 2010) by two-way ANOVA, using the GLM procedure. Data from color analysis were analyzed by Statistical Analysis System (version 9.2, SAS Institute, Cary, NC, USA). The PROC GLIMMIX procedure was used with bone and fat as a fixed factor and time as a random factor. When significance ($P < 0.05$) was indicated by ANOVA, LSMEANS and DIFF functions were used to separate the least squares means.

6.3. Results and discussion

6.3.1. Sample characterization

The non-aged samples had pH values of 5.60 ± 0.01 and 48.4 ± 5.5 N of Warner-Bratzler shear force. Moisture and fat content were 75.0 ± 0.2% and 2.15 ± 0.2%, respectively. The loins had on average 6.0 ± 0.4 mm-thick of subcutaneous fat, before trimming, measured between the 12^{th} and 13^{th} ribs.

6.3.2. Evaporation, trimming loss and yield

No interactions ($P > 0.05$; Table 6.1) between bone and subcutaneous fat were found for evaporation, trimming, and yield traits.

Boneless samples showed higher evaporation and trimming loss and lower yield, compared to bone-in samples ($P < 0.05$; Table 6.1). As expected, bone had a protective effect over lean beef, reducing losses in the dry aging process, consequently increasing yield. Other studies also reported higher evaporation and trimming loss for boneless compared to bone-in dry-aged beef (Lepper-Blilie et al., 2016; DeGeer et al., 2009). Similarly, samples without subcutaneous fat had higher evaporation and trimming loss, and lower total yield compared to samples with subcutaneous fat ($P < 0.05$; Table 6.1). These results indicated that subcutaneous fat also protects lean beef, reducing moisture evaporation and increasing dry aging yield. Although no study about the effects of subcutaneous fat on dry-aged beef was found, it is known that subcutaneous fat acts as a barrier during carcass chilling, reducing the carcass shrinkage and evaporation loss from the lean (Savell et al., 2005; Smith & Carpenter, 1973).

Thus, both bone and subcutaneous fat were considered important factors during the dry aging process, protecting lean beef from evaporation and reducing trimming loss, increasing yield.

Table 6. 1. Effects (Means ± SEM) of the presence or absence of bone and subcutaneous fat on evaporation loss, trimming loss and total yield (n=32).

Trait	Treatments				P-value		
	Bone		Subcutaneous Fat		Bone	Fat	Bone x Fat
	Bone-in	Boneless	With	Without			
Evaporation loss, %	$15.63 \pm 0.76_{b}$	$22.76 \pm 0.96_{a}$	$16.58 \pm 0.88_{y}$	$21.81 \pm 1.23_{x}$	<0.0001	<0.0001	0.249
Trimming loss, %	$8.16 \pm 0.55_{b}$	$18.18 \pm 0.65_{a}$	$11.70 \pm 1.21_{y}$	$14.64 \pm 1.53_{x}$	<0.0001	<0.0001	0.127
Total yield, %	$49.77 \pm 1.14_{a}$	$40.79 \pm 1.42_{b}$	$48.92 \pm 1.46_{x}$	$41.64 \pm 1.46_{y}$	<0.0001	<0.0001	0.749

$_{a,b}$ Means of bone effect in the same row with different superscript letters differ ($P < 0.05$)

$_{x,y}$ Means of subcutaneous fat effect in the same row with different superscript letters differ ($P < 0.05$)

6.3.3. pH, water activity, moisture and thiobarbituric acid-reactive substances (TBARS)

No interactions (P > 0.05; Table 6.2) between bone and subcutaneous fat were found for the pH, surface water activity, moisture content, and thiobarbituric acid-reactive substances (TBARS).

The presence or absence of bone did not affect the pH or TBARS values ($P > 0.05$; Table 6.2). Boneless samples had lower values of moisture and surface aw than bone-in samples ($P < 0.05$; Table 6.2). These results were expected as boneless loins had more exposed beef surface, which increased evaporation from the lean. Thus, bone influenced only traits related to water content and had no impact on the pH and lipid oxidation. Similar results were reported by DeGeer et al. (2009) who evaluated bone-in and boneless loin-cut, dry-aged for 21 and 28 days at 50% relative humidity, and found no differences in the pH and TBARS values between bone-in and boneless samples, and lower moisture content for boneless compared to bone-in samples.

Subcutaneous fat had no effect on the pH, TBARS, or moisture content values ($P > 0.05$; Table 6.2). Samples without subcutaneous fat showed lower surface aw values than samples with subcutaneous fat ($P < 0.05$; Table 6.2). The removal of subcutaneous fat exposes beef surface and increases evaporation from the lean, resulting in higher superficial dehydration, which explains the lower surface aw observed in this study.

Table 6. 2. Effects (Means ± SEM) of the presence or absence of bone and subcutaneous fat on pH, thiobarbituric acid-reactive substances (TBARS), moisture content, surface aw, cooking loss, pressed juice percentage and Warner-Bratzler shear force (WBSF) (n=32).

