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Ultrassom e redução de sódio em presunto cozido.

São José do Rio Preto
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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

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Dedico este trabalho à minha família:

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*“Sopre o vento,
enrole o tempo.
Agarre a chama,
seque a lama.
Faça o louco gostar.*

*Verdeje a grama,
contrate a grana.
Amarre o laço,
desenferruje o aço.
Faça a poeira voar.*

*Suje o lixo,
liberte o bicho.
Esquente o Sol,
fure o anzol.
Faça o pobre enriquecer.*

*Adoce o salgado,
condecure o soldado.
Extrapole o limite,
contrate o palpito.
Faça a chuva chegar.*

*Soltou. Pegue de volta.
Amarelou. Peça escolta.
O brilho empoeira, mas volta.*

*A conquista é sua,
o suor é seu.
O aplauso é pra ti,
a graça também.
Diz pra alma agradecer.*

*E no final,
não é fim.
É começo do novo, é mudança de tudo.
Ergue o volume e limpa as lentes.
E agora?
Tudo de novo.”*

Sons da mudança

*José Ricardo Barretto
(em fase de elaboração)¹*

¹ Poesia, de autoria de José Ricardo Barretto, a ser publicado, 2015.

RESUMO

Presunto cozido é um dos produtos cárneos mais consumidos do Brasil. O cloreto de sódio utilizado em sua fabricação é importante pois auxilia na extração das proteínas miofibrilares da carne e confere o sabor salgado característico deste produto. No entanto, o excesso de sódio na dieta está relacionado com o desenvolvimento de doenças cardíacas. Há, então, a necessidade em reduzir o teor de sódio sem prejudicar as características físico-químicas e a aceitação sensorial. Na primeira etapa foi realizada a determinação da composição química e a obtenção de isotermas de sorção para quatro amostras de presunto cozido comercial submetidas a condições simuladas de armazenamento. As isotermas foram determinadas pelo método gravimétrico. Os dados experimentais foram ajustados aos modelos matemáticos de Guggenheim, Anderson e de Boer; Brunauer, Emmett e Teller; Halsey; Henderson; e Peleg. O modelo de Guggenheim, Anderson e de Boer foi escolhido para descrever melhor as isotermas, pois tinha um ajuste muito bom. O aumento da temperatura reduziu o teor de umidade de equilíbrio do produto. O aumento da umidade relativa resultou num aumento no teor de umidade de equilíbrio do produto, independente da temperatura. Quanto maior o teor e a disponibilidade da proteína, ou quanto menor o teor de gordura, maior o teor de umidade de equilíbrio do produto. Na segunda etapa estudou-se os efeitos da redução de sal e a aplicação do ultrassom sobre as propriedades físico-químicas e microestrutura e a aceitação sensorial do presunto cozido. Quatro tratamentos com redução de sal, incluindo um com a aplicação de ultrassom (1,5; 1,12; 0,75 e 0,75% sal + ultrassom) foram produzidos. O uso de ultrassom diminuiu o total de líquido exsudado e aumentou a dureza. Para L^* , a amostra com 0,75% de sal e ultrassom não diferiu do controle no dia zero de armazenamento. O uso do ultrassom também aumentou os valores de a^* . O tratamento com ultrassom causou microfissuras nas miofibrilas. A aceitação sensorial do presunto cozido com 0,75% de sal foi melhorada com a aplicação de ultrassom. O ultrassom mostrou bom potencial para uso na produção de produtos cárneos mais saudáveis. Na terceira etapa avaliaram-se os efeitos do ultrassom e da adição de cloreto de potássio nas propriedades físico-químicas e sensoriais de presunto cozido com baixo teor de sódio. Foram preparados quatro tratamentos de presunto cozido com baixo teor de sódio: CT - tratamento controle; UsT - tratamento com ultrassom; KT - adição de 0,5% de KCl; UsKT - tratamento com ultrassom e adição de 0,5% de

KCl. O ultrassom reduziu o total de líquido exsudado e melhorou a aceitação sensorial para gosto e sabor salgado em comparação com a CT. A adição de KCl mostrou os melhores resultados para o líquido exsudado, para todos os parâmetros de aceitação sensorial, para dureza e mastigabilidade, e estes não diferiram dos resultados obtidos com a combinação do uso de ultrassom e adição de KCl. O uso de KCl sozinho ou o uso do ultrassom é uma alternativa tecnológica e sensorialmente viável para o presunto cozido com baixo teor de sódio.

Palavras-chave: Ultrassom, presunto cozido, redução de sódio, cloreto de potássio, cavitação.

ABSTRACT

Restructured cooked ham is one of the most consumed meat products in Brazil. The sodium chloride used in its manufacture is important because it helps in the extraction of myofibrillar proteins from meat and gives the characteristic salty taste of this product. However, excess dietary sodium is related to the development of heart disease. So, is necessary to reduce sodium content without impairing physicochemical characteristics and sensory acceptance. In the first stage was carried out the determination of the chemical composition and the experimental obtaining of sorption isotherms for four samples of commercial restructured cooked ham subjected to simulated commercial storage conditions. The isotherms were determined using the gravimetric method. The experimental data were adjusted to the mathematical models of Guggenheim, Anderson and de Boer; Brunauer, Emmett and Teller; Halsey; Henderson; and Peleg. The Guggenheim, Anderson and de Boer model was chosen to better describe the isotherms as it had a very good fit. The increase in temperature reduced the equilibrium moisture content of the product. Increased relative humidity resulted in an increase in equilibrium moisture content of the product regardless of storage temperature. The higher the content and availability of the protein or the lower the fat content, the higher the equilibrium moisture content of the product. In the second stage was studied the effects of salt reduction and the application of ultrasound on the physicochemical properties, the microstructure and the sensory acceptance of cooked ham. Four treatments with reduced salt including one with the application of ultrasound (1.5, 1.12, 0.75 and 0.75% salt + ultrasound) were produced. The use of ultrasound decreased the Total Fluid Release and increased the hardness in cooked ham. For lightness, the sample with 0.75% salt with the application of ultrasound did not differ from the control at day zero of storage. The use of ultrasound increased redness too. The ultrasound treatment caused micro fissures on the myofibrils. The sensory acceptance of cooked ham with 0.75% of salt was improved with ultrasound applied. The ultrasound showed good potential for use in the production of healthier meat products. In thirdy stage were evaluated the effects of ultrasound and the addition of potassium chloride in the physicochemical properties and sensorial acceptance of low sodium restructured cooked ham. Four treatments of low sodium restructured cooked ham (mean of 324.52 mg Na/100 g) were prepared: CT - Control Treatment; UsT - Ultrasound Treatment; KT - addition of 0.5% KCl; UsKT - Ultrasound Treatment and

addition of 0.5% KCl. Ultrasound application reduced the total fluid released and improved the sensory acceptance for salty taste and flavor compared to CT. The addition of KCl produced the best results for total fluid release, for all parameters of sensory acceptance and for hardness and chewiness and these were not different from the results obtained with the combination of the use of ultrasound and addition of KCl. The use of KCl alone or use of the ultrasound is a technologically and sensorially viable alternative to low sodium restructured cooked ham.

Keywords: Ultrasound; restructured cooked ham; sodium reduction; potassium chloride, cavitation.

LISTA DE FIGURAS

Capítulo II – *Water sorption isotherms of cooked hams as affected by temperature and chemical composition.*

Figura 1 – Sorption isotherms for Sample 4 (S4) of cooked ham fitted to the GAB model at different storage temperatures. 56

Figura 2 – Sorption isotherms for Samples 1, 2, 3 and 4 (S1, S2, S3 and S4) of cooked ham fitted to the GAB model at fixed temperature (16 °C). 56

Capítulo III - *Improving sensory acceptance and physicochemical properties by ultrasound application to cooked ham with salt (NaCl) reduction.*

Figura 1 – Scanning electron micrographs (a, b, c and d: 500 X; e, f, g and h: 1500 X) of cooked ham. CV: cavitation effect on muscle fiber. T100 = 1.5% NaCl; T75 = 1.12% NaCl; T50 = 0.75% NaCl; T50US = 0.75% NaCl and ultrasound. 83

Capítulo IV - *Impact of ultrasound and potassium chloride on the physicochemical and sensory properties in low sodium restructured cooked ham.*

Figura 1 – Ultrasound probe system set-up. 97

LISTA DE TABELAS

Capítulo I - Uso do ultrassom em carnes e produtos cárneos: aspectos físico-químicos e sensoriais

Tabela 1 – Efeitos do uso do ultrassom sobre alguns parâmetros físico-químicos de carne e produtos cárneos. 30

Tabela 2 – Efeitos do uso do ultrassom sobre algumas características sensoriais em carnes e produtos cárneos. 36

Capítulo II – *Water sorption isotherms of cooked hams as affected by temperature and chemical composition.*

Tabela 1 - Water activity of the salt solutions at different temperatures. 50

Tabela 2 – Models used to fit sorption isotherm data from cooked hams. 51

Tabela 3 - Average values (\pm standard deviation) of the percentage composition of the cooked hams. 52

Tabela 4 - Fitting parameters of GAB equation for all samples at the different temperatures. 54

Capítulo III - *Improving sensory acceptance and physicochemical properties by ultrasound application to cooked ham with salt (NaCl) reduction.*

Tabela 1 - Percentual of water and salt of restructured cooked ham. 70

Tabela 2- Proximate analysis, pH and sodium content of cooked ham with salt reduction including application of ultrasound (n=3). 76

Tabela 3 - Color parameters, TFR, TPA and the TBARS of cooked ham with salt reduction including application of ultrasound (n=3). 78

Tabela 4 - Sensory acceptance of restructured cooked ham with salt reduction including application of ultrasound. 85

Capítulo IV - *Impact of ultrasound and potassium chloride on the physicochemical and sensory properties in low sodium restructured cooked ham.*

Tabela 1 - Percentual of water and KCl in low sodium restructured cooked ham. 97

Tabela 2 - Proximate analysis, pH, sodium and potassium content of restructured cooked ham. 102

Tabela 3 - TFR, Color parameters and TPA of restructured cooked ham.	104
Tabela 4 - Sensorial acceptance of restructured cooked ham.	109

SUMÁRIO

1	INTRODUÇÃO GERAL	17
	REFERÊNCIAS	19
2	OBJETIVOS	21
2.1	Objetivo geral	21
2.2	Objetivos específicos	21
	CAPÍTULO I	22
	Uso do ultrassom em carnes e produtos cárneos: aspectos físico-químicos e sensoriais	23
	RESUMO	23
1	INTRODUÇÃO	24
2	ASPECTOS GERAIS SOBRE O ULTRASSOM	25
3	ULTRASSOM EM CARNES E PRODUTOS CÁRNEOS	27
3.1	Capacidade de retenção de água (CRA)	27
3.2	Cor instrumental	31
3.3	Textura instrumental	32
3.4	Características sensoriais	34
3.5	Considerações sobre o uso do ultrassom e redução de sódio em produtos cárneos	37
4	CONSIDERAÇÕES FINAIS	38
	Referências	39
	CAPÍTULO II	45
	Water sorption isotherms of cooked hams as affected by temperature and chemical composition.	46
	ABSTRACT	46
1	INTRODUCTION	47
2	MATERIALS AND METHODS	49
2.1	Sample preparation	49
2.2	Chemical composition	49
2.3	Obtaining the sorption isotherms	49
2.4	Modelling of sorption isotherms	51
3	RESULTS AND DISCUSSION	51

3.1	Chemical composition	51
3.2	Sorption isotherms	53
4	CONCLUSION	58
	References	58
	CAPÍTULO III	66
	Improving sensory acceptance and physicochemical properties by ultrasound application to cooked ham with salt (NaCl) reduction.	67
	ABSTRACT	67
1	INTRODUCTION	68
2	MATERIALS AND METHODS	69
2.1	Restructured cooked ham manufacture	69
2.2	Proximate analysis, pH and sodium content	71
2.3	Total fluid released (TFR)	71
2.4	Instrumental color	72
2.5	Texture profile analysis (TPA)	72
2.6	Thiobarbituric acid reactive substances (TBARS)	73
2.7	Microbiological evaluation	73
2.8	Sensory acceptance	74
2.9	Microstructure	74
2.10	Statistical analysis	75
3	RESULTS AND DISCUSSION	75
3.1	Physicochemical properties	75
3.2	Total fluid release (TFR)	77
3.3	Instrumental color	79
3.4	Texture profile analysis (TPA)	80
3.5	TBARS	81
3.6	Microstructure of restructured cooked ham	81
3.7	Microbiological analysis	84
3.8	Sensory acceptance	84
4	CONCLUSION	86
	References	87
	CAPÍTULO IV	92

Impact of ultrasound and potassium chloride on the physicochemical and sensory properties in low sodium restructured cooked ham.	93
ABSTRACT	93
1 INTRODUCTION	94
2 MATERIALS AND METHODS	96
2.1 Cooked ham manufacture	96
2.2 Proximate analysis, pH, sodium content and Total Fluid Release (TFR)	98
2.3 Instrumental Color and Texture Profile Analysis (TPA)	99
2.4 Microbiological evaluation	99
2.5 Sensory acceptance	100
2.6 Statistical Analysis	101
3 RESULTS AND DISCUSSION	101
3.1 Physicochemical properties	101
3.2 TFR	103
3.3 Instrumental color	105
3.4 Texture profile analysis	106
3.5 Microbiological analysis	108
3.6 Sensorial acceptance	108
4 CONCLUSION	110
References	111
CONCLUSÃO GERAL	117
APÊNDICE A	118
APÊNDICE B	119
ANEXO A	120
ANEXO B	121
ANEXO C	124

1 INTRODUÇÃO GERAL

A produção brasileira de carne suína atingiu a marca de 3,75 mil toneladas no ano de 2017, 25% maior que em 2005. Apenas 18,5% dessa produção destinou-se à exportação, sendo a maioria consumida pelo mercado interno. A principal forma de consumo de carne suína entre os brasileiros está na forma de produtos processados, uma vez que 89% dessa carne é industrializada em diversos tipos de produtos como salames, linguiças, apresuntados e presuntos (ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2018).

Presunto cozido é um dos produtos cárneos processados mais populares entre os consumidores (VÁLKOVÁ et al., 2007). Seu processamento cresceu consideravelmente no final do século 20 e início do século 21 em função de ser um produto pronto para consumo, versátil, prático e ter boa aceitação sensorial (NIELSEN, 2007). A legislação brasileira define presunto cozido como um produto cárneo industrializado obtido exclusivamente com o pernil de suínos, desossado, adicionado de ingredientes, e submetido a um processo de cozimento adequado (BRASIL, 2000).

O processamento tradicional de presunto cozido consiste na preparação da salmoura, que poderá ser injetada ou adicionada ao pernil suíno com o uso de massageadores ou *tumbler* seguido de embalagem, cocção e resfriamento adequados. A qualidade final do produto depende de muitos fatores, destacando-se a criação dos animais, composição das matérias-primas e as condições do processamento (VÁLKOVÁ et al., 2007). A salmoura utilizada em sua produção é composta por ingredientes que visam melhorar as características tecnológicas, como é o caso do cloreto de sódio que é utilizado para atribuir características sensoriais desejáveis, auxiliar na segurança e na estabilidade do produto devido a solubilização das proteínas miofibrilares e redução da atividade de água (ORDOÑEZ, 2005).

Contudo, a ingestão excessiva de sódio está associada ao desenvolvimento de algumas doenças crônicas não-transmissíveis (ISER et al., 2011). Nesse sentido há necessidade em reduzir o uso desse ingrediente em alimentos processados. Em 2010 foi firmado um acordo entre a Agência Nacional de Vigilância Sanitária (ANVISA) e a Associação Brasileira de Indústria de Alimentos (ABIA) cujo objetivo foi reduzir o teor

de sódio dos alimentos industrializados (BRASIL, 2010). Entretanto sua redução não deve ocorrer sem estudos efetivos, uma vez que em produtos cárneos o cloreto de sódio promove a estabilidade microbiológica, aumenta a capacidade de retenção de água e, conseqüentemente reduz as perdas por exsudação (TERRELL, 1983).

Tecnologias alternativas vêm sendo estudadas objetivando a promoção das propriedades tecnológicas de produtos alimentares. Nesse contexto, inserem-se novas tecnologias de processamento como o ultrassom (LEADLEY e WILLIAMS, 2008; CHEMAT et al., 2011). Vários trabalhos relatam (JAYASOORIYA et al., 2007; CÁRCEL, et al., 2012; MCDONNELL et al., 2013) que o ultrassom pode ser útil ao acelerar e intensificar a extração, esterilização e difusão, podendo reduzir o tempo de processamento. Cárcel et al. (2012) enfatizam que a tecnologia do ultrassom possibilita inovação na indústria por economizar energia e aumentar o rendimento e a qualidade dos produtos, fazendo com que esta tecnologia abra um novo campo no processamento de alimentos. Ondas ultrassônicas originam o fenômeno de cavitação, capaz de alterar algumas propriedades físicas e favorecer reações químicas (JAYASOORIYA et al., 2007). Diante disso, a tecnologia do ultrassom pode ser uma alternativa para colaborar no processamento de produtos cárneos com teores reduzido de sódio, podendo minimizar os efeitos negativos dessa redução no produto.

APRESENTAÇÃO DO TRABALHO

Este trabalho foi organizado em quatro capítulos para a melhor distribuição e entendimento dos assuntos abordados.

O capítulo I consiste em uma revisão bibliográfica sobre o tema abordado na tese. A revisão intitulada de “Uso do ultrassom em carnes e produtos cárneos: aspectos físico-químicos e sensoriais” foi redigida em português, na forma de um artigo científico. Será feita a versão deste capítulo para o inglês para ser submetido à publicação em periódico especializado na área de Ciência de Alimentos.

O capítulo II trata-se do artigo científico “*Water sorption isotherms of cooked hams as affected by temperature and chemical composition*” publicado na Revista *Food Science and Technology, ahead of print, Epub* 13 de dezembro de 2018, de autoria de Tiago Luis Barretto, Tiago Carregari Polachini, Andrea Carla da

Silva Barretto e Javier Telis-Romero. Este capítulo aborda a construção de isotermas de sorção a avaliação da composição química de presuntos cozidos comerciais produzidos no Brasil. Esta etapa foi importante, uma vez que permitiu conhecer e estudar mais detalhadamente sobre teores de umidade, proteína e gordura bem como condições de armazenamento sobre o produto objeto do estudo: o presunto cozido.