Trait	Treatments				P-value		
	Bone		Subcutaneous Fat		Bone	Fat	Bone x Fat
	Bone-in	Boneless	With	Without			
pH	5.44 ± 0.01	5.45 ± 0.01	5.46 ± 0.01	5.43 ± 0.01	0.326	0.069	0.912
TBARS, mg MDA/kg	0.19 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.691	0.588	0.325
Moisture content, %	74.06 ± 0.23$_a$	72.97 ± 0.21$_b$	73.63 ± 0.26	73.40 ± 0.26	<0.05	0.469	0.091
Surface aw	0.9409±0.0020$_a$	0.9324±0.0021$_b$	0.9396 ± 0.0020$_x$	0.9336 ±0.0024$_y$	<0.05	<0.05	0.177
Cooking loss, %	16.66 ± 0.58$_a$	14.06 ± 0.56$_b$	16.49 ± 0.39$_x$	14.23 ± 0.74$_y$	<0.05	<0.05	0.322
Pressed juice, %	33.19 ± 0.67	32.67 ± 0.87	33.06 ± 0.72	32.79 ± 0.83	0.645	0.811	0.292
WBSF, N	36.03 ± 1.98	36.46 ± 3.08	37.97 ± 3.14	34.52 ± 1.78	0.905	0.342	0.113

$_{a,b}$ Means of bone effect in the same row with different superscript letters differ ($P < 0.05$)

$_{x,y}$ Means of subcutaneous fat effect in the same row with different superscript letters differ ($P < 0.05$)

6.3.4. Cooking loss, Pressed juice percentage and Warner-Bratzler shear force (WBSF)

No interactions ($P > 0.05$; Table 6.2) between bone and subcutaneous fat were found for cooking loss, WBSF, and pressed juice percentage.

Bone-in samples had higher cooking loss values compared to boneless ($P < 0.05$; Table 6.2) whereas, WBSF and pressed juice percentage were not affected by bone-in and boneless treatments ($P > 0.05$; Table 6.2). This difference observed in cooking loss values could be associated to the higher moisture content on bone-in samples. Other studies also indicated no differences of WBSF values in bone-in and boneless samples (Lepper-Blilie et al., 2016; DeGeer et al., 2009). Likewise, samples with subcutaneous fat showed higher cooking loss than those without subcutaneous fat ($P < 0.05$; Table 6.2), and no differences were observed in WBSF and pressed juice percentage values ($P > 0.05$; Table 6.2). Cooking loss varies with meat composition (moisture and fat content) and cooking conditions, such as time and temperature (Gerber et al., 2009; Love & Prusa, 1992). Although no difference was observed in moisture content between samples with or without subcutaneous fat ($P > 0.05$; Table 6.2), the difference in cooking loss values are possibly related to the melting of subcutaneous fat during cooking, which could increase cooking loss values.

Regardless of the presence of bone and subcutaneous fat, the treatments were considered very tender, as the WBSF values were lower than 38.2 N (ASTM, 2011). Furthermore, after cooking, all treatments had similar juiciness level, measured by pressed juice percentage.

6.3.5. Instrumental Color

The instrumental color parameters (L*, a*, b*) were not affected by bone or subcutaneous fat ($P > 0.05$). However, they were affected by display time ($P < 0.05$). This result is related to discoloration during display, which decreases the instrumental color values. Other studies also showed a decrease of color parameters from 0 to 9 (Yu et al., 2017) or 11 days of display (Canto et al., 2015).

No interaction ($P > 0.05$) between bone, fat, and time were found for L* values (lightness) and no differences were observed between the treatments ($P > 0.05$). However, there was a time effect ($P < 0.05$) for L*, reducing values from 40.92 to 38.86 (SE: 0.536; data not shown in tabular form), comparing the first with the last day of display, respectively.

Additionally, there was a bone and time interaction ($P < 0.05$; Figure 6.1) for a* (redness) and b* (yellowness) parameters. Both bone-in and boneless treatments showed a decrease of a* values during display. However, after six days of display, bone-in treatments had a greater decrease of a* values compared to boneless ($P < 0.05$; Figure 6.1). Decreases of a* values indicated discoloration during display (Kim et al., 2017; Hui et al., 2005). The b* values were higher for bone-in treatments from the third to the sixth day of display compared to boneless ($P < 0.05$; Figure 6.1). No difference was found on the seventh day of display ($P > 0.05$; Figure 6.1), and after eight days of display, boneless showed higher b* values than bone-in treatments ($P < 0.05$; Figure 6.1). Thus, the results of instrumental color suggested that boneless treatments had slightly, but significantly, higher color stability than bone-in (Figure 6.2), as boneless showed higher a* and b* values after six and seven days of display, respectively.

In this study, the steaks assigned for color analysis were refrigerated at 4 ºC in the dark. In a supermarket display, discoloration can occur faster, due to the temperature fluctuations and light exposure. Lower temperature can increase display; however, retail display temperature is frequently up to 7 ºC (Hui et al., 2005). Furthermore, light exposure promotes the formation of metmyoglobin through photochemical autoxidation (Hui et al., 2005).

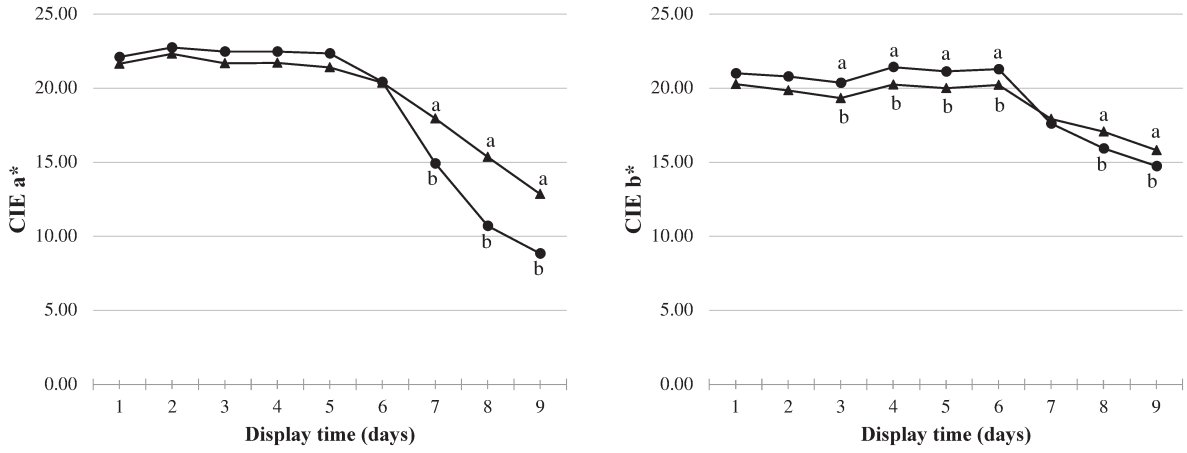

Figure 6. 1. Effects of bone and time interaction on CIE a* and b* color parameters during 9 days of display.

Standard error: 0.588 (CIE a*) and 0.331 (CIE b*). a, b letters between bone-in and boneless treatments within each day of display differ (P < 0.05). (—●—) Bone-in treatments; (—▲—) Boneless treatments.

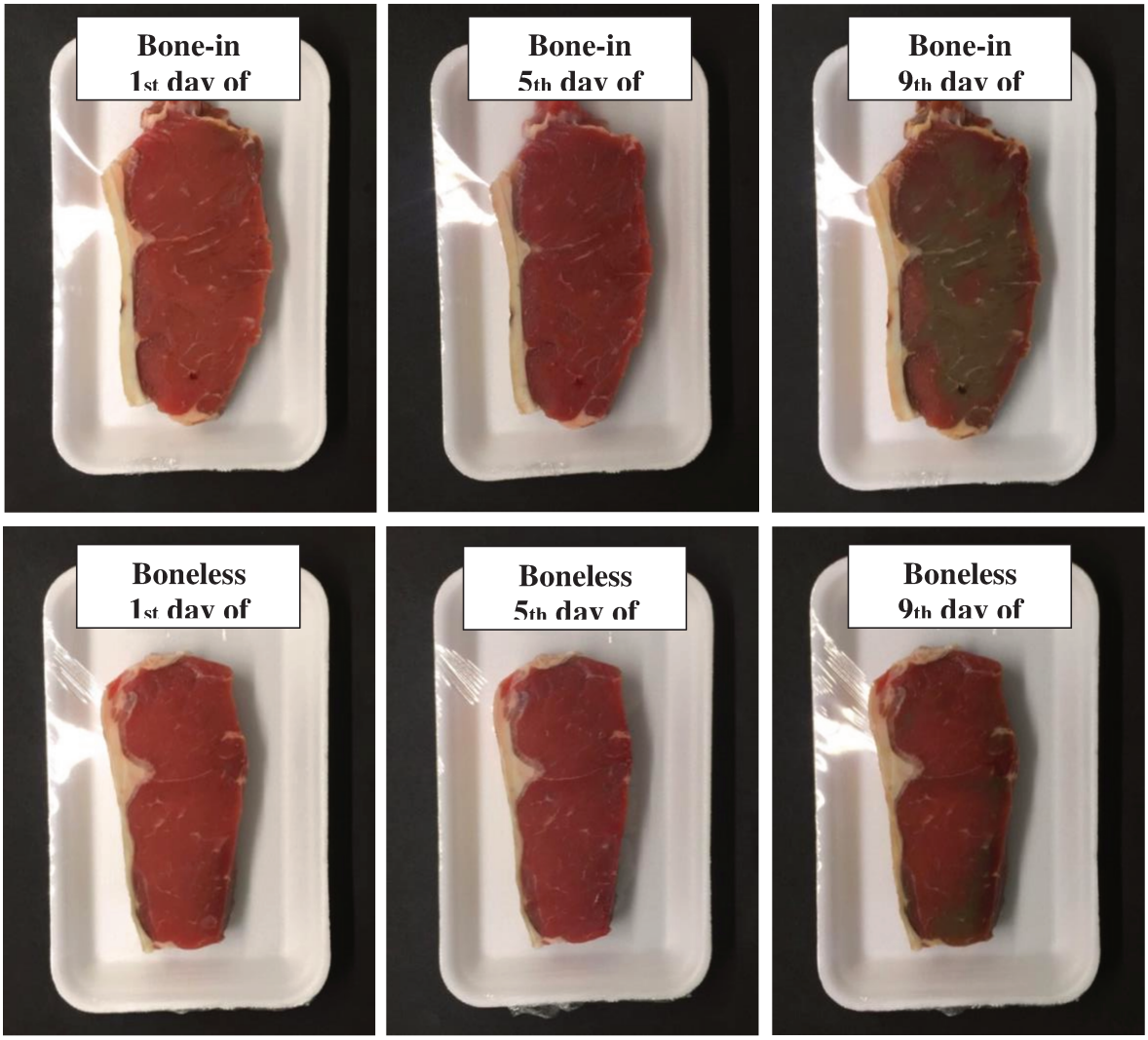

Figure 6. 2. Pictures illustrating the development of color discoloration on bone-in and boneless steaks during display.