O capítulo III trata-se do artigo científico “*Improving sensory acceptance and physicochemical properties by ultrasound application to cooked ham with salt (NaCl) reduction*” publicado na Revista *Meat Science*, volume 145, 2018, p. 55-62, de autoria de Tiago Luis Barretto, Marise Aparecida Rodrigues Pollonio, Javier Telis-Romero e Andrea Carla da Silva Barretto.

O capítulo IV trata-se do artigo científico “*Impact of ultrasound and potassium chloride on the physicochemical and sensory properties in low sodium restructured cooked ham*” submetido para publicação na Revista *Meat Science*, de autoria de Tiago Luis Barretto, Elisa Rafaela Bonadio Bellucci, Roger Darros Barbosa, Marise Aparecida Rodrigues Pollonio, Javier Telis Romero e Andrea Carla da Silva Barretto. Este capítulo complementa os estudos abordados no capítulo III.

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2 OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos do uso do ultrassom sobre as propriedades sensoriais e tecnológicas da redução de sódio em presunto cozido.

2.2 Objetivos específicos

Avaliar a influência da composição centesimal sobre as isotermas de sorção de presuntos cozidos comerciais;

Avaliar a influência da redução de sódio e do uso do ultrassom em presunto cozido sobre a microestrutura, aceitação sensorial, exsudação de líquido, composição centesimal, pH, teor de sódio, cor instrumental, perfil de textura, estabilidade microbiológica e oxidativa.

Avaliar os efeitos da adição de cloreto de potássio e do uso de ultrassom no presunto cozido com baixo teor de sódio sobre a aceitação sensorial, estabilidade microbiológica, pH, composição centesimal, cor instrumental, perfil de textura e exsudação de líquido.

CAPÍTULO I

USO DO ULTRASSOM EM CARNES E PRODUTOS CÁRNEOS: ASPECTOS FÍSICO-QUÍMICOS E SENSORIAIS

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USO DO ULTRASSOM EM CARNES E PRODUTOS CÁRNEOS: ASPECTOS FÍSICO-QUÍMICOS E SENSORIAIS

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RESUMO

O ultrassom é uma onda mecânica acústica e demanda um meio para se propagar. Em intensidades acima de 20 kHz, pode causar cavitação, fenômeno que ocorre quando a onda atravessa um meio líquido, produzindo pequenas bolhas e gerando energia. A energia pode acelerar reações químicas e/ou causar modificações na microestrutura do alimento. O objetivo deste estudo foi realizar uma revisão bibliográfica dos efeitos do uso do ultrassom sobre as propriedades físico-químicas e sensoriais em carnes e produtos cárneos. Com relação a Capacidade de Retenção de Água, há estudos que não observaram efeitos sobre esta propriedade com o uso do ultrassom. Entretanto, vários estudos destacam que o uso do ultrassom pode afetar a CRA da carne ou produto cárneo. Muitos autores discorrem sobre as modificações causadas na microestrutura da carne. A energia liberada pelo ultrassom pode destruir parcialmente as proteínas miofibrilares, deixando-as mais expostas facilitando sua ligação com a água. Também pode aumentar os espaços interfibrilares e reter maior quantidade de água. A transferência de massa ocasionada pelo uso do ultrassom também é relatada em diversos trabalhos. Com relação às propriedades de cor e textura instrumental é possível observar que o ultrassom pode ocasionar resultados diferentes, provavelmente em função da variabilidade das condições de processamento e dos produtos finais. Há, também, grande variação nas propriedades sensoriais relatadas em carnes e produtos cárneos submetidos ao ultrassom.

Algumas mudanças sensoriais são decorrentes das mudanças de microestrutura e capacidade de retenção de água. Há necessidade de ampliar-se as pesquisas dos efeitos do ultrassom sobre as propriedades das carnes e produtos cárneos contemplando maior variabilidade das condições de processamento e de produto finais.

Palavras-chave: Ultrassom, cavitação, capacidade de retenção de água, aceitação sensorial.

1. INTRODUÇÃO

A maneira de desenvolver e processar alimentos está cada vez mais pautada nas necessidades emergentes do consumidor. A grande demanda por alimentos processados que tenham qualidade e segurança comprovadas está ampliando as tecnologias utilizadas na indústria de alimentos (Chemat & Ashokkumar, 2017). Entre essas tecnologias, alguns estudos destacam o uso de alta pressão, pulsos elétricos, microfiltração, micro-ondas e ultrassom (Chemat, Zill, & Khan, 2011; Leadley & Willians, 2008, Chemat & Ashokkumar, 2017) no processamento industrial com objetivos de melhorar algumas propriedades funcionais nos alimentos, reduzir o tempo do processamento e o uso de água e energia e, minimizar a produção de efluentes e substâncias tóxicas. O ultrassom é considerado uma tecnologia emergente que pode atuar em controle e otimização de processos de produção de alimentos por promover reações químicas e físicas (Awad, Moharram, Shaltout, Asker & Youssef, 2012; Gallego-Juarez, 2010).

Ultrassom são ondas sonoras propagadas acima das frequências audíveis (>20 kHz). Essas ondas são classificadas como de alta e baixa energia. Ondas de alta energia têm a baixa frequência (20-100 kHz) desenvolvem maiores potências (10-1000 W.cm⁻²), suficientes para quebrar ligações intermoleculares. Intensidades >10 W.cm⁻² produzem o fenômeno da cavitação (Jayasooriya, Torley, d' Arcy, & Bhandari, 2007). A cavitação, responsável por grande parte dos efeitos do ultrassom no alimento, acontece quando a onda ultrassônica atravessa um meio líquido, produzindo bolhas no líquido. As bolhas oscilam entre os estados de compressão/rarefação até

ocorrer a sua implosão, proporcionando um aumento de pressão e temperatura localizado e a produção de microjatos que geram energia suficiente para desintegrar células e desnaturar enzimas (Chemat et al., 2011; Cárcel, García-Pérez, Benedito & Mulet 2012). Em algumas aplicações, as forças físicas e as reações químicas são úteis. Ojha, Keenan, Bright, Kerry, & Tiwari, 2016 verificaram que a cavitação provocada pelo ultrassom conduzido no processo de salga úmida de carne suína, aumentou a transferência de massa no sistema e, os autores concluíram que o ultrassom auxiliou na difusão de sal na carne, reduzindo o tempo de processamento. Paralelamente, outros trabalhos (Dolatowski & Stadnik, 2007; Stadnik & Dolatowski, 2011) verificaram que o ultrassom pode auxiliar no processo de cura da carne e pois no aumento na capacidade de retenção de água (CRA). Esses trabalhos ainda sugerem que o tratamento com o ultrassom acelera o processo de resolução do *rigor mortis* uma vez que observaram a fragmentação das estruturas das proteínas celulares. A utilização do ultrassom no processamento de carnes pode causar várias modificações na estrutura física e nas condições bioquímicas do produto. Nesse sentido, o objetivo deste artigo foi realizar uma revisão sobre os efeitos do uso do ultrassom sobre a CRA, cor e textura instrumental e sobre as propriedades sensoriais em carne e produtos cárneos.

2. ASPECTOS GERAIS SOBRE O ULTRASSOM

O ultrassom é uma energia gerada por uma onda mecânica longitudinal com vibração na frequência maior que 20.000 ciclos por segundo (20 kHz), que está acima do limite audível para seres humanos. O som é uma onda de pressão com propagação unidimensional e velocidade de um pulso ultrassônico depende das propriedades acústicas do meio em que se propaga. A velocidade é maior em sólidos do que em líquidos e maior em líquidos do que em gases (Kasaai, 2013). Em aplicações industriais e laboratoriais, o ultrassom é gerado a partir da energia elétrica fornecida a um transdutor, composto por um cristal pizoelétrico (Berlan, 1992). O cristal pizoelétrico transforma a energia elétrica em ondas mecânicas de frequência específica (Leighton, 1994). O transdutor é ligado a um aparato que transmite a vibração através do fluido. A energia transferida para a solução acarreta a propagação das ondas mecânicas. Parte dessa energia é convertida em calor e, muitas vezes, um

sistema de resfriamento é indicado, e o restante da energia pode produzir a cavitação (Alarcon-Rojo, 2019).

As ondas ultrassônicas são classificadas de acordo com sua frequência e intensidade. A frequência da onda ultrassônica é definida como o número de oscilações de onda que passam por um observador estacionário por segundo, é determinada pela fonte da onda e sua unidade é o Hertz (Hz) (Martin & Ramnarine, 2010). A intensidade de uma onda acústica é definida como a energia média transmitida através da unidade de área perpendicular à direção de propagação da onda (Mason & Lorimer, 2002).

Ultrassons de baixa energia são de alta frequência (2-20 kHz) e baixa intensidade ($<1\text{W.cm}^{-2}$) e não são destrutivos, tendo utilização em técnicas de imagens não invasivas, sensores e análises na medição da composição, maturação, eficácia de emulsificação e concentração ou dispersão de partículas em fluidos (Leadley & Williams, 2008). Para Prados, Garcia-Perez & Benedito (2017), a indústria de alimentos, em especial a de carnes e produtos cárneos exige técnicas de controle de qualidade não destrutivas que permitam determinar elementos da composição química do produto nas fases de recebimento de matéria-prima, processamento e pós-processamento para fins de controle de qualidade. Os referidos autores investigaram a viabilidade da aplicação de ultrassom como meio de realizar o monitoramento de salga seca em lombo e pernil suíno e para fins de predição do ganho final de sal pelos produtos. O uso da técnica ultrassônica é de grande potencial no monitoramento não destrutivo da salga seca para esse tipo de produto, bem como na predição do ganho de sal para fins de classificação, concluíram os autores.

Na tecnologia de alimentos, as ondas ultrassônicas são úteis em técnicas analíticas sobre propriedades físico-químicas e composição. Ondas ultrassônicas de alta energia apresentam baixas frequências (20-100 kHz) e desenvolvem níveis de potência mais altos ($10\text{-}1000\text{ W.cm}^{-2}$), essa energia é suficiente para romper ligações intermoleculares, sendo que intensidades superiores a 10 W.cm^{-2} origina a cavitação, capaz de alterar algumas propriedades físicas e favorecer reações químicas (Jayasooriya et al., 2007). A cavitação, responsável por grande parte dos efeitos do ultrassom no alimento, dá-se quando a onda ultrassônica atravessa um meio líquido, originando alternância de ondas de compressão (quando a pressão é positiva) e rarefação (quando a pressão é negativa), produzindo bolhas no líquido. Durante o

ciclo de expansão, ocorre a difusão de gases para o interior da bolha, promovendo sua expansão. Numa cavitação estável, a oscilação compressão/rarefação é regular e as bolhas resultam em microagitação no líquido sem implodir. Na cavitação instável, conhecida como transiente, as bolhas oscilam entre os estados de compressão/rarefação até ocorrer a sua implosão, proporcionando um aumento de pressão e temperatura localizado e a produção de microjatos que geram energia suficiente para desintegrar células e desnaturar enzimas (Chemat et al., 2011; Cárcel et al., 2012).

3. ULTRASSOM EM CARNES E PRODUTOS CÁRNEOS

Recentemente estudos têm reportado os efeitos da aplicação do ultrassom na carne e em processamento de produtos cárneos. A tabela 1 sintetiza os principais efeitos do uso do ultrassom sobre os parâmetros físico-químicos. A tabela 2 apresenta os efeitos do uso do ultrassom sobre as características sensoriais.

3.1 Capacidade de retenção de água (CRA)

A CRA configura-se como um dos parâmetros mais importantes que influenciam a qualidade da carne e do produto cárneo. Afeta a variação de peso no transporte e o armazenamento, perda no descongelamento, encolhimento durante o cozimento, a suculência e a maciez da carne (Gault, 1985; Lawrie, 1985). A CRA também influencia a cor, textura e dureza da carne *in natura* e pós-cozção (Hughes, Oiseth, Purslow, & Warner, 2014). A perda por exsudação acontece nos espaços entre os feixes de fibras musculares e na rede perimisial, e nos espaços entre as fibras musculares e a rede endomisial. As fibras se tornam menos fluidas com menor capacidade de reter a água após a instalação do *rigor mortis*, quando os músculos se transformam em carne. A exsudação excessiva por gotejamento acontece da combinação de rápido declínio do pH e alta temperatura no músculo *post mortem* (Joo, Kim, Hwang, & Ryu, 2013), podendo desnaturar proteínas.

Como a tecnologia de utilização do ultrassom em carnes e produtos cárneos é

relativamente nova, muitos trabalhos avaliam a CRA e as perdas de líquidos de carnes e produtos que sofreram esse tratamento. Alves et al. (2018) ao avaliar as perdas de peso em salame italiano tratado com ultrassom a $500\text{W}\cdot\text{cm}^{-2}$ de potência nominal, 25 kHz, durante 0, 3, 6 e 9 minutos não verificaram diferença significativa de peso em relação ao produto sem o uso do ultrassom. As perdas variaram de 30 a 40% em todos os tratamentos e, estão coerentes para este tipo de produto, afirmam os autores. Vimini et al. (1983) também não relataram nenhum efeito sobre a CRA de amostras de bolos de carne reestruturados tratados com ultrassom em comparação com o controle, sem uso dessa tecnologia.

Entretanto é importante discutir alguns trabalhos que reportam modificações na CRA de carnes e produtos cárneos tratados com ultrassom. Há trabalhos que relatam modificações na microestrutura do músculo causadas pela cavitação que afetam diretamente a CRA. Reynolds et al. (1978) reportaram aumento na CRA de presunto curado. Stadnik et al. (2008) ao aplicarem $2\text{W}\cdot\text{cm}^{-2}$ na maturação de carne bovina por 2 minutos a 45 kHz, também evidenciaram maiores valores para a CRA. Os autores justificam que a cavitação destrói parcialmente as proteínas miofibrilares e aumentam seu poder de ligar-se à água. Durante o processo de salga úmida de carne suína McDonnell, Allen, Morin & Lyng (2014b) aplicaram ultrassom com potência de 4.2, 11, 19 $\text{W}\cdot\text{cm}^{-2}$ a 20 kHz por 10, 25 e 40 minutos. Os autores relataram aumento da extração das proteínas, contudo não observaram diferenças na CRA entre as amostras, com e sem a utilização do ultrassom.

Em estudo recente, Barretto et al. (2018) descreveu a partir de imagens geradas por microscopia eletrônica que as bolhas geradas pela cavitação, resultante da aplicação do ultrassom a $600\text{W}\cdot\text{cm}^{-2}$ por 10 minutos, causou rupturas na superfície das proteínas miofibrilares no processamento de presuntos cozidos com baixo teor de sódio. Além das rupturas, os jatos de água provocados pela força da cavitação aumentaram o distanciamento do espaço interfibrilar. A maior exposição das proteínas e a abertura de canais entre as fibras aumentou significativamente a ligação da água com o produto, elevando a CRA. Comportamento semelhante foi reportado por Chemat, Zill, & Khan, 2011; Cárcel, García-Pérez, Benedito, & Mulet, 2012; Ozuna, Puig, García-Pérez, Mulet, and Cárcel, 2013; Siró et al., 2009; Zou, Zhang, Kang, Zhou, 2018. Após o tratamento com ultrassom a 40 kHz e $1500\text{W}\cdot\text{cm}^{-2}$ em carne bovina, Chang, Wang, Tang & Zhou (2015) visualizaram, por microscopia, que as

células musculares se romperam, os sarcômeros encolheram, o espaço extracelular aumentou, as cavidades intracelulares e os canais aumentaram. E concluíram que as mudanças na microestrutura do tecido podem ter algum efeito contributivo para amaciamento e aumento da CRA. Cichoski et al. (2019) aplicaram ultrassom no processamento de emulsões de carne suína. A potência utilizada foi de $1000\text{W}\cdot\text{cm}^{-2}$ na frequência de 25kHz por 5,5 minutos. Os autores verificaram que o uso do ultrassom melhorou significativamente o rendimento e a estabilidade da emulsão cárnea. Essa tecnologia reduziu a perda de gordura e de água em 30,59% a 30,48% respectivamente, comparados ao controle, sem uso do ultrassom. Os autores reiteram também que estes resultados podem ser recorrentes de possíveis modificações nas estruturas das proteínas miofibrilares, expondo os grupos polares, auxiliando na retenção de água e, aminoácidos não polares, que auxiliam na retenção de gordura.

O tempo e a potência de exposição ao ultrassom podem influenciar o comportamento da CRA no produto. Amiri, Sharifian & Soltanizadeh (2018) perceberam que o aumento da potência e do tempo de tratamento com ultrassom elevou a CRA em proteínas miofibrilares. O valor mais alto de CRA foi relacionado a amostra tratada por 30 minutos a potência de $300\text{W}\cdot\text{cm}^{-2}$. Os autores justificaram que a maior interação água-proteína como resultado do menor tamanho de partícula das proteínas miofibrilares, bem como maior pH após o tratamento com ultrassom aumentou a CRA.

O uso do ultrassom em carnes e produtos cárneos pode afetar a CRA. No entanto, o tipo do produto bem como a potência e tempo de aplicação podem interferir na quantidade da retenção. É possível perceber que as alterações na CRA são oriundas das modificações na estrutura das proteínas miofibrilares da carne e do produto cárneo causadas pela cavitação. O tipo de proteína e a dimensão do dano e modificação em sua estrutura nortearão o impacto do uso do ultrassom sobre a CRA do produto. Nesse sentido é importante a condução de mais estudos envolvendo diferentes carnes e produtos cárneos e, diferentes formatos de exposição às ondas ultrassônicas para aprofundar os conhecimentos sobre o impacto do ultrassom na CRA de carnes e produtos cárneos.

Tabela 1 - Efeitos do uso do ultrassom sobre alguns parâmetros físico-químicos de carne e produtos cárneos.