6.4. Conclusion

The data from the current study showed that both bone and subcutaneous fat had a similar protective effect on the lean beef, reducing evaporation from the loin sections and leading to a higher yield of dry-aged product, which could result in a more economically feasible process. In addition, even for beef of grass-fed zebu cattle, after the dry aging process the samples were considered very tender. However, sensory studies should further investigate consumers' acceptance of dry-aged beef from grass-fed zebu cattle in the Brazilian market. Therefore, regardless the feeding system and cattle breed, the use of bone-in loins with thicker subcutaneous fat is recommended to reduce losses of processing.

Conflict of interest

The authors declare no conflict of interest.

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7. DISCUSSÃO GERAL

Os resultados deste trabalho indicaram que o congelamento, tanto antes quanto após a maturação a seco, não afetou os valores de pH, TBARS e maciez instrumental. No entanto, as análises relacionadas ao teor de água apresentaram diferenças, sendo que os tratamentos com congelamento prévio à maturação a seco expressaram menores valores de atividade de água e umidade, comparado à ambos os tratamentos sem congelamento e com congelamento após a maturação a seco. Este resultado está diretamente relacionado à perda de peso durante o processo de maturação, já que as amostras com congelamento prévio à maturação a seco apresentaram um aumento de até 16% na perda de peso total do processo em comparação aos demais tratamentos. Em relação à cor instrumental, o congelamento reduziu a estabilidade da cor da carne durante a avaliação de display. Além disso, foi observado que o congelamento, antes e após a maturação a seco, resultou em alterações no perfil de compostos voláteis das amostras, o que é um indicativo de alterações no sabor da carne, porém outro estudo com avaliação sensorial das amostras deve ser realizado. E ainda, o congelamento prévio a maturação a seco não afetou a contagem microbiológica comparado ao tratamento sem congelamento.

Quanto aos resultados do estudo sobre os efeitos da presença de osso e gordura subcutânea na maturação a seco, foi observado que pH, TBARS, maciez e suculência instrumental não foram afetados pelos diferentes tratamentos. No entanto, as amostras sem osso apresentaram menores valores de atividade de água e umidade, enquanto as amostras sem gordura apresentaram menor atividade de água, comparados às amostras com osso e com gordura subcutânea, respectivamente. Estes resultados relacionados ao teor de água das amostras estão diretamente associados à maior perda de peso por evaporação durante o processo de maturação a seco, pois ambas as amostras sem osso e sem gordura subcutânea apresentaram maiores perdas por evaporação comparadas as amostras com osso e com gordura subcutânea, respectivamente. Além disso, como era esperado, os tratamentos com osso e gordura subcutânea apresentaram maiores rendimentos, sendo considerados fatores bastante relevantes para o processo de maturação a seco.

8. CONCLUSÃO GERAL

A perda de peso é considerada uma das maiores desvantagens relacionada à maturação a seco. Com este trabalho, concluiu-se que tanto o congelamento antes da maturação quanto a remoção dos ossos e/ou gordura subcutânea resultam em aumento da perda de peso, que consequentemente influencia outras características físico-químicas relacionadas ao teor de água, como umidade, atividade de água e perda de peso por cocção. Apesar do congelamento prévio à maturação a seco não ter afetado a contagem microbiológica esse processo não é indicado, já que resulta em perdas de peso significativamente maiores comparado a maturação de carnes não congeladas. Já o congelamento de bifes após a maturação a seco foi considerado um processo viável, pois não apresentou impactos negativos sobre a qualidade físico-química da carne. Em relação à presença de osso e gordura subcutânea, ambos foram considerados fatores importantes para reduzir a perda de peso durante a maturação a seco resultando em aumento de rendimento do processo. Portanto, sugere-se que a maturação a seco seja realizada com carne não congelada, ainda com osso e gordura subcutânea, visando maior rendimento de processo.

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ANEXO I

Parecer do Comitê de Ética em Pesquisa

UNICAMP - CAMPUS CAMPINAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: PROCESSOS DE MATURAÇÃO DE CARNE BOVINA: AVANÇOS TECNOLÓGICOS PARA IMPLEMENTAR SISTEMAS E AVALIAR A QUALIDADE DA CARNE

Pesquisador: Sergio Bertelli Pflanzer Junior

Área Temática:

Versão: 1

CAAE: 69320317.6.0000.5404

Instituição Proponente: Faculdade de Engenharia de Alimentos

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.203.053

Apresentação do Projeto:

Introdução: A carne é um alimento rico em proteínas de alta qualidade, ferro e vitamina B (ABERLE et al., 2001). Além de sua importância na alimentação, ela também representa para o Brasil uma fonte de renda e grande valor econômico. O Brasil ocupa um lugar de destaque na pecuária bovina mundial. Possui o segundo maior rebanho, com cerca de 219,2 milhões de cabeças; é o segundo maior produtor desta fonte proteica, oferecendo ao mercado aproximadamente 9,2 milhões de toneladas de carne; e destina para o exterior 1,85 milhão de toneladas, se colocando como maior exportador, juntamente com a Índia (USDA, 2016). Mesmo com todos esses números positivos, o Brasil é carente quanto falamos da qualidade organoléptica da carne bovina produzida. Não é incomum nos depararmos com consumidores insatisfeitos em relação a algumas dessas características, como a suculência, o sabor e, principalmente, a maciez. O contrafilé bovino, formado principalmente pelo músculo Longissimus dorsi , é o corte de preferência a ser analisado na maioria dos trabalhos científicos (DESTEFANIS et al., 2008; SASAKI et al. , 2010; GOMES et al., 2014), sendo considerado um corte representativo das carnes para assar, fritar ou grelhar, conhecidas como de preparo rápido, ou "carne de primeira" (FELÍCIO, 1999). Vários estudos demonstram que a maciez é a característica sensorial que mais influencia na aceitação da carne durante seu consumo, pois no momento da compra, a cor é o fator determinante (MORGAN et al.,

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1991; KOOHMARAIE, 1996; ENFALT et al., 1997; KOOHMARAIE et al., 2002; PLATTER et al., 2003; KOOHMARAIE et al., 2006). São vários os fatores que influem na maciez da carne. Esses fatores podem estar relacionados com a genética do bovino, com as condições de criação, com alguns procedimentos durante o abate e com o resfriamento das carcaças, com as condições de armazenamento da carne (maturação) e, por fim, com o método de preparo para consumo (LAWRIE, 1985). Dentre os fatores citados anteriormente, a maturação merece posição de destaque pela intensa melhoria dos aspectos sensoriais da carne. A maturação da carne se tornou essencial nos serviços de varejo e alimentação para atender à demanda e expectativa de se consumir um alimento excepcional (LASTER et al., 2008). De acordo com Devine (2004), maturação é o nome dado ao processo de amaciamento da carne que ocorre devido à ação de enzimas musculares endógenas, presentes no músculo vivo, que assumem outra função na carne post mortem. A maturação consiste em um método que promove o aumento da maciez e o desenvolvimento de sabor da carne fresca e é realizada, tradicionalmente, através da estocagem da carne em um refrigerador durante o período desejado para o amaciamento e desenvolvimento do sabor característico de maturação (WARREN E KASTNER, 1992). Diversas alterações bioquímicas ocorrem no músculo esquelético durante seu estoque post mortem e várias enzimas, presentes naturalmente no músculo, compõem um sistema enzimático envolvido na proteólise muscular, gerando tais alterações (TOLDRÁ et al.,1995). O processo de maturação envolve, principalmente, enzimas endógenas, sendo as principais as calpaínas (I e II) e catepsinas (LAMARE et al., 2002). Entretanto, a enzima catepsina seria incapaz de participar das reações que culminariam no aumento da maciez do músculo e não apresentaria papel significativo na proteólise da carne (KOOHMARAIE et al., 1988). Como resultado, o sistema enzimático responsável pelas alterações das proteínas miofibrilares durante a maturação envolveria as enzimas calpaína I e calpaína II. Durante o processo de proteólise, as enzimas causam um desarranjo das subunidades musculares, proveniente da fragmentação da linha-Z e da degradação de proteínas como titina, nebulina, desmina e troponina-T (KOOHMARAIE et al., 1988), o que leva a um aumento da maciez da carne. A proteólise que ocorre durante o período post mortem envolve quatro componentes principais: íons cálcio, calpaína I, calpaína II e calpastatina; e sofre grande influência de dois parâmetros: pH e temperatura (KOOHMARAIE, 1992). A proteólise é mais intensa em temperaturas mais altas e conforme o pH reduz a enzima calpaína II é favorecida sobre a calpaína I (DRANSFIELD, 1992). A liberação de grande quantidade de íons cálcio juntamente com a atividade da calpaína I leva a uma intensa proteólise nos primeiros dois dias, porém a enzima perde grande parte da sua atividade após 24 horas (aproximadamente 60%). Contudo, o processo de proteólise continua, agora pela