Produto	Aplicação (intens. /freq. /tempo)	Efeito do ultrassom	Autor
Salame Italiano	500W.cm ⁻² ; 25kHz; 3, 6 e 9 min.	Sem modificações nos parâmetros de cor instrumental; Sem efeito na perda de peso;	Alves et al. (2018).
Carne suína curada	4, 2, 11 e 19W.cm ⁻² ; 20kHz; 10, 25 e 40 min.	Sem modificações nos parâmetros de cor instrumental;	McDonnell et al. (2014).
Linguiça	200W.cm ⁻² ; 25 kHz; 10.5 min.	Sem modificações nos parâmetros de cor instrumental;	Cichoski et al. (2015).
Presunto cozido	600W.cm ⁻² ; 25kHz; 10 min.	Aumento do valor de a* e redução do valor de L*; Modificações na microestrutura; Aumento da CRA.	Barretto et al. (2018)
Presunto cozido	10-20W.cm ⁻² ; 40 kHz; 2-15 min.	Sem modificações no valor de L* e redução no valor de a*.	Ferrentino & Spilimbergo (2016)
Carne seca fermentada	480W.cm ⁻² ; 40kHz; 10 min.	Sem modificações no valor de L* e redução no valor de a*.	Wójciak et al. (2019)
Presunto cru curado	-	Modificações na microestrutura; Aumento da CRA.	Reynolds et al. (1978)
Carne bovina durante maturação	2W.cm ⁻² ; 45kHz; 2 min.	Modificações na microestrutura; Aumento da CRA.	Stadnik et al. (2008)

Carne bovina temperada	400-1000W.cm ⁻² ; 20kHz; 80-120 min.	Modificações na microestrutura; Aumento da CRA.	Zou et al. (2018)
Emulsão de carne suína	1000W.cm ⁻² ; 25kHz; 5.5 min.	Aumento do rendimento, maior estabilidade da emulsão (redução das perdas por exsudação); Aumento da coesividade, dureza e mastigabilidade.	Cichoski et al. (2019)
Carne de coelho	110W.cm ⁻² ; 40kHz; 0 a 120 min.	Aumento do valor de a*, redução de b* aumento da absorção de sal durante a salga.	Gómez-Salazar et al. (2018).

Fonte: elaborado pelo autor

3.2 Cor instrumental

A cor da carne está relacionada com os estados químicos da mioglobina no sarcoplasma. Ela depende de fatores *antemortem*: dieta, carcaça, genética; e de fatores *post-mortem*: cadeia do frio, processamento, ingredientes (Mancini, 2009). A cor da carne e de produtos cárneos é uma das características mais importantes que influencia o consumidor no momento da compra (Gracia & de Magistris, 2013; Ngapo, Martin, & Dransfield, 2007). Nesse sentido as transformações que a cor da carne e de produtos cárneos podem sofrer durante o processamento são de extrema importância para a indústria.

Alguns estudos se preocupam com o comportamento da cor instrumental em carnes e produtos cárneos submetidos ao tratamento com ultrassom. Alves et al. (2018) ao produzirem salame italiano submetidos ao tratamento com ultrassom a 500W.cm⁻² de potência nominal, 25 kHz, durante 0, 3, 6 e 9 minutos não reportaram nenhuma mudança nos parâmetros de cor instrumental. Resultado semelhante foi obtido por McDonnell, Lyng & Allen (2014a) que não observaram mudanças em L* e nem em a* de carne suína com cura acelerada pelo uso de ultrassom com 4, 2, 11 e 19 W.cm⁻² de potência nominal, 20kHz, por 10, 25 e 40 minutos. Para Sikes, Mawson,

Stark & Warner (2014) a cor da carne submetida ao ultrassom é pouco afetada, uma vez que o calor gerado pelo tratamento não é suficiente para desnaturar proteínas e pigmentos da carne. Cichoski et al. (2015) também não reportaram modificações nos parâmetros de cor instrumental de salsichas submetidas com ultrassom de potência $200\text{W}\cdot\text{cm}^{-2}$, 25 kHz por 10.5 minutos.

Em contrapartida, outros trabalhos destacam mudanças nos parâmetros de cor instrumental de carnes e produtos cárneos submetidos ao tratamento com ultrassom. Barretto et al. (2018) reportaram que o uso do ultrassom no processamento de presuntos cozidos com baixo teor de sódio aumentou os valores de a^* , enquanto baixou os valores de L^* . Ferrentino & Spilimbergo (2016) mostraram que a combinação de aplicação de ultrassom e alta pressão de dióxido de carbono em presunto cozido não modificou o valor de L^* , contudo diminuíram os valores para a^* . Wójciak et al. (2019) ao submeterem carne seca fermentada a $480\text{W}\cdot\text{cm}^{-2}$ por 10 minutos não relataram diferenças no parâmetro L^* , contudo evidenciaram uma redução para os valores de a^* até 93 dias de maturação do produto. O ultrassom acelera as mudanças totais na cor da carne e do produto cárneo, limita a formação de oximioglobina e retarda a formação da metamioglobina (Stadnik & Dolatowski, 2011). Gómez-Salazar, Ochoa-Montes, Cerón-García, Ozuna & Sosa-Morales (2018) ao avaliarem os efeitos do ultrassom de $110\text{W}\cdot\text{cm}^{-2}$ e 40 kHz sobre a cor instrumental de carne de coelho durante a salga úmida, perceberam que o ultrassom e a salga reduziram o valor de a^* , contudo com o uso do ultrassom, a redução de a^* foi menor comparada com a salga estática mostrando um efeito protetor do ultrassom para cor instrumental durante a salga úmida.

Com base nesses resultados da pesquisa, é possível afirmar que os efeitos do uso do ultrassom sobre a cor instrumental são bastante variáveis de acordo com o produto tratado e com as especificações do tratamento, nesse sentido é necessário ampliar os estudos sobre este efeito nesses tipos de produtos.

3.3 Textura instrumental

A textura é um dos atributos mais importantes da carne e produtos cárneos, ela pode influenciar a aceitação e o preço final do produto. O consumidor está disposto a

pagar mais pela garantia de uma carne mais tenra. Logo, o conhecimento, o controle e a previsão da textura em carnes, especialmente o atributo referente a dureza, tem sido extensivamente pesquisado (Miller et al., 2001).

Como há evidências de modificações causadas pela cavitação na microestrutura e na CRA de carnes e produtos cárneos, alguns trabalhos atentam-se para avaliar os efeitos da aplicação do ultrassom sobre a textura instrumental de carnes e produtos cárneos. Os resultados relatados mostram-se bastante variáveis e, provavelmente são condicionados às variações experimentais.

Ao aplicarem ultrassom no processo de salga de carne suína, Ozuna et al. (2013) relataram aumento da dureza da carne. Barretto et al. (2018) e Ojha et al. (2016) também observaram mesmo comportamento ao aplicarem ultrassom durante o processo de produção de presunto cozido e de salga úmida de carne suína respectivamente. Para esses autores, os efeitos do ultrassom na textura da carne podem estar associados à intensificação da difusão de NaCl provocado pela cavitação, que provoca mudanças estruturais nas proteínas da carne e contribuem com o aumento da dureza. Cichoski et al. (2019) evidenciaram aumento nos parâmetros de dureza, coesividade e mastigabilidade de emulsões cárneas tratadas com ultrassom. Para esses autores, a aplicação do ultrassom favoreceu uma formação do gel com maior força entre as proteínas. Um gel resistente consiste em interações de proteína de alta qualidade, com um consequente aumento da coesividade, dureza e mastigabilidade da emulsão.

Pinton et al. (2019) usaram o ultrassom para reduzir o teor de fosfato em emulsões cárneas, e perceberam que o ultrassom afetou a textura das emulsões. Eles relataram uma diminuição na dureza e mastigabilidade com aplicação de ultrassom durante nove minutos nas amostras sem redução de fosfato e após dezoito minutos de aplicação de ultrassom houve redução da dureza e mastigabilidade nas amostras com até 75% de redução de fosfato. Para os autores, este comportamento pode ser devido à maior retenção de água e gordura das amostras submetidas ao ultrassom quando comparadas às amostras não tratadas nos mesmos níveis de fosfato.

Siró et al. (2009) concluíram que diferentes tempos de aplicação e intensidades distintas do ultrassom podem resultar parâmetros da textura diferentes. Em um estudo sobre salga em lombo suíno, os autores concluíram que a aplicação de ultrassom

afetou a textura, em particular a maciez das amostras do produto. Sob intensidade mais baixa (2.0 W.cm^{-2}) não foi observado efeito sobre a textura, uma vez que o ultrassom não foi suficiente para enfraquecer a estrutura do tecido e, assim, não resultou em sua sensibilidade. À medida que se aumentou a intensidade (2.5 e $3,0 \text{ W.cm}^{-2}$), foi observado uma diminuição da dureza da carne. Segundo os autores, a natureza destrutiva da cavitação e a vibração das ondas ultrassônicas podem levar ao enfraquecimento físico da estrutura da carne, aumentando sua maciez. Por outro lado, intensidades ultrassônicas mais altas ($3,5$ e $4,0 \text{ W.cm}^{-2}$) causaram a desnaturação da proteína, baixando a capacidade de retenção de água, consequentemente levando ao enrijecimento do tecido da carne. Este resultado tornou-se mais pronunciado com o aumento do tempo de tratamento, concluíram os pesquisadores. Resultado não corroborado por Li, Kang, Zou, Xu, & Zhou, (2015) que relataram que o aumento tempo (40 minutos) sob alta intensidade (300 W.cm^{-2}) de aplicação de ultrassom em emulsão de peito de frango com sal a 1% ou 1,5% diminuiu significativamente a dureza do produto em comparação com os controles. Para esses autores, a diminuição da dureza é atribuída à deterioração funcional da proteína da carne.

São nítidas as diferenças nos resultados sobre as influências da aplicação do ultrassom sobre os parâmetros de textura da carne e de produtos cárneos. A multiplicidade de variáveis aqui contidas podem ser os fatores norteadores dessas diferenças. Não apenas a variação do processo (tempos e intensidades do ultrassom) mas também a natureza da matéria-prima. A textura da carne é determinada pela estrutura, quantidade e estado de seus diferentes componentes, como proteína, água, gordura e tecido conjuntivo. Assim, a composição e organização estrutural, juntamente com mudanças bioquímicas *post-mortem* e condições de armazenamento e processamento, são elementos fundamentais para descrever os mecanismos que definem a textura final da carne (Cardenas & Oliveira, 2016).

3.4 Características sensoriais

Os atributos sensoriais são fatores muito importantes para a qualidade na indústria de carnes e são responsáveis pelas escolhas de produtos pelos consumidores (Mandour, Bashari, Lagnika, He & Sun). Ao considerar o uso de tecnologias emergentes na carne, como o ultrassom, os mecanismos de ação e os

efeitos na transformação nos atributos sensoriais devem ser conhecidos (Alarcon-Rojo et al. 2019). Peña-González et al. (2017) submetem carne bovina ao ultrassom de potência de $11\text{W}\cdot\text{cm}^{-2}$, 40kHz por 60 minutos e avaliaram os efeitos sensoriais durante a maturação do produto. Os autores concluíram que a aplicação do ultrassom pode ser uma maneira viável para melhorar as propriedades sensoriais da carne, pois reduziu significativamente o tempo de maturação. A carne tratada com ultrassom apresentou, para os provadores, maior maciez e maior suculência comparada ao controle, sem uso dessa tecnologia. O ultrassom pode, também, auxiliar no processo de maturação da carne. O período de maturação pode ser reduzido, preservando os parâmetros de qualidade da carne (Dolatowski, Stadnik & Stasiak, 2007).

Barretto et al. (2018) avaliaram sensorialmente presunto cozido com baixo teor de sódio submetidos a ultrassom de $600\text{W}\cdot\text{cm}^{-2}$, 20kHz por 10 minutos. Os autores destacaram que o uso do ultrassom melhorou a aceitação sensorial para sabor, textura e aceitação global do produto. A melhor difusão do sal, que foi adicionado no processamento, provocada pela cavitação melhorou a extração das proteínas miofibrilares e, conseqüentemente aumentou a capacidade de retenção de água. Estes efeitos físico-químicos possivelmente contribuíram para aumentar as notas atribuídas pelos provadores na aceitação sensorial, comparadas a amostra sem o uso do ultrassom. Pinton et al. (2019) conduziram análise de aceitação sensorial de mortadela com adição reduzida de fosfato e tratamento com ultrassom com $1000\text{W}\cdot\text{cm}^{-2}$ de potência, 25 kHz durante 18 minutos. Os autores verificaram melhores notas para o atributo textura para o produto com 50% de redução de fosfato que foi submetido ao ultrassom, comparado ao seu controle sem o uso dessa tecnologia.

González-González et al. (2017) conduziram processo de salga úmida em músculo bovino assistido com ultrassom a 40 kHz numa potência de $11\text{W}\cdot\text{cm}^{-2}$ durante 20, 40 e 60 minutos. As amostras foram armazenadas a 4°C durante 7 dias. Os autores estudaram a transferência de massa e homogeneidade da transferência e a aceitação sensorial de vinte e quatro consumidores do produto e concluíram que o ultrassom pode oferecer uma alternativa viável às técnicas tradicionais de salga úmida. Contudo a carne submetida ao ultrassom não aumentou a transferência de massa, apenas apresentou uma distribuição mais homogênea dos solutos de salmoura e, não aumentou a aceitabilidade da carne de acordo com a análise sensorial. A aceitabilidade do produto marinado depende da maior transferência de

massa para a carne, e não da homogeneidade e heterogeneidade da distribuição do soluto. Uma análise sensorial quantitativa descritiva com painel treinado deve ser considerada para avaliar a percepção de propriedades específicas na carne em distribuições homogêneas e heterogêneas da salmoura, complementaram os autores.

Yeung & Huang (2017) ao submeterem lombo suíno ao ultrassom de 2200W.cm^{-2} , 15 kHz, durante zero a seis minutos perceberam que não houve mudança nas notas de aceitação sensorial para aparência e sabor. No entanto constataram que à medida que foi aumentado o tempo do ultrassom, aumentou-se também as notas atribuídas para maciez do produto. Os autores concluíram que para esse produto e nas condições que o ultrassom foi utilizado, ele representa uma alternativa viável para amaciar carnes e não impactar em sua aparência e sabor.

Como observado para os parâmetros físico-químicos (CRA, cor e textura instrumental) há grande variabilidade no comportamento sensorial de carnes e produtos cárneos submetidos ao ultrassom, uma vez também que a aceitação sensorial pode estar associada às mudanças físico-químicas. Como já exposto, os parâmetros sensoriais são muito importantes para a indústria da carne e, nesse sentido pesquisas sobre o impacto na aceitação sensorial de carnes e produtos cárneos são fundamentais no desenvolvimento da aplicação da tecnologia do ultrassom nesses tipos de produtos.

Tabela 2 - Efeitos do uso do ultrassom sobre algumas características sensoriais em carnes e produtos cárneos.

Produto	Aplicação (intens. /freq. /tempo)	Efeitos sensoriais do ultrassom	Autor
Carne bovina	11 W.cm^{-2} ; 40kHz; 60 min.	Aumento da maciez e suculência.	Peña-González et al. (2017).
Presunto cozido	600 W.cm^{-2} ; 20kHz, 10 min.	Melhora do sabor, textura e aceitação global.	Barretto et al. (2018).
Mortadela	1000 W.cm^{-2} ; 25kHz, 18 min.	Melhora da textura.	Pinton et al., (2019)

Carne bovina	11 W.cm ⁻² ; 40kHz, 20, 40 e 60 min.	Não foram observadas diferenças na aceitação sensorial.	González-González et al. (2017).
Lombo suíno	2200 W.cm ⁻² ; 15kHz; 0 a 6 min.	Não foram observadas diferenças na aparência, sabor e textura. Aumento da maciez com o aumento do tempo de ultrassom.	Yeung & Huang (2017)

Fonte: elaborado pelo autor

3.5 Considerações sobre o uso do ultrassom e redução de sódio em produtos cárneos

Alguns autores atribuem que quando há adição e ou maior concentração de sal durante o tratamento com ultrassom, a capacidade da carne em reter a água é maior, elevando assim a CRA (Kang et al., 2016; Kang et al., 2017). Ojha, Keenan, Bright, Kerry & Tiwari (2016) investigaram a difusão de sal em carne suína tratada com diferentes intensidades de ultrassom e concluíram que o coeficiente de distribuição de sal na carne foi significativamente maior que o controle sem o uso de ultrassom. A melhor difusão do sal ocasionada pelo uso do ultrassom, facilitou a extração das proteínas miofibrilares e sua ligação à água (Zou et al., 2018). Ao submeterem carne bovina temperada ao tratamento com ultrassom na potências de 0, 400, 600, 800 e 1000W.cm⁻², frequência de 20 kHz, por 80, 100, 120 minutos, Zou et al. (2018) mostraram que o ultrassom pode aumentar significativamente o teor de sal na carne e, aumentar a CRA, reduzindo significativamente a perda de água livre, melhorando o teor de água imobilizada. Quanto à microestrutura, os autores relataram que as miofibrilas de carne bovina foram rompidas pelo tratamento ultrassônico, juntamente com as linhas Z, levando ao edema muscular.

A tecnologia do ultrassom para reduzir o sal adicionado nos processos na indústria da carne é baseado na compreensão desses processos de transferência de

massa e como eles podem modificar as membranas celulares ajudando processos como a cura, marinação, secagem e amaciamento do tecido. O ultrassom durante o salga, por exemplo, pode auxiliar a distribuição de sal no tecido, proporcionando uma maior percepção sensorial do sal, mesmo quando o conteúdo global de cloreto de sódio adicionado é menor (Alarcon-Rojo, Janacua, Rodriguez, Paniwnyk, & Mason, 2015). A possibilidade de uma tecnologia de cura rápida, associada com o aumento da difusão de sal, pode respaldar a redução da adição de cloreto de sódio na solução de salmoura e obter maior controle sobre o amaciamento enzimático e sobre danos estruturais do produto (Tao & Sun, 2015).

Ozuna, Puig, Garcia-Perez, Mulet, & Carcel, 2013 afirmam que a tecnologia de ultrassom pode aumentar a transferência de massa de sal em processos como salga úmida de carne. A salga é um dos principais processos utilizadas na fabricação de produtos cárneos, uma vez que aumenta a vida útil, melhora o sabor, a suculência e a maciez dos produtos. Muitos estudos têm evidenciado que a aplicação do ultrassom à carne, a taxa de ganho de cloreto de sódio aumenta em comparação com a carne submetida à salga estática tradicional (Cárcel et al., 2007, Siro et al., 2009). Um estudo sobre a aplicação industrial do processo de salga na fabricação de presuntos com auxílio do ultrassom, utilizou amostras de carne suína tratadas com diferentes intensidades ultrassônicas. Para todas as amostras, o teor desejado de cloreto de sódio foi atingido dentro de duas horas. A amostra controle, sem o uso do ultrassom, exigiu 4 horas para atingir o mesmo teor. O tratamento com ultrassom também não mostrou nenhum efeito negativo sobre o rendimento do produto, sobre os teores de umidade ou perfil de textura instrumental (McDonnell et al., 2014b, McDonnell et al., 2014c).