atividade das enzimas calpaina I e II e, após 6 dias, a calpaina II é a única atuando sobre as estruturas do músculo (DRANSFIELD, 1992; KOOHMARAIE, 1987). As calpaínas (I e II) estão presentes no músculo in vivo, porém, não expressam atividade proteolítica dado ao pH elevado e a atividade da calpastatina. A queda do pH, durante o post mortem, favorece a atividade proteolítica das calpaínas e diminui a capacidade inibitória da calpastatina (COTTIN et al., 1981), garantindo que o processo proteolítico continue na carne durante esta fase (DEVINE, 2004). Animais com genética de gado zebuíno (bos indicus) apresentam uma maior atividade da calpastatina, com a consequente diminuição da atividade das calpaínas post mortem, gerando assim uma carne mais dura (RUBENSAM et al., 1998; WHIPPLE et al., 1990). A última etapa da proteólise ocorrida na maturação é a geração de aminoácidos livres (TOLDRÁ et al., 1995).. O estudo de Nishimura et al. (1988) relacionou o aumento de aminoácidos livres em carne estocada post mortem com a melhora no sabor. De fato, a maturação deixa os alimentos mais saborosos (YAMAGUCHI E NINOMIYA, 1999). A pesquisa desenvolvida por Polak et al., (2007) encontrou um aumento na concentração de aminoácidos livres em carne bovina com períodos maiores de maturação incluindo um aumento no teor de glutamato (glu). O glutamato está presente em diversos alimentos como pescados, queijos e carnes, nos quais ele melhora o gosto e a palatabilidade (BAGNASCO et al., 2014). Segundo Yamaguchi e Ninomiya (1999), o gosto umami, relacionado com uma descrição de saboroso, cárneo e "similar a caldo de carne", é transmitido, entre outros compostos, por glutamato. Dessa forma, a melhora do sabor na carne maturada, que se associa ao aumento do teor de aminoácidos livres, pode estar relacionada ao aumento de compostos geradores de gosto umami como o glutamato. Fundamentalmente, existem dois tipos de maturação: a maturação úmida ou "Wet aging" na qual a carne é embalada a vácuo em uma embalagem com baixa permeabilidade a gases e vapor da água durante o período de maturação em estoque resfriado; e a maturação a seco ou "Dry aging" que implica na exposição da carne, sem nenhuma embalagem, às condições impostas pelo equipamento de refrigeração com controle de temperatura, umidade e fluxo de ar (AHNSTRÖM et al., 2006, CAMPBELL et al., 2001). Independente do processo utilizado, a maciez sensorial ou instrumental parece não ser afetada (VILELLA et al., 2016). O método de maturação a seco é conhecido por melhorar o sabor da carne, gerando um produto de sabor único e distinto em relação à carne maturada por "Wet-aging" (LI et al., 2014; DEGEER et al., 2009). Um sabor de "carne assada" tende a ser atribuído à carne maturada a seco, enquanto a carne de maturação úmida é conhecida por apresentar sabores "de sangue" e "metálico", considerados menos desejáveis (CAMPBELL et al., 2001; WARREN E KASTNER, 1992). Embora a maior parte da carne maturada consista de produtos obtidos pela maturação úmida, os

fornecedores de carne maturada a seco relatam que o desenvolvimento do sabor particular é o principal motivo para a produção desse produto (AHNSTRÖM et al., 2006). No entanto, o processo de maturação a seco também possui desvantagens: a carne sofre uma maior taxa de encolhimento e perdas de peso devido à perda de umidade durante a maturação e a remoção das aparas (consistindo da retirada das superfícies ressecadas da peça) causando o aumento dos custos de produção e, consequentemente, do preço final da carne (STENSTRÖM et al., 2014; PARRISH et al., 1991). Dessa forma, a maturação úmida ganhou popularidade devido a sua maior conveniência, melhor rendimento e vida de prateleira mais extensa enquanto o produto maturado a seco se tornou um item para mercados especiais ou gourmet (WARREN E KASTNER, 1992). Os trabalhos científicos têm mostrado resultados variados quanto ao desenvolvimento de sabor diferenciado da carne maturada a seco em relação a maturação úmida, sendo que alguns deles confirmam essa diferença (STENSTRÖM et al., 2014; LI et al., 2014; WARREN E KASTNER, 1992) e outros não (LASTER et al., 2008; PARRISH et al., 1991). Esses experimentos procuraram discernir as diferenças entre cames de maturação a seco e úmida através de análises sensoriais. Apesar de haver trabalhos que descrevam a diferenciação sensorial entre carne bovina maturada a seco e úmida, não foram encontrados estudos que elucidem o mecanismo do desenvolvimento de sabor característico da carne maturada a seco, entretanto, analisando os dados apresentados, podemos ressaltar alguns pontos importantes: conforme descrito anteriormente, os provadores do painel treinado do estudo de Li et al. (2014) determinaram que a carne maturada a seco possuiu mais gosto umami em relação a carne de maturação úmida; Nishimura et al. (1988) descreveram uma relação do aumento de aminoácidos livres em carne estocada post mortem com um melhoramento de sabor; Polak et al. (2007) relataram ter encontrado um aumento na concentração de aminoácidos livres na carne, incluindo Glutamato (glu), durante o processo de maturação. Avaliando o tempo ótimo de maturação da carne pelo processo seco, Fumiko et al. (2016) constataram que 40 dias foi ideal para a maciez, suculência, sabor umami e de carne com elevados teores de gordura intramuscular. Um novo processo de maturação, que consiste no acondicionamento da carne em uma embalagem com alta permeabilidade à umidade ("special bag"), vem sendo estudado (DIKEMAN et al., 2013). Esse sistema simula o processo de maturação seca, entretanto, traz benefícios, como a diminuição das perdas de processo e da contaminação superficial, aumentando rendimento, sem prejudicar os atributos sensoriais caraterísticos do processo a seco (AHNSTRÖM et al., 2006; DEGEER et al,. 2009). Independente do sistema de maturação a ser empregado, as condições de armazenamento, como temperatura, umidade e tempo de estocagem devem ser controladas, visando garantir a estabilidade da cor e o baixo

desenvolvimento microbiano. Diante do exposto, o objetivo deste trabalho é avaliar a carne de bovinos zebuinos submetidas aos três sistemas de maturação existentes (úmido, seco e "special bag"), controlando as condições de armazenamento como congelamento, temperatura e tempo de estocagem, dentre outros, visando desenvolver protocolos de maturação específicos para cada tipo de produto/mercado, a serem utilizados pela indústria frigorífica nacional. Hipótese: Os provadores treinados deverão ter a capacidade de detectar e avaliar os seguintes aspectos sensoriais presentes nas amostras: intensidade de sabor, maciez, suculência e aceitação global. Acredita-se que com esta análise se encontre diferenças significativas entre os diferentes tipos e tempos de maturação, temperaturas e umidades de armazenamento, entre outros aspectos analisados. Os diferentes tipos de maturação possuem diferenças sensoriais já demonstradas em outros estudos realizados para avaliação da palatabilidade. Sabe-se que as carnes que passam pelo processo de maturação apresentam uma maior maciez, entretanto, espera-se que, tanto provadores treinados quanto consumidores, percebam diferenças nesse atributo. A equipe treinada poderá apresentar uma percepção de que as amostras maturadas a seco, em relação a amostras maturadas úmidas, possuem maior intensidade em certos atributos como o gosto umami e sabor amanteigado, de carne e assado (lembrando a utilização de caldos de carne comercializados). Os consumidores poderão demonstrar uma aceitação maior para as amostras maturadas a seco, visto que estas, normalmente, geram um sabor mais intenso de carne assada e apresentam maior maciez do que as carnes submetidas à maturação úmida ou não maturadas. As amostras armazenadas sob maiores valores de umidade (85%) e temperatura (7C) poderão obter maiores notas para o atributo suculência, visto que não haverá tanta perda de exsudato quanto as amostras armazenadas sob a umidade de 60%. Após, no mínimo, 14 dias de maturação seca, sugere-se que haja uma diferença sensorial mais perceptível em relação à maciez e suculência, pois um tempo de maturação seca por menos tempo talvez não seja suficiente para modificar os atributos sensoriais a ponto de serem perceptíveis durante as análises, até mesmo para os provadores treinados.

Objetivo da Pesquisa:

O objetivo do estudo é avaliar a carne de bovinos zebuinos submetidas aos três sistemas de maturação existentes (úmido, seco e "special bag"), controlando as condições de armazenamento como congelamento, temperatura, umidade e tempo de estocagem, visando desenvolver protocolos de maturação específicos para cada tipo de produto/mercado, a serem utilizados pela indústria frigorífica nacional. Objetivos específicos: Definir qual temperatura é a mais indicada

para os processos de maturação úmida e seca; Definir, para os processos a seco e "special bag", qual umidade ideal de maturação; Definir qual o tempo mínimo e máximo para o processo de maturação a seco; Avaliar o efeito da maturação a seco quando realizada em carne previamente maturada pelo processo úmido; Avaliar o efeito da desossa prévia aos processos de maturação a seco; Avaliar o efeito do congelamento/descongelamento em processos de maturação úmida e seca.

Avaliação dos Riscos e Benefícios:

Segundo informações do pesquisador: não há riscos previstos na participação dessa pesquisa salvo voluntários que possuam algum tipo de alergia a carne bovina. Os sujeitos da pesquisa serão submetidos a testes sensoriais envolvendo amostras de carne bovina obtidas de frigoríficos. Estes estabelecimentos são devidamente fiscalizados por órgão públicos (a Vigilância Sanitária gerenciada pelo Ministério da Saúde e o Serviço de Inspeção Federal do Ministério da Agricultura) responsáveis pelo controle higiênico e sanitário e garante um produto final seguro e próprio para o consumo.

Segundo informações do pesquisador, não há benefícios diretos para os participantes da pesquisa (provadores e consumidores), porém argumenta que a carne é um alimento rico em proteínas de alta qualidade, vitamina B e ferro, componentes extremamente importantes para a mantença de uma vida saudável.

Comentários e Considerações sobre a Pesquisa:

Este protocolo se refere ao projeto de pesquisa intitulado "Processos de maturação de carne bovina: avanços tecnológicos para implementar sistemas e avaliar a qualidade da carne", cujo pesquisador responsável é o Prof. Dr. Sérgio Bertelli Pflanzer. A pesquisa embasará a dissertação de mestrado (aluno/pesquisador colaborador não citado). A Instituição Proponente é a Faculdade de Engenharia de Alimentos da UNICAMP. Segundo as Informações Básicas do Projeto, a pesquisa tem orçamento estimado em R$ 3.000,00 (três mil reais), mas não foi informada a fonte de financiamento, e o cronograma apresentado contempla início dos experimentos em 19/06/2017, com término da pesquisa previsto para 21/12/2018. O número de participantes da pesquisa é de 135, sendo 15 provadores treinados (análise sensorial) e 120 consumidores (análise sensorial de aceitação). Os critérios do recrutamento para a equipe de provadores treinados serão: interesse e disponibilidade em participar do estudo, facilidade de se expressar e de trabalhar em grupo, avaliar as amostras sem tempero e não serem fumantes. Após o treinamento da equipe será feita uma nova seleção, com o objetivo de selecionar os provadores que: possuem capacidade de discriminar as amostras; que apresentem boa reprodutibilidade e que apresentem resultados

consensuais com os demais membros da equipe sensorial. Em relação aos consumidores,os mesmos serão convidados a participarem do estudo, tendo como critério de inclusão apenas o interesse e disponibilidade, avaliar as amostras sem tempero e não serem fumantes.