4 CONSIDERAÇÕES FINAIS

O ultrassom mostra-se uma tecnologia emergente na indústria de alimentos e apresenta possíveis vantagens, particularmente à indústria de carnes e produtos cárneos. Por tratar-se de uma tecnologia nova, carece de pesquisas envolvendo suas condições de aplicação em diferentes matérias-primas e produtos. A partir dos estudos já conduzidos, é possível observar que o uso do ultrassom pode ser uma

alternativa viável do ponto de vista tecnológico e sensorial no processamento de carnes e produtos cárneos. A capacidade de retenção de água, cor e textura instrumental bem como a aceitação sensorial podem ser melhoradas com a utilização dessa tecnologia. A intensificação dos estudos envolvendo ultrassom em carnes pode no futuro tornar essa tecnologia industrialmente aplicável.

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CAPÍTULO II

WATER SORPTION ISOTHERMS OF COOKED HAMS AS AFFECTED BY TEMPERATURE AND CHEMICAL COMPOSITION

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Artigo Original

WATER SORPTION ISOTHERMS OF COOKED HAMS AS AFFECTED BY TEMPERATURE AND CHEMICAL COMPOSITION

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ABSTRACT

This study was focused on the determination of the chemical composition and the experimental obtaining of sorption isotherms for four samples of commercial cooked ham subjected to simulated commercial storage conditions. The isotherms were determined using the gravimetric method. The experimental data were adjusted to the mathematical models of Guggenheim, Anderson and de Boer; Brunauer, Emmett and Teller; Halsey; Henderson; and Peleg. The Guggenheim, Anderson and de Boer model was chosen to better describe the isotherms as it had a very good fit. The increase in temperature reduced the equilibrium moisture content of the product. Increased relative humidity resulted in an increase in equilibrium moisture content of the product regardless of storage temperature. The differences in chemical composition between the samples affect the desorption isotherms. The higher the content and availability of the protein or the lower the fat content, the higher the equilibrium moisture content of the product.

PRACTICAL APPLICATION

The sorption isotherms of cooked ham can be influenced by storage temperature, by environmental activity water, and by the chemical composition of the product. The higher the storage temperature, the lower the equilibrium moisture content. Increased

environmental activity water, results in an elevated equilibrium moisture content. And the lower the Moisture/Protein ratio or the lower the fat content, the higher its equilibrium moisture content. This information can help the meat industry to maintaining the cooked ham humidity and increasing its shelf-life.

Keywords: Mathematical modeling, equilibrium moisture content, water activity, protein, fat.

1. INTRODUCTION

Cooked ham is one of the most popular processed meat products among Brazilian and European consumers (Válková et al., 2007). The growing consumption of cooked ham is linked to the recent efforts that are focused on the increase of its acceptability through the evaluation of physical and sensory characteristics, such as appearance, texture, flavor and color (Ávila et al., 2014; Barbieri, et al., 2016; Delahunty, et al. 1997; Tomović et al., 2013).

The processing of cooked ham consists basically of brine incorporation in the pork by tumbling and massaging, followed by cooking to extract proteins and adequate cooling to take the ham out of the mold (Talens et al., 2013). The final product is then transported and maintained under controlled low temperatures until posterior use. As a consequence of all these steps, the final quality of the hams will depend on many factors, including the origin and composition of ingredients and the processing conditions (Valková et al., 2007).

The selection of the ingredients is considered essential in ensuring the sensory quality from storage up to the product consumption (Toldrá, et al., 2010). The ingredient composition can affect the way water molecules bind the food matrix, causing changes on water activity and, consequently, on food stability (Rizvi, 2005). In the specific case of meat products, these alterations can influence not only the microbial growth, but also the meat texture. Enzymes such as proteases and lipases may have their activity affected by different aw conditions (Toldrá, 2006).

The sorption isotherms are an interesting approach to provide information about the water activity and equilibrium moisture content of a product at a certain

temperature and ambient relative humidity (Ahmat, et.al., 2014). Best processing and storage conditions can be reached by understanding water sorption behavior in food products (Brett, 2009; Sharma, et al., 2018). This method can be carried out at different temperatures, avoiding unwanted changes and increasing shelf life of foodstuff (Staudt, et al., 2013).

Water sorption isotherms of different meat products have been studied by a number of authors, considering the effect of temperature (Clemente, et al., 2009; Delgado & Sun, 2002; Comaposada, et al., 2000; Cortés & Chejne, 2010; Lind & Rask, 1991; Lopes Filho et al., 2002). However, scarce information has been specifically reported about the sorption isotherms of cooked ham.

Several models (empirical, semi-empirical and theoretical) can be used to mathematically describe sorption isotherms for meat. It is consolidated in literature that the most of the food sorption isotherms can be expressed analytically by the Guggenheim-Anderson-de Boer (GAB) equation (Al-Muhtaseb, et al., 2004; Bizot, 1983; Chirife & Iglesias, 1978; Lewicki, 1997.). Peleg (1993) proposed a dual power expression which, when compared to the GAB equation, produces a good or better fit to the experimental data. On the other hand, the parameters of the GAB model give insights about the interactions between the sorbent-sorbate, as well as the multilayer-monolayer moisture (Quirijns, et al., 2005).

The modeling of water sorption isotherms plays an important role on food storage. A well-fitted model can predict the gain or loss of water through the equilibrium moisture content. For example, Comaposada et al. (2000) stated that if the water activity of pork on the surface is high, an increase in temperature and/or a decrease in relative humidity can produce a significant loss of moisture content. This phenomenon may increase the dehydration speed inside the meat product, which is not always desirable. The water loss during storage can prejudice not only the meat quality, but also the commercial value of the product as they are commonly sold according to the weight.

In this way, the aim of this study was to study the influence of the chemical composition on the sorption isotherms of four brands of commercial cooked ham. For this, the cooked hams were firstly characterized according to the chemical composition. Then, the corresponding water sorption isotherms were obtained at different common storage temperatures for a wide range of relative humidities. Experimental data were

fitted by the different proposed models and the differences among the samples were evaluated.

2. MATERIALS AND METHODS

2.1. Sample preparation

Cooked hams of four different brands (Sample 1 – S1, Sample 2 – S2, Sample 3 – S3 and Sample 4 – S4) were purchased at a local store in São José do Rio Preto, São Paulo, Brazil. For each brand, three different lots were bought and used in this study. Each lot purchased was chopped and homogenized. The samples, after homogenization, were packed in polyethylene bags (0.15 mm of thickness) and vacuum packed. They were wrapped in aluminum sheet and stored in a freezer (-12 °C) until its use. To conduct the analysis, the cooked ham samples were thawed in cold storage at 5 °C.

2.2 Chemical composition

The chemical composition analysis was done in triplicate for the four samples using the methods described by AOAC (2007). Moisture content was determined in an oven, protein by the micro-Kjeldahl method, ashes by incineration in a muffle furnace and fat contents using the Soxhlet method. The total carbohydrates were determined by difference.

2.3 Obtaining the sorption isotherms

The sorption isotherms of ham samples were determined by the static gravimetric method (Jowitt et al., 1983), following the procedures for obtaining desorption isotherms at 2, 9, 16, 24 and 30 °C. To produce and maintain the relative humidity between 6.1% and 92%, saturated solutions of LiBr, LiCl, LiI, MgCl₂, NaI, NaBr, KI, NaNO₃, NaCl and KCl were prepared by dissolving sufficient quantities of each these salts (Sigma-Aldrich, St. Louis, MO, USA) in deionized water. At the equilibrium, the water

activity (a_w) of salt solutions in Table 1 corresponds to the air relative humidity. These values were obtained by the study of Labuza (1963) at different temperatures.

Table 1 - Water activity of the salt solutions at different temperatures.

Salt solution	Temperature (°C)				
	2	9	16	24	30
LiBr	0.076	0.072	0.068	0.064	0.061
LiCl	0.112	0.112	0.112	0.112	0.112
LiI	0.224	0.209	0.194	0.180	0.167
MgCl ₂	0.334	0.334	0.331	0.327	0.322
NaI	0.434	0.428	0.415	0.396	0.374
NaBr	0.642	0.624	0.603	0.581	0.559
KI	0.740	0.724	0.708	0.693	0.679
NaNO ₃	0.789	0.775	0.760	0.745	0.729
NaCl	0.756	0.755	0.754	0.753	0.752
KCl	0.881	0.871	0.861	0.851	0.842

Labuza (1963)

For each measurement, three repetitions of about 1 g of sample of ham were placed in small plastic containers, which were in turn placed on a support in each jar to avoid contact with the salt solution. The jars were then placed in a chamber with a controlled temperature (BOD, Model TE-391, TECNAL, Brazil) at temperatures of 2, 9, 16, 24 and 30 ° C. The weights of the samples were monitored until the moisture content, on a dry weight basis, did not exceed 0.1% (after approximately five weeks) at the point of equilibrium reached. For each batch of ham, the initial moisture content was determined according to the AOAC method (2007) for posterior determination of the equilibrium moisture content (X_{eq}). The values for X_{eq} as a function of a_w at the fixed temperatures were used to construct sorption isotherms curves for the cooked ham samples.

2.4 Modelling of sorption isotherms

Five isotherm models (Table 2) were chosen to fit the experimental data of equilibrium moisture content and a_w at all temperatures studied. Non-linear regressions were carried out using the OriginPro 8.0 software (OriginLab Corporation, Northampton, MA, USA) to adjust the mathematical models. The accuracy of fit of each model was evaluated based on the adjusted coefficient of determination (R_{adj}^2) and the Root Mean Squared Error (RMSE). Coefficients of determination greater than 0.98 indicates a good fit, and the RMSE close to zero shows fidelity to the experimental data (Cantu-Lozano, et al., 2013; Mclaughlin & Magee, 1998; Mcminn, 2006).

Table 2 - Models used to fit sorption isotherm data from cooked hams.

Model	Equation
BET (Brunauer, Emmett & Teller, 1938)	$X_{eq} = \frac{X_m C a_w}{(1 - a_w)(1 - a_w + C a_w)} \quad (1)$
GAB (Chirife & Iglesias, 1978)	$X_{eq} = \frac{X_m C_g K a_w}{(1 - K a_w)(1 - K a_w + C_g K a_w)} \quad (2)$
Henderson (Henderson, 1952)	$X_{eq} = \left(\frac{-1}{H_1} \ln(1 - a_w) \right)^{1/H_2} \quad (3)$
Halsey (Van Den Berg & Bruin, 1981)	$X_{eq} = (-h_1 \ln(a_w))^{(-1/h_2)} \quad (4)$
Peleg (Peleg, 1993)	$X_{eq} = k_1 a_w^{n_1} + k_2 a_w^{n_2} \quad (5)$

C_g , K , K_1 , K_2 , M_1 , M_2 , h_1 , h_2 , n_1 and n_2 are constants, a_w is the water activity (relative humidity of salt solutions, decimal), X_{eq} is the equilibrium moisture content (dry basis), and X_m is the monolayer moisture content (dry basis). Fonte: elaborado pelo autor

3 RESULTS AND DISCUSSION

3.1 Chemical composition

The samples composition was experimentally determined. In general, the samples presented very similar composition to the ones published in literature (Del Campo et al., 1988; Desmond et al., 2000; Talens et al., 2013). In order to verify the significant differences among the samples, triplicates were subjected to the analysis of variance and to the Tukey test at 95% of confidence. The results of chemical composition are shown in Table 3.

All samples could be differentiated ($p < 0.05$) with respect to fat content, presenting values between 1.06 and 2.58%. These differences in fat are considered low and are inherent to the raw material (leg of pork) composition that may have small changes in moisture content, fat and final protein. S4 had a lower amount of fat, which was considered statistically different ($p < 0.05$) from the other samples. According to Dutra et al. (2012), meat products with lower fat contents tend to present softer texture and poor binding properties.

Table 3 - Average values (\pm standard deviation) of the percentage composition of the cooked hams.

%	Samples			
	S1	S2	S3	S4
Fat	2.18 \pm 0.01 ^c	2.58 \pm 0.01 ^a	2.38 \pm 0.01 ^b	1.06 \pm 0.01 ^d
Protein	19.19 \pm 0.06 ^c	19.12 \pm 0.04 ^c	19.67 \pm 0.10 ^b	19.86 \pm 0.14 ^a
Moisture	74.26 \pm 0.33 ^a	73.76 \pm 0.46 ^a	74.09 \pm 0.46 ^a	74.32 \pm 0.33 ^a
Ash	2.76 \pm 0.01 ^b	3.18 \pm 0.01 ^a	3.06 \pm 0.01 ^a	2.98 \pm 0.01 ^a
Water/Protein mean ratio	3.87	3.86	3.77	3.74
Carbohydrates*	1.61	1.36	0.80	1.78

Fonte: elaborado pelo autor

Averages followed by the same letter in the same line do not show significant difference in the Tukey test at 95% of confidence.

*Calculated by difference from the average fat, protein, moisture and ash contents.

For the amount of protein, the results were between 19.12 and 19.86%, with significant statistical differences. However, the values are considered to be close. According to Brazilian legislation (Brazil, 2000), the minimum protein content for this product is 14%, so all samples are in compliance. In the same legislation, a maximum water/protein (W/P) ratio of 5.35 is suggested, being also in accordance to

the presented data. These differences are probably a result of variations on the quality and integrity of the ham muscles, as well as on the amount and concentration of the injected brine (Casiraghi et al., 2007). As S4 had the highest protein content ($p < 0.05$), the relationship W/P presented lower values when compared to the other samples. In addition, Spanish legislation characterizes these cooked hams in the extra category, which must have W/P less than 4.13 (Talens et al., 2013).

The carbohydrate contents were also compliant, since the upper limit is 2% according to the Brazilian legislation. These carbohydrates can be represented by dextrose, which is used to confer taste to cooked hams (Toldrá et al., 2010).

3.2 Sorption isotherms

Experimental data of equilibrium moisture content showed that water desorption occurred for all samples. The values of X_{eq} ranged from 5.4% up to 63.4% in dry basis (5.1% and 38.9%, respectively, in wet basis), which were lower than the initial moisture content. They increased at lower temperatures and higher a_w , being in accordance to the adsorption and desorption behavior of different foodstuff such as dairy, powdered beverages, vegetable and fruits, residues of agroindustry and specially meat products (Ahmat et al., 2014; Comaposada et al. 2000; Cortés & Chejne, 2010; Delgado & Sun, 2002; Gabas et al., 2007; Kaymak-Ertekin & Gedik, 2004; Lomauro et al., 1985; Singh, et al., 2001).

In order to represent adequately sorption behavior, non-linear regressions were carried out to fit experimental data. The best fits to the experimental data were obtained by the Peleg model (Eq. 5, table 2) and the GAB model (Eq. 2, table 2). Considering all the samples of cooked ham at the temperatures studied, the R_{adj}^2 for the Peleg model ranged from 0.9960 to 0.9999 and the $RMSE$ from 0.0075 to 0.0001. Meanwhile, for the GAB model, R_{adj}^2 ranged from 0.9854 to 0.9939 and the $RMSE$ from 0.3137 to 0.0086. The Henderson model (Eq. 3, table 2) had the lowest R_{adj}^2 , implying that this model does not closely describe the experimental data for cooked ham. Halsey model (Eq. 4, table 2) showed good accuracy, mainly for lower temperatures. However, at higher temperatures, this model showed slight higher lack of fit than GAB model. With respect to Brunauer, Emmett and Teller (BET) model, GAB

model also presented slight higher accuracy, which might be attributed to the limited range of water activity (up to 0.3–0.4) that BET model is able to fit (Timmermann et al., 2001).

Despite the Peleg model having a greater R_{adj}^2 value and lower $RMSE$, the GAB model was chosen to better describe the sorption isotherms of cooked ham at different storage temperatures. The fitting parameters of GAB equation is displayed in Table 4 for all samples and temperatures. The GAB model is considered the most versatile model available in the literature for isothermal adjustment of food (Al-Muhtaseb et al., 2004; Telis et al., 2000). On the same way, Moraes and Pinto (2012) recommended the GAB model to describe the water sorption in food matrices.

Table 4 - Fitting parameters of GAB equation for all samples at the different temperatures.

	Temperature °C				
	2	9	16	24	30
Sample 1					
X_m	0.0754	0.0688	0.0677	0.0559	0.0536
C_G	26.7943	25.9835	27.7396	153.6238	94.7108
K	0.9831	0.9968	0.9986	1.0204	1.0357
R_{adj}^2	0.9912	0.9839	0.9820	0.9913	0.9895
$RMSE$	0.0126	0.0160	0.0154	0.0090	0.0102
Sample 2					
X_m	0.0755	0.0712	0.0679	0.0629	0.0576
C_G	23.9674	31.1278	41.8241	55.7274	76.1910
K	0.9902	0.9995	1.0082	1.0183	1.0254
R_{adj}^2	0.9903	0.9909	0.9918	0.9929	0.9939
$RMSE$	0.0341	0.0337	0.0337	0.0325	0.0307
Sample 3					
X_m	0.0787	0.0726	0.0699	0.0664	0.0645
C_G	23.6260	31.2144	41.6545	56.2088	50.8047
K	0.9899	0.9994	1.0095	1.0174	1.0170
R_{adj}^2	0.9903	0.9910	0.9920	0.9928	0.9913
$RMSE$	0.0146	0.0127	0.0113	0.0099	0.0099
Sample 4					
X_m	0.0818	0.0736	0.0791	0.0690	0.0638
C_G	27.2917	28.7607	26.1043	53.7948	77.4053
K	0.9911	1.0002	0.9951	1.0225	1.0185

R_{adj}^2	0.9926	0.9898	0.9854	0.9930	0.9933
RMSE	0.0133	0.0138	0.0160	0.0105	0.0086

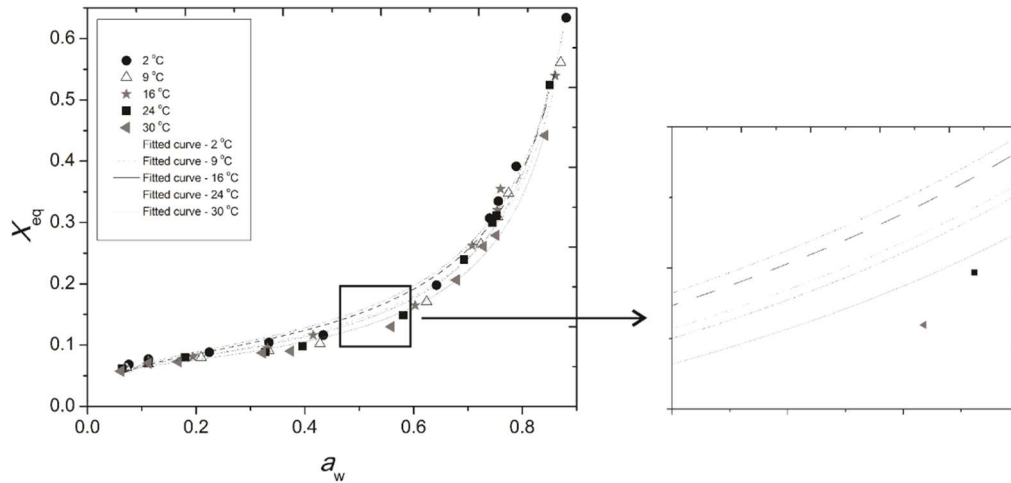
Fonte: elaborado pelo autor

This theoretical triparametric model is suitable for applications in food engineering and allows a very good fit for almost all types of food in the a_w ranging between 0.1 and 0.9 (Anderson, 1946; Saravacos et al., 1986). The biggest advantage is that their parameters have physical meaning, different from empirical and semi-empirical models. These parameters can also provide important information on the state of the water in food. In the GAB equation, for example, the concept of monolayer moisture content X_m is taken into account (Maroulis et al., 1988). It is related to the stability and shelf-life of the product (Rosa et al., 2010; Yan et al., 2012). The values of X_m extracted from the GAB equation (Eq. 2, table 2) showed a decreasing trend with increasing temperature. This behavior is reported in literature since the studies of Iglesias and Chirife (1976a,b) until the recent reports for different food matrices (Freitas et al., 2016; Owo et al., 2016; Polachini et al., 2016). It is a consequence of the variations on the number of active sorption sites due to changes on temperature and on the chemical composition of the samples.

The graphical representation of GAB adjustments is shown at Figures 1 for S4 at all temperatures and at Figure 2 for all samples at fixed temperature. It is clear that the curves assumed a sigmoidal behavior, typical of type II isotherms (Brunauer et al., 1940). Type II isotherms were also obtained by Ahmat et al. (2014) when studying the desorption isotherms of fresh beef. This type of isotherm can be divided into three regions: the first corresponds to the monolayer moisture strongly attached to the product matrix; the second follows an almost linear path, which corresponds to the multilayers moisture; and the third part is related to the free water available for reaction (Mathlouthi, 2001).

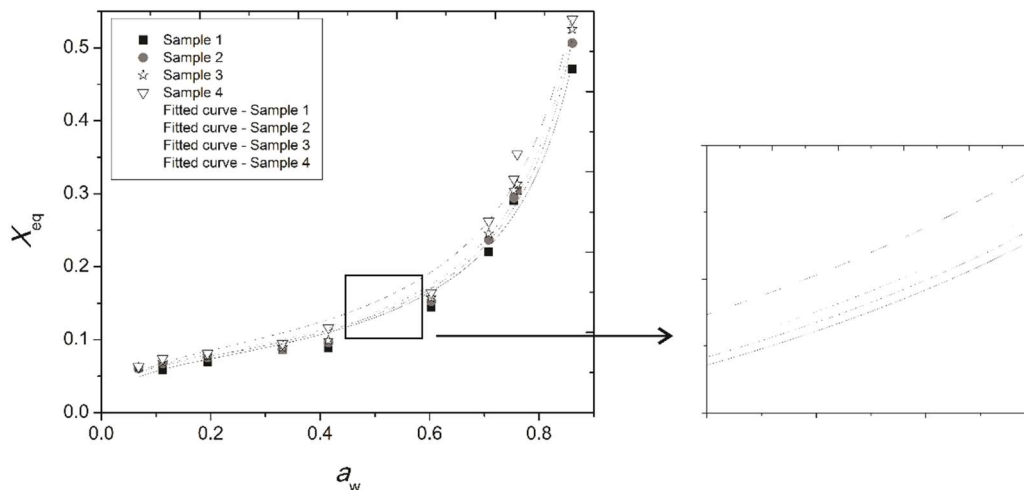
It can also be seen in Figure 1 that, for a_w between 0.45 and 0.6 (the highlighted and magnified area in that figure), there is the area of greater distance between the curves. In this range of a_w , the decreases in X_{eq} due to the increase in storage temperature was greater in relation to the other a_w intervals studied. At an average relative humidity of 50%, the values of predicted equilibrium moisture content could vary up to 23% among the samples, depending on the storage temperature.

Figure 1 - Sorption isotherms for Sample 4 (S4) of cooked ham fitted to the GAB model at different storage temperatures.



Fonte: elaborado pelo autor

Figure 2 - Sorption isotherms for Samples 1, 2, 3 and 4 (S1, S2, S3 and S4) of cooked ham fitted to the GAB model at fixed temperature (16 °C).



Fonte: elaborado pelo autor

When analyzing Figure 2, the S4 isotherm features a higher X_{eq} when compared to the other samples, for all ranges of a_w studied. In particular for sample S4, the X_{eq} in the a_w range between 0.45 and 0.6 has become even higher than in the other tracks, as can be seen by a greater displacement between its curve and the curves of other

samples. This phenomenon also occurred to the isotherms obtained at other temperatures (2, 9, 24 and 30 °C). Such behavior is possibly due to differences in the chemical composition of the studied cooked ham. S4 showed higher protein content and thus a lower W/P ratio. Chou and Morr (1979) highlighted the binding properties between water-protein. They reinforced the great capacity of water binding to polar amino groups of protein, contributing to a significant increase in monolayer moisture. This fact is in accordance with the encountered values of X_m for the different samples. Sample S4 had higher values of X_m when compared to samples S1 and S2, which presented lower protein contents. Analogously, as the W/P ratio reduced, the higher the X_{eq} became for all ranges of a_w , studied, regardless of the product storage temperature.

Meat proteins, particularly myofibrillar protein, are excellent gelling agents. They are better able to retain water in the system, and are largely responsible for the structural characteristics and texture of meat products (Robe & Xiong, 1993; Xiong, 1993). The interactions between the protein and the water are of great importance to the water retention capacity and the congelation in meat products and, consequently, affect the technological properties of the product (Puolanne & Halonen, 2010).

Schut (1976) confirmed that the fat can also affect the water retention capacity in meat systems. The author claims that both fat and water are connected to the meat because they are trapped in the protein matrix. So, by reducing the fat content of the system, there must be an increase in the water retention capacity, since more protein is available to bind to water (Trout & Schmidt, 1986). This phenomenon was also seen in this study, since S4 showed an increased availability of protein due to the lower W/P ratio and lower fat content. This possibly contributed to water retention and hence kept X_{eq} higher for all a_w , when compared to the other three samples at all temperatures studied. Iglesias and Chirife (1982) also reported that increasing fat content promotes a decrease in equilibrium moisture content based on the assumption that fat does not sorb water. It is in close agreement to what is shown in the Figure 2. Samples S2 and S1, which contain more fat, had lower equilibrium moisture at specific water activity.

4 CONCLUSION

By the present study, cooked hams were chemically characterized at the first moment, presenting more significant differences according to proteins and fat contents. Desorption isotherms were obtained and the GAB model was well-fitted to the experimental data of sorption isotherms of cooked ham in storage simulation at various temperatures. The sorption isotherms had a sigmoidal shape and were classified as Type II for all samples at all temperatures. The storage temperature caused a decrease in the product X_{eq} with increasing temperature. At the same storage temperature, increased relative humidity resulted in an elevated X_{eq} . Comparing desorption isotherms of the different samples, it could be seen a maximum variation of 23% at specific relative humidity. In this way, the chemical composition of cooked ham demonstrated significant influence on the water desorption isotherms. The lower the W/P ratio or the lower the fat content, the higher its X_{eq} . Cooked ham is generally a product with a high moisture content of over 70%. Once removed from the original packaging and sliced during marketing, a low storage temperature at high relative humidity reduces water desorption. A cooked ham with low W/P ratio and low fat content can also assist in maintaining the product humidity and sensory aspects, besides increasing its shelf-life.

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CAPÍTULO III

IMPROVING SENSORY ACCEPTANCE AND PHYSICOCHEMICAL PROPERTIES BY ULTRASOUND APPLICATION TO COOKED HAM WITH SALT (NaCl) REDUCTION

BARRETTO, T. L., POLLONIO, M. A. R., TELIS-ROMERO, J. & BARRETTO, A. C. S.
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Artigo Original

Há direitos de utilização deste artigo, como autor, na composição da
tese. (ANEXO A)

IMPROVING SENSORY ACCEPTANCE AND PHYSICOCHEMICAL PROPERTIES BY ULTRASOUND APPLICATION TO COOKED HAM WITH SALT (NaCl) REDUCTION

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ABSTRACT

The objective of this research was to study the effects of salt reduction and the application of ultrasound (nominal current of 600Wcm^{-2} for 10min) on the physicochemical properties, the microstructure and the sensory acceptance of restructured cooked ham. Four treatments with reduced salt including one with the application of ultrasound (1.5, 1.12, 0.75 and 0.75% salt + ultrasound) were produced. The treatment with 0.75% salt provided a reduction of about 30% in the sodium content. The use of ultrasound decreased the Total Fluid Release and increased the hardness. For lightness, the sample with 0.75% salt with the application of ultrasound did not differ from the control at day zero of storage. The use of ultrasound increased redness too. The ultrasound treatment caused micro fissures on the myofibrils. The sensory acceptance of restructured cooked ham with 0.75% of salt was improved with ultrasound applied. The ultrasound showed good potential for use in the production of healthier meat products.

Key words: Sodium reduction, pork, microstructure, cavitation, meat products.

1. INTRODUCTION

Most pork consumed in Brazil is in processed products, since 89% of this meat is industrialized in various products such as salami, sausages, restructured cooked ham and cooked restructured pork shoulder (ABPA, 2016). On the world stage, restructured cooked ham also appears as one of the most popular meat products among consumers (Válková, Saláková, Buchtová, & Tremlová, 2007). Restructured cooked ham is produced exclusively from boned prime leg of pork with added ingredients and subjected to a suitable cooking process (Brazil, 2000). The ingredients used in the formulation aim to improve the product characteristics, such as salt (NaCl), which plays an essential role in the water holding capacity, the lipid retention properties, color, flavor and texture as well as playing a part in the extraction of myofibrillar proteins and contributing to the shelf life of meat products (Ruusunen & Puolanne, 2005).

Sodium chloride is the major source of dietary sodium (Wentzel-Viljoen, Steyn, Ketterer, & Charlton, 2013), and excessive sodium intake is related to increased blood pressure. This increases the risk of strokes and death from vascular diseases (Iser, Claro, Moura, Malta, & Neto, 2011; Vollmer et al., 2001). In this sense, there is a need to reduce the use of salt in processed meat products. However, salt reduction should not occur without effective studies, due to its technological and sensory importance for the development of meat products (Ruusunen & Puolanne, 2005). Some studies (Bis et al., 2016; Carraro, Machado, Espindola, Campagnol, & Pollonio, 2012) tested reformulations of meat products, partially substituting sodium chloride by potassium chloride and the results showed the possibility of producing products with lower sodium content without compromising the technical and sensory qualities. However, when used principally on its own, some studies report that KCl can damage the sensory acceptance of meat products by promoting bitterness, astringency and metallic taste (Askar, El-Samahy, & Tawfic, 1994; Geleijnse, Kok, & Grobbee, 2003; Gelabert, Gou, Guerrero, & Arnau, 2003).

Alternative technologies have been studied aiming to promote the functional properties of food products, added to the convenience of less processing time, less water, less energy expenditure and less production of effluents and toxic substances. In this context, new processing technologies such as microwaves, pulsed electric

fields, high hydrostatic pressure, ultraviolet light, ohmic heating and ultrasound are inserted (Chemat, Zill, & Khan, 2011; Leadley & Willians, 2008). With respect to ultrasound, several studies report (Cárcel, García-Pérez, Benedito, & Mulet, 2012; Jayasooriya, Torley, D'arcy, & Bhandari, 2007; McDonnell, Lyng, & Allen, 2014b; Vimini, Kemp, & Fox, 1983) that this technology can be useful to accelerate and intensify the extraction and diffusion of sodium and, thus, reduce the processing time. Cárcel, García-Pérez, Benedito, and Mulet (2012) emphasizes that the technology of ultrasound enables innovation in the industry by saving energy and increasing the yield and quality of the final products, meaning that this technology opens up a whole new field in food processing.

Ultrasound waves can be classified as high and low energy. Those of high energy have low frequency (20–100kHz) and develop higher power levels (10–1000W·cm⁻²), sufficient to break intermolecular bonds, with intensities > 10W·cm⁻² producing a cavitation phenomenon, able to change some physical properties, catalyze chemical reactions (Jayasooriya, Torley, D'arcy, & Bhandari, 2007) and improve mass transfer processes (Ojha, Keenan, Bright, Kerry, & Tiwari, 2016). So, the objective of this study was to study the effects of salt reduction and the use of ultrasound on the physicochemical properties, microstructure and sensory acceptance of restructured cooked ham.

2. MATERIALS AND METHODS

2.1 Restructured cooked ham manufacture

Topside ham (*semimembranosus and adductor muscles*) (70.1% moisture content, 21.43% protein content and 6.9% lipid content) was obtained from a local slaughterhouse (Slaughterhouse “Olhos D’água”, Ipuã, Sao Paulo, Brazil). The meat was received frozen at -18°C, ground using a kidney plate into pieces measuring 3x3cm, vacuum packed and maintained in this condition for 24h. Thawing occurred in a refrigerator at 4°C for 48h before the start of the processing of the ham.

The amount of sodium chloride in Brazilian restructured cooked ham is variable typically around 1.5% - 2%. So, 1.5% was chosen as the salt content used as the

control. Four treatments were made for restructured cooked ham - T100: 1.5% NaCl; T75: 1.12% NaCl; T50: 0.75% NaCl and; T50US: 0.75% NaCl and subjected to ultrasound 10min in a nominal current of $600W \cdot cm^{-2}$. The topside ham was mixed with brine, which was made by homogenizing of 1.5% soybean isolate protein (Bremil, Lajeado, Brazil), 0.94% sodium-free California condiment (Fego, Goiânia, Brazil), 0.1% monosodium glutamate (Ajinomoto, São Paulo, Brazil), 0.02% cochineal carmine dye (Christian Hansen, Hoersholm, Denmark), 0.28% curing salt (Kraki, Santo André, Brazil - 10% sodium nitrite and 90% sodium chloride), 0.19% sodium erythorbate (NewMax, Americana, Brazil), 0.47% sodium tripolyphosphate (NewMax, Americana, Brazil), 0.47% sucrose (União, Sertãozinho, Brazil), 0.24% carrageenan (Indukern, Jundiaí, Brazil), 0.28% maltodextrin (Ingredion, Mogi Guaçu, Brazil). All the ingredients for the brine were mixed in a homogenizer (Fisatom, São Paulo, Brazil). The percentages of water and salt (sodium chloride) for each treatment are shown in Table 1. After the topside ham and brine had been mixed, T100, T75 and T50 were massaged in a tumbler (Frigomaq, Chapecó, Brazil) for 60min (15rpm).

Table 1 - Percentual of water and salt of restructured cooked ham.

Ingredients	Treatments (%)			
	T100	T75	T50	T50US
Water	31.52	31.89	32.27	32.27
Salt – NaCl	1.5	1.12	0.75	0.75

T100=1.5% NaCl; T75=1.12% NaCl; T50=0.75% NaCl; T50US=0.75% NaCl and ultrasound.

Fonte: elaborado pelo autor

T50US was subjected to ultrasound and then also massaged in the same tumbler under the same conditions as the other treatments. To apply the ultrasound in T50US, the incorporated ingredients were packed in a cylindrical stainless steel vat (21cm diameter, 42cm high) that was immersed in an ice bath and then subjected to the ultrasound waves. The system comprised a VCX-1500 ultrasound processor (Sonics & Materials Inc., Newtown, USA) which emits waves at a frequency of 20kHz. The processor was equipped with a Ti-6Al-4V titanium probe that emits ultrasound in both the axial and radial directions (Sonics & Materials Inc., Newtown, USA). The probe was immersed in the mixture and positioned in the center of the vessel.

0.8 kg portions of each treatment were embedded in a 95mm plastic casing (Viscofan, Navarra, Spain) and accommodated in stainless steel molds for ham. They were kept at rest at 4°C for 60 min. Subsequently, the molds were immersed in water at 25°C in a cooking tank (Frigomaq, Chapecó, Brazil). After immersion, the temperature was adjusted to 80°C. The cooking cycle ended when the ham reached 72°C at its thermal center and it was promptly cooled by immersion in iced water for sixty minutes. The hams were stored at 4°C until the beginning of the analyses. Ten restructured cooked hams were prepared per treatment in each batch. Three independent batches of the restructured cooked hams were prepared on three different days.

2.2 Proximate analysis, pH and sodium content

Moisture, ash and protein contents were determined according to the AOAC (2007) method. The lipid content was determined, following the methodology described by Bligh and Dyer (1959). Total carbohydrates were determined by a difference calculation. The pH was measured in triplicate for each treatment using a PG 1800 digital pH meter (Gehaka, Sao Paulo, Brazil) that was calibrated with two standard solutions (pH4 and pH7) at room temperature, at zero-day cold storage used by inserting the probe in the piece of restructured cooked ham. The sodium content was analyzed using dry digestion (Horwitz, 2010). The homogenized samples were weighed, pre-calcinated on a hot plate and incinerated in a muffle furnace at 450°C until the ash, free of black spots, formed. The ash was quantitatively transferred to a volumetric flask with nitric acid solution 5% (v/v) and the reading was performed using a DM-62 flame photometer (Digimed Analytical Group, São Paulo, Brazil). All assays were performed in triplicate.

2.3 Total fluid released (TFR)

This evaluation shows the water retention capacity in the period after cooking and also during cold storage. The hams were partially opened at one end and placed

over a 250 ml becker for 10 minutes to collect all the exuded liquid. TFR (%) was measured in triplicate after zero and sixty days of refrigerated storage by subtracting the weight of the ham after cooking and draining (W_2) from the total weight of the ham before cooking (W_1), expressed as a percentage of the total weight before cooking, according to Eq. (1).

$$\text{TFR (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

2.4 Instrumental color

Instrumental color was measured using a Colorflex EZ 45/0 spectrophotometer (HunterLab, Reston, US) calibrated using the black glass and white tile provided. Samples were analyzed in a 2.5 in. glass sample cup, with a 1.25 in. port insert, illuminant D65, at room temperature. The color was expressed in L^* for lightness, a^* for redness and b^* for yellowness. Four measurements were obtained for each parameter at equidistant points, by rotating the capsule. The mean of the measurements represented the reading for each sample. The measurements were performed after zero and sixty days of refrigerated storage.

2.5 Texture profile analysis (TPA)

The TPA was done with a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, England) and using its propriety Exponent program, version V.5.1.1.0 (Stable Micro Systems, Godalming, England), following the method described by Bourne (1978). The parameters determined were: Hardness ($\text{N}\cdot\text{cm}^{-2}$): maximum force required to compress the product; Cohesiveness: extent to which the sample could be deformed prior to rupture; Springiness: ability of sample to recover its original form after the deforming force was removed; and Chewiness: work required to masticate the sample for swallowing. The ham samples were molded with a circular stainless steel cutter (diameter 2cm) and a cylindrical probe was used (3.6cm diameter), with

50% compressions, test speed 1mm/s, n=10. The measurements were taken after zero and sixty days of refrigerated storage.

2.6 Thiobarbituric acid reactive substances (TBARS)

The thiobarbituric reactive substances (TBARS) values were determined as described by Bruna, Ordonez, Fernández, Herranz, and La Roz (2001) after zero and sixty days of refrigerated storage. The amount of TBARS was expressed as mg of malonaldehyde per kilogram of sample ($\text{mg MDA} \cdot \text{kg}^{-1}$ sample). Concentrations were determined at 532 nm and a standard curve was prepared using 1,1,3,3 tetraethoxypropane. Because the restructured cooked ham has added sodium nitrite in the formulation, 0.5% sulfanilamide in 20% HCl v/v was added to minimize the interference of this compound in the reaction with thiobarbituric acid (Zipser & Watts, 1962).

2.7 Microbiological evaluation

Microbiological analyses were carried out on samples of the restructured cooked ham after zero and sixty days of refrigerated storage and the results evaluated in accordance with the microbiological standards established by Brazilian legislation (Brazil, 2001). The restructured cooked ham packaging was opened aseptically and 25 g of sample were diluted in 225 ml of sterile peptone water (HIMEDIA, Mumbai, India) for analysis of thermo-tolerant fecal coliforms, coagulase-positive *Staphylococci* and sulfite-reducing *Clostridia*. To investigate *Salmonella* spp., 25g of the sample was diluted with lactose broth (HIMEDIA, Mumbai, India) (Horwitz, 2010). The thermo-tolerant coliforms were investigated using the fermentation technique in multiple tubes and were expressed in most probable number (MPN) per gram of sample. Coagulase-positive *Staphylococci* were investigated by inoculating Baird-Parker Agar (HIMEDIA, Mumbai, India) enriched with egg yolk and 1% potassium tellurite. *Clostridia* sulfite-reducers were analyzed by inoculating the samples with SPS Agar (HIMEDIA, Mumbai, India) incubated anaerobically, in duplicate, and expressed as log colony-forming units (CFU) per gram of sample.

2.8 Sensory acceptance

Sensory analysis was conducted after obtaining the results of the microbiological analysis, after fifteen days of refrigerated storage of the product. A group of one hundred and fifteen untrained restructured cooked ham consumers, composed of students and staff of the Institute of Biosciences, Letters and Exact Sciences of the São Paulo State University (UNESP/Ibilce), made up the team for the acceptance analysis. All the treatment samples, sliced on a plate, were coded randomly with three-digit numbers and presented to the consumers randomly in a sequential monadic manner as described by Meilgaard, Civille, and Carr (1999). The sensory test was held in one session with four samples. All consumers evaluated one sample of all treatments in a randomized order, in single cabins with day light in the Sensory Analysis Laboratory of the Food Engineer and Technology Department UNESP. Unsalted crackers and water at room temperature were provide to clean the palate between each sample. Using a structured 9-point hedonic scale (APÊNDICE A), anchored at the extremes by 9 – extremely desirable and 1 – extremely undesirable, consumers evaluated the following attributes: color, taste, texture and overall acceptance. And on a 5-point scale, anchored at the extremes by 1 – certainly would by and 5 – certainly would not by, they showed their intention of purchasing the product. To conduct the sensory analysis, this project had been previously approved by the Research Ethics Committee of UNESP/Ibilce (no.58925516.9.0000.5466) (ANEXO B).

2.9 Microstructure

The microstructure of the restructured cooked ham samples was obtained after fifteen days of refrigerated storage using a LEO 450 VPi scanning electron microscope - SEM (Zeiss, Oberkochen, Germany) following the procedure described by Totosaus and Pérez-Chabela (2009) with some modifications. The samples were cut into fillets of 4mm x 2mm x 2mm taken from inside the product and immersed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 24h for fixation. The samples were washed with 0.1M sodium phosphate buffer solution and remained in a refrigerator for 2h in a solution of osmium tetroxide in 0.1M phosphate buffer. They were subsequently dehydrated in a series of increasing concentrations of ethanol (30%, 50%, 70%, 90%

and 100% ethanol). They were then dried by the critical point method using CO₂, arranged on aluminum stubs and coated with gold. Observations were then made using the SEM.

2.10 Statistical analysis

The results were expressed as the mean values and the standard error of the mean. Each triplicate was included as a random term, and different time were included as fixed term. The data obtained on physicochemical properties (instrumental color, texture profile analysis, TFR and TBARS) were analyzed statistically using mixed model ANOVA analyses and the means were compared using the Tukey test ($P < .05$).

For the sensory test, the treatment was considered as the main effect and consumers as random variable. The data obtained in the sensorial analysis were analyzed statistically using mixed model ANOVA and the means were compared using the Tukey test ($P < .05$).

The results were expressed as the mean values and standard error of mean. All statistical analysis was performed using Statistica 7.0 software (Statsoft Inc., USA).

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties

The proximate analysis, pH and sodium content are shown in Table 2. In all treatments, the amounts of moisture and lipid were similar ($P > .05$). The protein content of all treatments was within the standards established by Brazilian legislation - a minimum of 14% (Brazil, 2000). The average values of protein content were from 16.42 to 17.40%. This variation is due to innate differences in the raw material. However, the T100 sample had less protein than the T75 and T50US ($P < .05$), but did not differ ($P > .05$) from the T50 samples. Stanley, Bower, and Sullivan (2017) found no differences in protein content between samples of pork sausage patties with reduction of sodium and Yotsuyanagi et al. (2016) reported the same on reducing the NaCl in frankfurters.

For the ash content, the T100 sample was higher ($P < .05$) than the other treatments, probably due to the higher salt content used in the formulation of this sample. Similar results were obtained by Yotsuyanagi et al. (2016) who showed that, by increasing the NaCl in frankfurters by 57%, there was an 18% increase in the ash content produced.

Table 2 - Proximate analysis, pH and sodium content of cooked ham with salt reduction including application of ultrasound (n=3).

	Treatments				SEM	Sig.
	T100	T75	T50	T50US		
Proximate composition (%)						
Moisture	76.64 ^a	76.35 ^a	76.91 ^a	76.31 ^a	0.217	n.s.
Fat	3.57 ^a	3.60 ^a	3.78 ^a	3.77 ^a	0.282	n.s.
Protein	16.42 ^b	17.40 ^a	16.81 ^{ab}	17.35 ^{ab}	0.233	sig.
Ash	3.05 ^a	2.38 ^b	2.17 ^b	2.05 ^b	0.075	sig.
Carbohydrate	0.36	0.27	0.33	0.52		
pH	6.10 ^{ab}	6.25 ^a	6.02 ^b	6.01 ^b	0.047	sig.
Sodium (mg sodium/100g)	976.54 ^a	837.60 ^b	676.84 ^c	715.34 ^c	8.700	sig.

^{a-c} Mean values in the same line not followed by a common letter differ significantly ($P < .05$).

SEM: standard error of the mean.

T100=1.5% NaCl; T75=1.12% NaCl; T50=0.75% NaCl; T50US=0.75% NaCl and ultrasound.

Fonte: elaborado pelo autor

T50 did not differ ($P > .05$) from each other, since both had the same percentage of NaCl in their formulations. T75 showed a reduction of 16% of sodium, T50 of 32% and T50US of 28.5%. Therefore, reductions of 25% and 50% of salt in the formulations of restructured cooked ham products produced reduced sodium contents of 16% and 28.5% respectively. Similar results were obtained by Carraro, Machado, Espindola, Campagnol, and Pollonio (2012) who studied the effects of lowering NaCl in bologna sausage, where they reported that 50% salt gave a reduction of 31% in the sodium

content in the final product. Yotsuyanagi et al. (2016) reported that a reduction of 57% NaCl in frankfurters led to a 36% reduction in the sodium content of the final product.

3.2 Total fluid release (TFR)

The reduction in salt affected ($P < .05$) the TFR in restructured cooked ham (Table 3). Sodium chloride aids in the extraction and solubility of myofibrillar proteins by increasing the ionic strength, improving its properties as an emulsifier, binder and also its water retention capacity (Ruusunen & Puolanne, 2005). In this context, the salt reduction in restructured cooked ham means an increase in the TFR. Li, Kang, Zou, Xu, and Zhou (2015) worked with chicken breast meat batter with reduced salt and observed a reduction in the water retention capacity when the salt concentration was decreased from 2% to 1%. On the other hand, the use of ultrasound technology has reduced the TFR since T50US is not different ($P > .05$) from the T100 and T75 on day zero or from T75 after sixty days. McClements (1995) reported that ultrasound helped with the extraction of myofibrillar proteins, which have the property of linking themselves to water, increasing thereby the water holding capacity. The increase in water holding capacity improved the TFR results.

An increase in the salt concentration in the pork during curing, following treatment with high power ultrasound, has been reported by several studies (Cárcel, Benedito, Bon, & Mulet, 2007; Ojha, Keenan, Bright, Kerry, & Tiwari, 2016; Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013). Siró et al. (2009) observed more diffusion of salt due to treatment with ultrasound compared with curing under static conditions. The authors also concluded that the diffusion coefficient increased along with the increase of ultrasonic intensity ($2\text{--}4\text{W}\cdot\text{cm}^{-2}$) at a frequency of 20kHz. In a study on the effect of ultrasound on salt distribution in salted meat, Cárcel, Benedito, Bon, and Mulet (2007) reported that intensities above $51\text{W}\cdot\text{cm}^{-2}$ showed significant mass transfer of NaCl in the meat. Increased diffusion of salt in the meat matrix assists in the extraction of myofibrillar proteins, which increases the bonding power with water, there by reflecting a decrease in the TFR. All the treatments increased the TFR ($P < .05$) during the storage.

Table 3 - Color parameters, TFR, TPA and the TBARS of cooked ham with salt reduction including application of ultrasound (n=3).

	Day	Treatments				SEM	P value
		T100	T75	T50	T50US		
L*	0	64.14 ^{b,A}	63.03 ^{b,B}	69.16 ^{a,A}	64.92 ^{b,A}	0.372	<0.01
L*	60	60.79 ^{b,B}	64.57 ^{a,A}	65.85 ^{a,B}	64.57 ^{a,B}	0.385	<0.01
P value		<0.01	<0.01	<0.01	<0.01		
a*	0	12.18 ^{a,B}	12.05 ^{a,A}	9.37 ^{c,B}	10.92 ^{b,A}	0.204	<0.01
a*	60	13.61 ^{a,A}	11.83 ^{b,B}	10.85 ^{c,A}	11.41 ^{bc,A}	0.172	<0.01
P value		<0.01	<0.01	<0.01	0.14		
b*	0	8.31 ^{b,B}	8.63 ^{ab,B}	9.64 ^{a,A}	9.31 ^{a,A}	0.095	<0.01
b*	60	9.67 ^{a,A}	9.21 ^{b,A}	9.32 ^{ab,A}	9.23 ^{ab,A}	0.065	0.03
P value		<0.01	<0.01	0.15	0.71		
TFR	0	0.03 ^{b,B}	0.32 ^{b,B}	2.74 ^{a,B}	0.56 ^{b,B}	0.411	<0.01
TFR	60	0.21 ^{c,A}	0.65 ^{bc,A}	3.12 ^{a,A}	1.17 ^{b,A}	0.421	<0.01
P value		<0.01	<0.01	<0.01	<0.01		
Hardness (N)	0	21.01 ^{a,A}	17.14 ^{ab,B}	12.61 ^{b,B}	22.83 ^{a,B}	1.253	<0.01
Hardness (N)	60	26.97 ^{ab,A}	26.70 ^{ab,A}	23.28 ^{b,A}	35.26 ^{a,A}	1.497	0.05
P value		0.06	<0.01	<0.01	0.02		
Cohesiveness	0	0.31 ^{a,A}	0.36 ^{a,A}	0.30 ^{a,B}	0.40 ^{a,A}	0.017	0.11
Cohesiveness	60	0.38 ^{a,A}	0.44 ^{a,A}	0.40 ^{a,A}	0.36 ^{a,A}	0.015	0.26
P value		0.12	0.16	<0.01	0.42		
Springness	0	0.67 ^{a,B}	0.70 ^{a,A}	0.67 ^{a,A}	0.64 ^{a,A}	0.013	0.52
Springness	60	0.77 ^{a,A}	0.71 ^{ab,A}	0.66 ^{b,A}	0.65 ^{b,A}	0.012	<0.01
P value		<0.01	0.87	0.75	0.79		
Chewiness	0	4.59 ^{ab,B}	4.27 ^{b,B}	2.57 ^{b,B}	7.69 ^{a,A}	0.514	<0.01
Chewiness	60	8.00 ^{a,A}	8.52 ^{a,A}	6.38 ^{a,A}	9.91 ^{a,A}	0.667	0.35
P value		<0.01	<0.01	<0.01	0.42		
TBARS*	0	0.05 ^{a,B}	0.03 ^{a,B}	0.03 ^{a,B}	0.05 ^{a,B}	0.005	0.09
TBARS*	60	0.14 ^{a,A}	0.16 ^{a,A}	0.14 ^{a,A}	0.17 ^{a,A}	0.007	0.34
P value		<0.01	<0.01	<0.01	<0.01		

^{a-c} Mean values in the same line not followed by a common letter differ significantly ($P < .05$). ^{A-B} Mean values in the same column not followed by a common letter differ significantly ($P < .05$). SEM: standard error of the mean. *expressed in mg MDA.Kg⁻¹ sample. T100=1.5% NaCl; T75=1.12% NaCl; T50=0.75% NaCl; T50US=0.75% NaCl and ultrasound. Fonte: elaborado pelo autor

3.3 Instrumental color

The reduction of 50% of salt increased the lightness on day 0 of storage (Table 3). A similar result was found by Horita, Messias, Morgano, Hayakawa, and Pollonio (2014) who worked with sausages with salt reduction from 2% to 1.5 and 1% salt. With the use of ultrasound in restructured cooked ham with 50% reduced salt, no difference was found ($P < .05$) for lightness compared with T100, showing that ultrasound positively contributes ($P < .05$) to this parameter. After sixty days of storage, T100 had a lower lightness ($P < .05$) than the other treatments, and there was no difference ($P > .05$) between treatments with reduced salt and with the use of ultrasound showing that, for all treatments with reduced salt, the lightness increased. The lightness was reduced after sixty days of storage ($P < .05$), except for T75 for which the lightness had increased after sixty days of storage.

The instrumental color data showed that redness significantly ($P < .05$) decreased in restructured cooked ham when salt was reduced by 50% (T50) (Table 3). However the redness was similar ($P > .05$) when the salt reduction was 25% (T75). These values are in agreement with other authors (Dimitrakopoulou, Ambrosiadis, Zetou, & Bloukas, 2005; Pires et al., 2017) who observed a decrease in redness with salt reduction. Dimitrakopoulou, Ambrosiadis, Zetou, and Bloukas (2005) showed that restructured pork shoulder decreased its redness when the added salt was reduced from 2% to 1%. Pires et al. (2017) showed that the reduction of 20, 40 and 60% of sodium in bologna sausage decreased the redness when compared to the control sample. The use of ultrasound in restructured cooked ham with 50% salt reduction (T50US) increased redness on day 0, when compared to T50, since, at this time of storage, T50US was lower ($P > .05$) to T100 and T75. However, after 60 days, T50 and T50US showed similar values for redness ($P > .05$) and they were both lower ($P < .05$) than T100. Different results were obtained by Ferrentino and Spilimbergo (2016), who analyzed the combined treatment of high-pressure carbon dioxide and high power ultrasound on restructured cooked hams. They reported that samples with and without treatment did not differ ($P > .05$) for redness at 0 weeks of storage. However, after 1 and 4 weeks of storage, the samples subjected to the processing had lower ($P < .05$) values for redness. The ultrasound can enhance the salt diffusion into the meat matrix because of NaCl mass transfer (Ojha, Keenan, Bright, Kerry, & Tiwari, 2016) and this

can increase the extraction of myofibrillar proteins (Vimini, Kemp, & Fox, 1983). The 50% reduction of salt increased yellowness at day 0 of storage (with or without ultrasound). After 60 days of storage, more yellowness was observed for T100. The use of ultrasound did not affect the yellowness during storage. Other studies have not confirmed the color effect when ultrasound was used (Jayasooriya, Torley, D'arcy, & Bhandari, 2007; McDonnell, Lyng, & Allen, 2014b; Stadnik & Dolatowski, 2011).

3.4 Texture profile analysis (TPA)

Significant changes to hardness were observed in T50US (Table3) at day 0 and after 60days of refrigerated storage. The treatment with ultrasound significantly increased ($P < .05$) the hardness in restructured cooked ham with 50% salt reduction and it was similar to the control treatment (T100) and T75. There was no difference between T75 and T50 ($P > .05$). Similarly, Ojha, Keenan, Bright, Kerry, and Tiwari (2016) observed a significant improvement in the Warner Bratzler shear force in pork meat when ultrasound was used. These same authors observed that ultrasound can improve the texture while enhancing salt diffusion. The reduction of salt showed significant ($P < .05$) reduction in the hardness of the restructured cooked ham on day 0 when compared to T100. According to Çarkcioğlu, Rosenthal, and Candoğan (2016), changes in hardness or other parameters in meat products are associated with differences in the water holding capacity. Salt is used to solubilize myofibrillar proteins in meat systems, increasing the water holding capacity and improving the texture (Desmond, 2006). Other studies have shown the opposite effect on hardness (Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014). The hardness increased ($P < .05$) after 60 days of storage even when salt was reduced from 75 to 50%. The highest values observed for TRF after 60 days of storage, possibly contributed to the increased hardness of these samples after 60 days of storage, due to the loss of water.

There were no significant differences ($P > .05$) among the treatments in cohesiveness (Table 3). Similar results were reported by Barekat and Soltanizadeh (2017), who evaluated the texture of meat subjected to ultrasound and there were no differences in the cohesiveness of the meat compared to the control. In another study, McDonnell, Lyng, and Allen (2014b), when evaluating the texture profile of pork with curing accelerated by ultrasound, observed a small reduction in cohesiveness in the

samples subjected to ultrasound. For the springiness, there were no significant differences ($P > .05$) between the treatments on day zero of storage but, after sixty days, the lowest springiness values were for T50 and T50US, i.e. the salt reduction resulted in decreased springiness. In studies of sodium reduction in frankfurters, the parameters of hardness, springiness, cohesiveness and chewiness were not affected by the other ingredients in the formulation, such as increased soy protein to increase gel formation (Yotsuyanagi et al., 2016).

Chewiness was not affected by the reduction of salt in restructured cooked ham during refrigerated storage (Table 3). The use of ultrasound significantly ($P < .05$) increased the chewiness at day 0 and it was higher than T50, but after 60 days there was no significant difference.

3.5 TBARS

The salt reduction at 25% and 50% and treatment with ultrasound did not affect the oxidative stability of restructured cooked ham after 0 and 60 days of storage (Table 3). The values found in this study ranged from 0.03 to 0.17 mg malonaldehyde / kg sample, showing that there wasn't any alteration to the flavor originating from lipid oxidation. According to O'Neill, Galvin, Morrissey, and Buckley (1998), TBARS values from 0.5 to 2.0 mg.kg⁻¹ do not affect the sensory acceptance. The low percentage of the lipids in restructured cooked ham and the controlled temperature during processing in the present research may have contributed to such oxidation results. The storage time showed an increase ($P < .05$) in the values of TBARS for all treatments. Coombs et al. (2018) also reported an increase in TBARS levels on lamb *M. longissimus lumborum* after four weeks of refrigerated store.

3.6 Microstructure of restructured cooked ham

The SEM technique showed (Fig. 1) the effect of the ultrasound on the restructured cooked ham microstructure. The micrograph of the T50US sample (Fig. 1d), treated for 10 min with a nominal current of 600W.cm⁻² shows ruptures and greater dispersion of connective tissue fibers caused by the application of ultrasound when compared to images of the treatments without the use of this technology (1a, 1b and

1c). Some micro fissures on myofibril were identified in the same Fig. (1d) caused by acoustic energy of ultrasound, i.e. cavitation. Cavitation occurs when the ultrasonic wave passes through a liquid medium, causing alternating compression and rarefaction waves, producing bubbles in the liquid until implosion occurs, providing a localized pressure and temperature rise and the production of micro jets that generate sufficient power to disrupt cells (Chemat, Zill, & Khan, 2011.; Cárcel, García-Pérez, Benedito, & Mulet, 2012).

The micro jets, caused by imploding bubbles, collide with the surface of the myofibrils, causing micro fissures and changing the product structure. Similar effects were described by Ozuna, Puig, García-Pérez, Mulet, and Cárcel (2013) when evaluating the effect of ultrasound treatment on the static moist curing of pork. In Fig. 1h, note the greater spacing between the myofibrils compared to other images (Fig. 1e, f and g) which did not have the ultrasound treatment. Siró et al. (2009) also observed a greater distance between the myofibrils in pork loins subjected to static moist curing and treated with ultrasound. For these authors, cavitation caused by the ultrasound opens channels between the myofibrils, which facilitate the penetration of brine, called the “sponge effect”. Larger interfibrillar spaces allow more water retention in the product, which could minimize the weight loss during storage. This sponge effect, observed in restructured cooked ham with reduced salt and with the use of ultrasound, can also be explained by the lower TFR obtained in this treatment (Table 3) as previously mentioned.

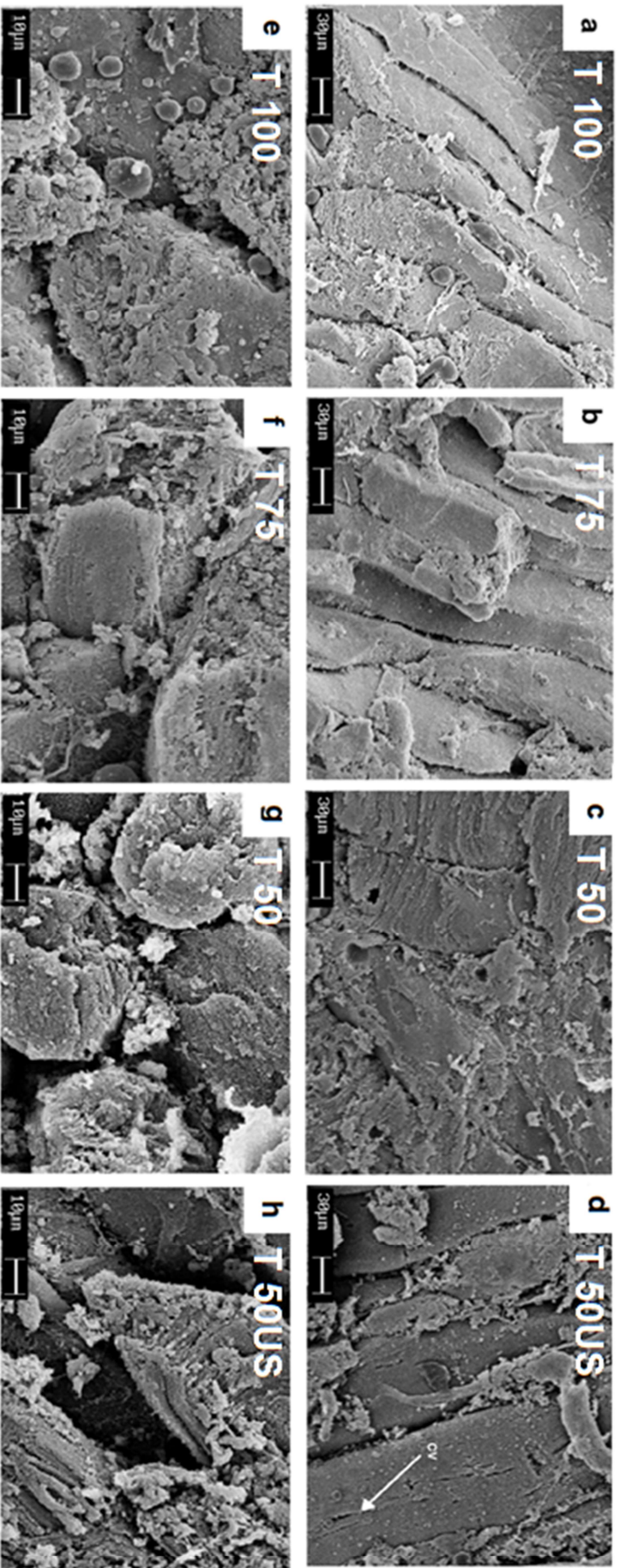


Fig. 1 - Scanning electron micrographs (a, b, c and d: 500 X; e, f, g and h: 1500 X) of cooked ham. T: cavitation effect on muscle fiber. T100 = 1.5% NaCl; T75 = 1.12% NaCl; T50 = 0.75% NaCl; T50US = 0.75% NaCl and ultrasound.

Fonte: elaborado pelo autor

3.7 Microbiological analysis

The results of the microbiological evaluations showed no differences between periods of zero and sixty days of storage. For all treatments in both periods, the growth of coliforms at 45°C was < 3 NMP/g, coagulase positive *Staphylococci* was < 10² CFU/g, anaerobic count of sulfite reducing *Clostridia* at 46°C was 101CFU/g and *Salmonella* sp. was absent in 25 g of sample. The results are within the limits established in Brazilian legislation for this product (Brazil, 2001).

3.8 Sensory acceptance

Regarding the sensory color, T100 had a higher score ($P < .05$) than T50 and T50US and did not differ ($P > .05$) from T75 (Table 4). The reduction of 50% NaCl reduced the score for color in restructured cooked ham. Similar results were reported by Zanardi, Ghidini, Conter, and Ianieri (2010) that, by reducing the NaCl concentration of Italian salami by 50%, there were lower scores for color compared to the control. The treatment with ultrasound (T50US), did not improve the color scores for restructured cooked ham when compared to T50, with the same NaCl concentration (Table 4). With regard to taste, T50 showed a lower score, differing from all other treatments ($P < .05$), showing that the use of ultrasound improved this attribute. The reduction of 50% of salt produced a lower score ($P < .05$). Yotsuyanagi et al. (2016) also observed a significant reduction in score for the taste when studying frankfurters with 1% salt compared to 1.3% and 1.75% salt. T50US, T75 and T100 were not different ($P > .05$) in terms of taste for sensory acceptance. The restructured cooked ham with 50% sodium chloride solution subjected to ultrasound provided the same sensory acceptance for taste in relation to the restructured cooked ham without sodium reduction, probably due to better diffusion of salt in the meat matrix.

For texture, T 50 was less accepted ($P < .05$) than T100 and T75, indicating that the reduction of NaCl decreased the score for texture. However, T50US did not differ ($P > .05$) from T100 and T75, showing that the use of ultrasound contributed to scores for texture similar to higher salt levels in restructured cooked ham formulations. The 50% salt sample (T50) had significantly reduced ($P < .05$) global acceptance (Table 4). These results are in agreement with the results obtained in other studies (Horita,

Messias, Morgano, Hayakawa, & Pollonio, 2014; Pires et al., 2017; Yotsuyanagi et al., 2016). This effect is due to the fact that salt reduction in meat products decreases the extraction of myofibrillar proteins, reducing the ionic strength and consequently reducing the water retention capacity of the system, affecting the formation of the protein network with heat treatment and maybe increasing drip losses during storage and thus affecting product texture (Tamm, Bolumar, Bajovic, & Toepfl, 2016).

Table 4 - Sensory acceptance of restructured cooked ham with salt reduction including application of ultrasound

	Treatments				SEM	P value
	T100	T75	T50	T50US		
Sensorial acceptance						
Color	7.83 ^a	7.70 ^a	7.30 ^b	7.32 ^b	0.059	<0.01
Taste	7.54 ^a	7.32 ^a	6.66 ^b	7.21 ^a	0.063	<0.01
Texture	7.67 ^a	7.40 ^a	6.97 ^b	7.31 ^{ab}	0.065	<0.01
Global acceptance	7.57 ^a	7.37 ^a	6.76 ^b	7.28 ^a	0.060	<0.01
Purchase intention	4.07 ^a	3.95 ^a	3.49 ^b	3.97 ^a	0.041	<0.01

^{a-b} Mean values in the same line not followed by a common letter differ significantly ($P < .05$). SEM: standard error of the mean. T100=1.5% NaCl; T75=1.12% NaCl; T50=0.75% NaCl; T50US=0.75% NaCl and ultrasound.

Fonte: elaborado pelo autor

In this study, ultrasound treatment has improved the sensory acceptance of taste, texture and global acceptance and increased the purchase intent of restructured cooked ham with 50% salt reduction, possibly due to better diffusion of the salt which is produced by the use of ultrasound. McDonnell, Lyng, Arimi, and Allen (2014a) produced restructured cooked ham with 2.25% sodium chloride and subjected three treatments to ultrasound (10.7; 17.1 and 25.4W.cm⁻²) and a control, after 2, 4 and 6h. On applying the sensory analysis, the authors reported significant improvement in taste with increasing intensity of the ultrasound. Ozuna, Puig, García-Pérez, Mulet, and Cárcel (2013) corroborate this fact by finding improved diffusion of NaCl and moisture in the meat, caused by cavitation, a result of the acoustic treatment. Ojha, Keenan, Bright, Kerry, and Tiwari (2016) investigated the NaCl diffusion in pork subjected to different strengths of ultrasound. The authors concluded that with 54.9W.cm⁻² power,

the diffusion coefficient of NaCl in the meat was significantly higher than the control without the use of ultrasound. McClements (1995) reported that ultrasound also facilitated the extraction of myofibrillar proteins, which have properties linked to water, increasing thereby the water retention capacity. All the ultrasound properties described above contributed to increasing the acceptance of texture and also the global acceptance in this study.

4 CONCLUSION

The ultrasound helps by improving the quality of restructured cooked ham with reduced sodium chloride at low levels, showing good potential for application in the development of other healthier meat products. Restructured cooked ham was produced with a reduction of 50% in the added salt and with sodium content reduced by 32% with ultrasound application. There was no impairment of the physicochemical and sensory properties. The use of ultrasound improved the physicochemical properties in restructured cooked ham with reduced sodium, such as reducing total fluid release, increasing the yield, improving the color and without negatively affecting the oxidative stability. The ultrasound modified the microstructure of restructured cooked ham by producing microfissures in the muscle fibers and this improved sensory acceptance for taste and texture parameters and for global acceptance.

Chemical compounds used in this research

Sodium acetate (PubChem CID: 517045); Methanol (PubChem CID: 887); Chloroform (PubChem CID: 6212); Sodium Carbonate (PubChem CID: 10340); Thiobarbituric acid (PubChem CID: 2723628); Glacial acetic acid (PubChem CID: 176); Sodium hydroxide (PubChem CID: 14798); Trichloroacetic acid (PubChem CID: 6421); Tetraethoxypropane (PubChem CID: 67147); Boric acid (PubChem CID: 7628).

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CAPÍTULO IV

IMPACT OF ULTRASOUND AND POTASSIUM CHLORIDE ON THE PHYSICOCHEMICAL AND SENSORY PROPERTIES IN LOW SODIUM RESTRUCTURED COOKED HAM

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**IMPACT OF ULTRASOUND AND POTASSIUM CHLORIDE ON THE
PHYSICOCHEMICAL AND SENSORY PROPERTIES IN LOW SODIUM
RESTRUCTURED COOKED HAM**

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ABSTRACT

The objectives of this study were to evaluate the effects of power ultrasound (nominal intensity 600 W.cm⁻² for 10 min.) and the addition of potassium chloride (KCl) in the physicochemical properties and sensorial acceptance of low sodium restructured cooked ham. Four treatments of low sodium restructured cooked ham (mean of 324.52 mg Na/100 g) were prepared: CT - Control Treatment; UsT - Ultrasound Treatment; KT - addition of 0.5% KCl; UsKT - Ultrasound Treatment and addition of 0.5% KCl. Ultrasound application reduced the total fluid released and improved the sensory acceptance for salty taste and flavor compared to CT. The addition of KCl produced the best results for total fluid release, for all parameters of sensory acceptance and for hardness and chewiness and these were not different from the results obtained with the combination of the use of ultrasound and addition of KCl. The use of KCl alone is a technologically and sensorially viable alternative to low sodium restructured cooked ham.

Key words: Low sodium, potassium chloride, ultrasound, total fluid release.

1. INTRODUCTION

The quality and safety of meat products may be adversely affected if sodium chloride (NaCl) content is reduced without the adoption of strategies to reduce any negative impact (Delgado-Pando et al., 2018). NaCl assists in the extraction of myofibrillar proteins during processing, which plays an important role in water retention capacity, lipid retention properties, color, taste and texture (Ruusunen & Puolanne, 2005).

Studies have explored the strategies of using other salts to minimize the impact of adding less NaCl to meat products without impairing technological and sensory properties, such as the addition of non-chlorinated salts such as phosphates and lactates (Ruusunen & Puolanne, 2005), and also chlorinated salts such as potassium chloride (KCl), calcium chloride (CaCl₂) and magnesium chloride (MgCl₂) as partial substitutes for NaCl (Horita et al., 2016). Stanley, Bower & Sullivan (2017) and Alves et al. (2017) tested partial replacement of NaCl by KCl in pork sausages and pork bologna, respectively. Both studies concluded that KCl represents an alternative for formulations of meat products with low sodium content, without affecting the physicochemical and sensorial properties of the product.

KCl is one of the best substitutes for NaCl due to its molecular similarity. However, its use may be limited by negative sensory properties (Horita et al., 2014). When used alone, some studies have shown that KCl can decrease the sensory acceptance of meat products, since it promotes bitterness, astringency and a metallic flavor (Geleijnse, Kok and Grobbee, 2003; Gelabert, Gou, Guerrero, & Arnau, 2003). The chloride anion is responsible for extracting the myofibrillar protein which helps increase the binding with water. Protein extraction can be maintained with 50% NaCl replacement by KCl but even this level of replacement may negatively affect the flavor of the product (Horita et al., 2014). Bis et al. (2016) found that the replacement of 35% NaCl by a commercial (mostly KCl) additive in ready-to-eat roasted beef was viable, since no changes in sensory and technological properties were observed compared to the product with no reduction in NaCl. Alves et al. (2017) observed that it was possible

to replace 50% of the NaCl by KCl in the production of bologna-type sausages without affecting the technological characteristics, while the addition of 1% lysine and/or 0.1% liquid smoke to the product reduced the taste defects caused by the replacement of that amount of KCl.

Alternative processing technologies, such as the use of ultrasound, have been explored in meat processing studies, showing significant improvements in the extraction process of myofibrillar proteins and contributing to the technological and sensorial properties (Barretto, Pollonio, Telis-Romero & Barretto, 2018; Majid, Nayik, & Nanda, 2015; Jayasooriya, Bhandari, Torley, & D'arcy, 2004). For Saleem & Ahmad (2016) ultrasound is an innovative technology that has been shown to improve on important characteristics such as texture, color, flavor and yield of meat and meat products.

High-energy ultrasonic waves have a low frequency (20-100 kHz) and can develop higher power levels (10-1000 W.cm⁻²). This energy can disrupt intermolecular bonds and produce cavitation, enough to modify physical properties, catalyze chemical reactions (Jayasooriya, Torley, D'arcy, & Bhandari, 2007) and improve mass transfer processes (Ojha, Keenan, Bright, Kerry, & Tiwari, 2016). Cichoski et al. (2019) evaluated the technological quality of meat emulsions submitted to ultrasound with 1000 W.cm⁻² of nominal power, 25 kHz for 5.5 minutes. The authors reported improvement in emulsion yield and stability, as well as good values for cohesiveness, hardness and chewiness. Barretto et al. (2018), when applying ultrasound (600 W.cm⁻², 20kHz for 10 minutes) in the processing of restructured cooked ham with reduced sodium content, reported that the color, cooking loss and sensory acceptance were better compared to the treatment without ultrasound. And the use of ultrasound in restructured cooked ham with reduced sodium content showed similar results for the technological and sensorial properties of the product without sodium reduction.

Studies involving the development of low sodium meat products that do not negatively affect the physicochemical properties and sensory acceptance are necessary in order to satisfy current consumer demands and the recommendations of the health agencies. The objective of this study was to evaluate the effects of the addition of KCl and the use of ultrasound on the physicochemical properties and sensorial acceptance in low sodium restructured cooked hams.

2. MATERIALS AND METHODS

2.1 Cooked ham manufacture

Topside ham (*semimembranosus and adductor muscles*) (68.5% moisture, 22.9% protein and 6.6% lipid) was obtained from a local market (São José do Rio Preto, São Paulo, Brazil). The freshly boned meat was ground using a kidney plate into pieces of approximately 3 x 3 cm, cooled to 4 °C and vacuum packed in a polyethylene bag. Immediately after packing, the raw material was frozen at -18 °C and maintained at this temperature for up to 48 hours prior to processing. Thawing occurred in a refrigerator at 4 °C for 48 hours before processing.

To provide low sodium content, 0.5% NaCl was added in the production of the four treatments of restructured cooked ham: CT – Control Treatment; UsT – Ultrasound Treatment: ultrasound application with nominal intensity of 600 W cm⁻²; KT – KCl Treatment: addition of 0.5% KCl; UsKT – Ultrasound and KCl Treatment: addition of 0.5% KCl and ultrasound application with nominal intensity of 600 W cm⁻². The topside ham was incorporated into the homogenized brine which was composed of 1.5% soybean isolate protein (Kraki, Santo André, Brazil), 0.94% sodium-free californian condiment (Fego, Goiânia, Brazil), 0.1% monosodium glutamate (Ajinomoto, São Paulo, Brazil), 0.02% cochineal carmine dye (Christian Hansen, Hoersholm, Denmark), 0.28% curing salt (Kraki, Santo André, Brazil - 10% sodium nitrite and 90% sodium chloride), 0.19% sodium erythorbate (NewMax, Americana, Brazil), 0.47% sodium tripolyphosphate (NewMax, Americana, Brazil), 0.47% sucrose (União, Sertãozinho, Brazil), 0.24% carrageenan (Kraki, Santo André, Brazil), 0.28% maltodextrin (Ingredion, Mogi Guaçu, Brazil). All the brine ingredients were homogenized in a blender (Fisatom, São Paulo, Brazil). The percentage of water and KCl in the brine of each treatment is shown in Table 1. After addition of the brine to the topside ham, CT and KT treatments were massaged in a tumbler (Frigomaq, Chapecó, Brazil) for 60 min (15 rpm). The UsT and UsKT treatments were submitted to ultrasound and then also massaged in the same tumbler under the same conditions. To apply the ultrasound to UsT and UsKT, the incorporated ingredients were packed in a cylindrical stainless steel vat (21 cm diameter, 42 cm height) which was immersed in an ice bath and then subjected to the ultrasonic waves for 10 minutes. The system

consisted of a VCX-1500 ultrasonic processor (Sonics & Materials Inc., Newtown, EUA) which emits waves at a frequency of 20 kHz. The processor was equipped with a Ti-6Al-4V titanium probe that emits ultrasound in both the axial and radial directions (Sonics & Materials Inc.,Newtown,USA). The ultrasound probe was immersed in the mixture and positioned in the center of the vat, as shown in Figure 1. A thermocouple was attached to the center of the bottom of the vat for temperature monitoring during processing. The temperature was controlled not to exceed 8°C.

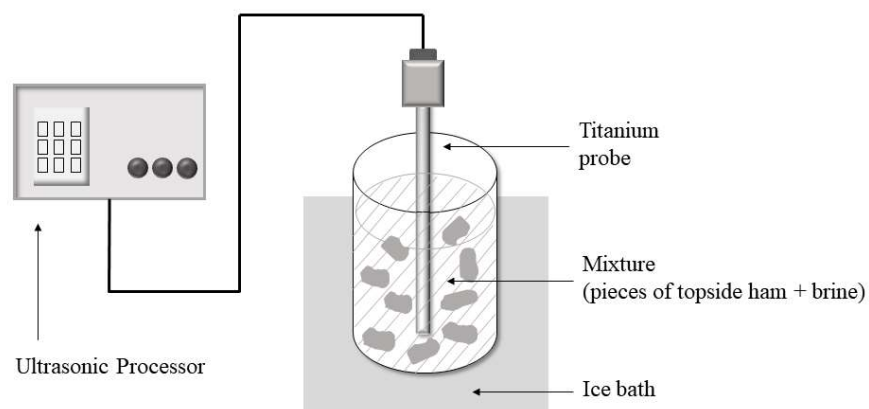
Table 1 - Percentual of water and KCl in low sodium restructured cooked ham.

Ingredients	Treatments (%)			
	CT	UsT	KT	UsKT
Water	32.51	32.51	32.01	32.01
NaCl	0.50	0.50	0.50	0.50
KCl	-	-	0.50	0.50

CT – Control Treatment; UsT – Ultrasound Treatment; KT – KCl Treatment; UsKT – Ultrasound and KCl Treatment.

Fonte: elaborado pelo autor

Figure 1 - Ultrasound probe system set-up.



Fonte: elaborado pelo autor

Then, 800g portions of each treatment were embedded in 95 mm plastic casing (Viscofan, Navarra, Spain), and put into stainless steel molds for ham. They were kept at 4°C for 60 minutes for the curing process. Subsequently the molds were immersed in the cooking tank with the water initially at 25 °C. After immersion, the temperature was increased to 80 °C. The cooking cycle ended as soon as the hams reached 72 °C in their geometric center and then they were immediately cooled in a water and ice bath. The hams were stored at 4 °C until further analysis. Ten packets of restructured cooked ham were produced for each treatment. Three independent batches were prepared on different days.

2.2. Proximate analysis, pH, sodium content and Total Fluid Release (TFR)

Moisture, ash and protein contents were determined according to AOAC (2007), lipid content according to Bligh & Dyer (1959) and carbohydrates determined by difference. For pH, a PG 1800 digital pH meter (Gehaka, São Paulo, Brazil) was calibrated with two buffer solutions (pH 4 and pH 7) at room temperature. The pH for each treatment was measured by inserting the probe in each restructured cooked ham. This was done at the beginning of the refrigerated storage. For the sodium and potassium content, the analysis was conducted by dry digestion, according to Horwitz (2010). The homogenized samples were weighed, pre-calcined and incinerated in a muffle oven at 450 °C until clear ash was obtained. The ashes were quantitatively transferred to a volumetric flask with 5% (v/v) nitric acid solution and the reading was taken using a DM-62 flame photometer (Digimed Analytics, São Paulo, Brazil). All assays were performed in triplicate. Total Fluid Release (TFR) shows the water retention capacity in the period after cooking and during refrigerated storage. This was measured according to Barretto et al. (2018). The samples of restructured cooked ham were opened and placed on a 250 ml beaker for 10 minutes to collect all the exuded liquid. TFR (%) was measured in triplicate after zero and thirty days of refrigerated storage, by subtracting the weight after cooking and draining (W2) from the total weight of the product before cooking (W1), expressed as a percentage of the total weight before cooking, according to Equation 1.

$$\text{TFR (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

2.3 Instrumental Color and Texture Profile Analysis (TPA)

To obtain the instrumental color parameters, a Colorflex EZ 45/0 spectrophotometer (HunterLab, Reston, US) was used. The equipment was calibrated with standard black and white tiles. The samples were cut into 0.5 cm thick discs and packed in a 2.5 inch glass capsule with a 1.25 inch port insert, D65 illuminant, at room temperature. The instrumental color was expressed in L* for lightness, a* for redness and b* for yellowness. Four measurements were obtained for each parameter at equidistant points from the capsule. The mean of the measurements was calculated for each sample. Measurements were performed at zero and after thirty days of refrigerated storage. TPA analysis was conducted with a TA-XT Plus texturometer (Stable Micro Systems, Godalming, UK) using Exponent Software version V. 5.1.1.0 (Stable Micro Systems, Godalming, UK), according to the method described by Bourne (1978). Based on the force *versus* strain data, the hardness parameter ($\text{N}\cdot\text{cm}^{-2}$) (maximum force required for sample compression); cohesiveness ($\text{N}\cdot\text{cm}^{-2}\cdot\text{s}$) (measure of deformation before rupture, determined as the area below the compression curve); springiness ($\text{s}\cdot\text{s}^{-1}$) (recovery speed between the end of the first compression and the beginning of the second compression); and chewiness (corresponding to work for grinding food, determined as the product of hardness, cohesiveness and springiness) were obtained. The restructured cooked ham samples were taken using a circular stainless steel cutter (diameter 2 cm) and a cylindrical test probe was used (3.6 cm diameter) for a cycle of two compressions of 50% of the sample height. The test speed was $1 \text{ mm}\cdot\text{s}^{-1}$ ($n = 10$). The analyses were performed after zero and thirty days of refrigerated storage.

2.4 Microbiological evaluation

Microbiological analyses were carried out on all treatments of restructured cooked ham after ten days of refrigerated storage and the results were evaluated according to microbiological standards established by Brazilian legislation (Brazil,

2001). The package was aseptically opened, and 25 g of sample were diluted in 225 mL of sterile peptone water (Himedia, Mumbai, India) for thermo-tolerant fecal coliforms, coagulase-positive Staphylococci and sulfite-reducing Clostridia. For analysis of *Salmonella* spp., 25 g of the sample were diluted with lactose broth (Himedia, Mumbai, India) (Horwitz, 2010). The thermo-tolerant fecal coliforms were analyzed by the fermentation technique in multiple tubes and were expressed in the most probable number (MPN) per gram of sample. Coagulase-positive Staphylococci were analyzed by inoculating Baird-Parker agar (Himedia, Mumbai, India) with egg yolk and 1% potassium tellurite. The sulfite-reducing Clostridia was analyzed by inoculating the samples with SPS agar (Himedia, Mumbai, India) incubated anaerobically, in duplicate, and expressed as log colony-forming units (CFU) per gram of sample.

2.5 Sensory acceptance

The sensorial analysis in the restructured cooked ham was carried out after seventeen days of refrigerated storage, once the results of the microbiological analysis had proved its safety. A group of 105 untrained consumers of restructured cooked ham, composed of members of the academic community of the Institute of Biosciences, Letters and Exact Sciences of the State University of São Paulo (UNESP/IBILCE), formed the team for acceptance sensorial analysis. The samples of each treatment were sliced in a meat slicer (Bermar, São José do Rio Preto, Brazil) and arranged on plastic plates, randomly coded with three-digit numbers and presented to consumers in a random, monadic and sequential manner, as described by Meilgaard, Civille & Carr (1999). All consumers evaluated a sample of all treatments in random order in a single session, in individual cabins under white light, in the Laboratory of Sensory Analysis of the Department of Engineering and Food Technology of UNESP-IBILCE. Unsalted biscuits and water at room temperature were provided to clean the palate between each sample. From a structured hedonic scale of 9 points (APÊNDICE B), ranging from 1- extremely disliked to 9 - extremely liked, consumers evaluated the following attributes: color, flavor, salty taste, texture, global acceptance. And on a 5-point scale, ranging from 1 – certainly would not buy to 5 – certainly would buy, they showed their intention of purchasing the product. To conduct

the sensory analysis, this project had been previously approved by the Research Ethics Committee of UNESP-IBILCE 83944018.5.0000.5466 (ANEXO C).

2.6 Statistical Analysis

The results were expressed as the mean values and the standard error of the mean. Each triplicate was included as a random term, and the different times were included as fixed term. The data obtained on the physicochemical properties were analyzed statistically using mixed model ANOVA and the means were compared using the Tukey test ($P < 0.05$). For the sensory test, the treatment was considered as the main effect and consumers as a random variable. The data obtained in the sensory analysis were analyzed statistically using mixed model ANOVA and the means were compared using the Tukey test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties

The proximate analysis, pH, sodium and potassium contents are presented in Table 2. No differences ($P > 0.05$) were observed for moisture, fat, protein and ash contents between treatments. Probably the same raw material and similar formulations among the treatments provided products with no difference in the proximate analysis. Stanley et al. (2017) also did not find any differences in moisture, protein and lipid content in pork sausage patties with different NaCl and KCl content, while Barretto et al. (2018) observed differences in protein content between restructured cooked ham with reduced sodium and ultrasound treatment, these differences were attributed to inherent properties of the raw material. According to the results in the current study, the addition of the KCl and ultrasound treatment did not affect the proximate analysis of restructured cooked hams. Brazilian legislation (Brazil, 2000) recommends a minimum of 14% in protein content and a maximum of 5.35 in the moisture/protein ratio, thus all treatments are within the permitted levels.

Table 2 - Proximate analysis, pH, sodium and potassium content of restructured cooked ham.

	Treatments				SEM	P value
	CT	UsT	KT	UsKT		
Proximate composition (%)						
Moisture	77.33 ^a	77.51 ^a	77.42 ^a	77.54 ^a	0.07	0.16
Lipid	2.75 ^a	3.01 ^a	2.79 ^a	3.18 ^a	0.10	0.49
Protein	17.19 ^a	16.71 ^a	17.22 ^a	16.65 ^a	0.24	0.07
Ash	2.19 ^a	2.28 ^a	2.15 ^a	2.25 ^a	0.02	0.25
Carbohydrate	0.54	0.49	0.42	0.38		
pH	6.33 ^a	6.33 ^a	6.37 ^a	6.36 ^a	0.01	0.27
Sodium (mg/100g)	330.39 ^a	318.26 ^a	326.74 ^a	322.68 ^a	1.92	0.11
Potassium (mg/100g)	282.53 ^b	286.98 ^b	555.47 ^a	544.75 ^a	40.1	< 0.01

^{ab} Mean value in the same line not followed by a common letter differ significantly ($P < .05$). SEM: standard error of the mean. CT – Control Treatment; UsT – Ultrasound Treatment; KT – KCl Treatment; UsKT – Ultrasound and KCl Treatment.

Fonte: elaborado pelo autor

There was no significant difference in the sodium content between treatments (Table 2), with a mean of 324.52 mg of sodium per 100 grams of product, a value considered low when compared to previous studies with cooked ham. Barretto et al. (2018) and Paula et al. (2019) related the development of cooked ham with 996.52 and 1513 mg of sodium/100 g of product, starting from the addition of 1.5 and 2.2% of NaCl, respectively. These values show reductions of around 66% in the sodium content of the restructure cooked ham in the present study. Coherent results were found for potassium content, where KT and UsKT did not differ ($P > 0.05$) from each other, and both of which presented the highest values as a result of the addition of 0.5% potassium chloride in their formulations. It should be noted that potassium consumption has not been associated with the development of hypertension or cardiovascular disease and may be associated with healthier products (Geleijnse et al., 2007, Kimura et al., 2004). Ultrasound application and addition of KCl did not affect the pH of low sodium restructured cooked ham (Table 2). Stanley et al. (2017) also

reported no differences in the pH of pork sausage patties with KCl added as a partial NaCl substitute. Barretto et al. (2018) found that the use of ultrasound on low sodium restructured cooked ham also did not alter the final pH of the product.

3.2 TFR

Table 3 presents the TFR values after zero and thirty days of refrigerated storage. The refrigerated storage time did not influence this parameter for all treatments studied. In both periods, the control treatment (TC) had the highest values ($P < 0.05$) for TFR, showing lower liquid retention capacity after processing and after thirty days of refrigerated storage. With the application of ultrasound in UsT, the TFR values for all periods of refrigerated storage were smaller ($P < 0.05$) than CT, showing that ultrasound application contributed significantly to lower fluid released within thirty days of refrigerated storage. Cichoski et al. (2019) reported that ultrasound application in normal mode with 1000 W.cm^{-2} of nominal intensity for 5.5 minutes significantly reduced the fluid losses in meat emulsions. The authors attribute this to the structural changes in the product caused by cavitation, which leave the myofibrillar proteins more exposed, facilitating their binding with the water and, thus, reducing losses by exudation. Pinton et al. (2019), when evaluating meat emulsions with different phosphate content, observed that the application of ultrasound at 1000 W.cm^{-2} of intensity, 25 kHz, for 18 minutes, significantly reduced the water losses in all treatments with different phosphate contents, compared to the control treatment with no ultrasound. For the authors, the improvement of the technological quality and the lower fluid released in the emulsion submitted to ultrasound suggest that there were modifications in the myofibrillar protein structure, leading to a greater interaction between the polar and non-polar groups of water. In addition, cavitation may have improved the diffusion of salt and phosphate in the meat emulsion, thus contributing to a greater solubilization of myofibrillar proteins. Barretto et al. (2018) also showed that cavitation caused by the use of ultrasound in the production of restructured cooked ham causes micro fissures on the surface of myofibrillar proteins, facilitating their contact with water and the energy generated by cavitation opened interfibrillar channels that allowed a greater retention of water in the product. Kang et al. (2016) and González-González et al. (2017) highlighted the ability of ultrasound to promote the diffusion of sodium in meat systems, the energy produced by cavitation being able

to diffuse the added sodium in a more homogeneous way. The better diffusion probably improves the extraction of myofibrillar proteins and increases their binding with water, reducing TFR.

Table 3 - TFR, Color parameters and TPA of restructured cooked ham.

	Day	Treatments				SEM	P value
		CT	UsT	KT	UsKT		
TFR	0	12.85 ^{a,A}	6.23 ^{b,A}	2.08 ^{c,A}	1.89 ^{c,A}	0.912	<0.01
TFR	30	13.89 ^{a,A}	7.33 ^{b,A}	2.89 ^{c,A}	3.35 ^{c,A}	0.868	<0.01
<i>P</i> value		0.653	0.344	0.346	0.158		
L*	0	62.32 ^{a,A}	63.07 ^{a,B}	63.27 ^{a,A}	62.54 ^{a,A}	0.253	0.514
L*	30	63.06 ^{ab,A}	65.03 ^{a,A}	62.95 ^{ab,A}	62.02 ^{b,A}	0.308	0.004
<i>P</i> value		0.404	0.004	0.642	0.544		
a*	0	10.67 ^{a,A}	10.50 ^{a,A}	10.00 ^{a,A}	10.15 ^{a,A}	0.128	0.170
a*	30	10.62 ^{a,A}	9.85 ^{a,A}	10.33 ^{a,A}	10.74 ^{a,A}	0.130	0.067
<i>P</i> value		0.866	0.090	0.377	0.147		
b*	