Tamanho da Amostra no Brasil: 135
provadores treinados 15 análise sensorial
consumidores 120 análise sensorial de aceitação

Considerações sobre os Termos de apresentação obrigatória:

Foram analisados os seguintes documentos de apresentação obrigatória:

1 - Folha de Rosto Para Pesquisa Envolvendo Seres Humanos: Foi apresentado o documento "folhaderosto_preenchida.pdf" devidamente preenchido, datado e assinado.

2 - Projeto de Pesquisa: Foram analisados os documentos "projeto-2.pdf" e "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_913495.pdf". Adequado.

3 - Orçamento financeiro e fontes de financiamento: Informações sobre orçamento financeiro incluídas no documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_913495.pdf", porém não foi esclarecido qual será a fonte do recurso para financiamento do projeto. Não adequado.

4- Cronograma: Informações sobre o cronograma incluídas nos documentos "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_913495.pdf" e "projeto-2.pdf". Adequado.

5 - Termo de Consentimento Livre e Esclarecido: apresentados 2 (dois) TCLE, "TCLE_maturacao_consumidores.pdf" e "TCLE_maturacao_provadores.pdf". Adequado.

Recomendações:

Considerar as pendências abaixo.

Conclusões ou Pendências e Lista de Inadequações:

1 - Solicitamos que o pesquisador esclareça a fonte de financiamento do projeto de pesquisa.

2 - O arquivo "projeto-2.pdf" cita que este será "um projeto para mestrado". Solicita-se que o nome do aluno envolvido no projeto seja incluído como participante do projeto.

3- Declarar no TCLE em riscos, indenização ou outro item que considerar conveniente que :

* que o participante terá acompanhamento gratuito e pelo tempo necessário em caso de evento adverso na participação da pesquisa,

* em caso de danos terá direito a indenização.A Resolução 466/12 (item IV.3) define que "os participantes da pesquisa que vierem a sofrer qualquer tipo de dano resultante de sua participação

na pesquisa,previsto ou não no TCLE, têm direito à indenização, por parte do pesquisador, patrocinador e das instituições envolvidas". Cabe enfatizar que a questão da indenização não é prerrogativa da Resolução 466/12, estando prevista no código civil.

Considerações Finais a critério do CEP:

Conforme compromisso assumido pelo mesmo com o cumprimento da resolução 466/2012, item IX.1 letra a. Quando for submeter respostas às pendências, verificar se o cronograma de realização da pesquisa, descrito na plataforma Brasil e no projeto anexado, está contemplando o início da coleta de dados APÓS a liberação do projeto pelo CEP.

Apresentar carta resposta ao CEP declarando quais as informações alteradas, quais as respostas as pendências apresentadas destacando-as no documento pertinente.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_913495.pdf	01/06/2017 19:43:05		Aceito
Projeto Detalhado / Brochura Investigador	projeto.pdf	01/06/2017 19:41:59	Sergio Bertelli Pflanzer Junior	Aceito
Declaração de Pesquisadores	Sergio_Pflanzer.jpg	01/06/2017 19:40:56	Sergio Bertelli Pflanzer Junior	Aceito
Folha de Rosto	folhaderosto_preenchida.pdf	05/05/2017 19:03:16	Sergio Bertelli Pflanzer Junior	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_maturacao_provadores.pdf	05/05/2017 13:50:01	Sergio Bertelli Pflanzer Junior	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_maturacao_consumidores.pdf	05/05/2017 13:49:22	Sergio Bertelli Pflanzer Junior	Aceito

Situação do Parecer:

Pendente

Necessita Apreciação da CONEP:

Não

CAMPINAS, 05 de Agosto de 2017

Assinado por:
Maria Fernanda Ribeiro Bittar
(Coordenador)

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

ANEXO II

Declaração de Cadastro do Projeto no SisGen

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A3DF80B

A atividade de acesso ao Conhecimento Tradicional Associado, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A3DF80B**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Conhecimento Tradicional Associado**
Finalidade do Acesso:	**Pesquisa**

Espécie

Não há

Fonte do CTA

CTA de origem não identificável

Título da Atividade:	**PROCESSOS DE MATURAÇÃO DE CARNE BOVINA: AVANÇOS TECNOLÓGICOS PARA IMPLEMENTAR SISTEMAS E AVALIAR A QUALIDADE DA CARNE**

Equipe

Sergio Bertelli Pflanzer Junior	**UNICAMP**
Ana Paula da Silva Bernardo	**Unicamp**
Maristela da Silva do Nascimento	**Unicamp**
Astrid Caroline Muniz da Silva	**Unicamp**

Data do Cadastro:	**16/10/2018 15:37:36**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **15:38** de **16/10/2018**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN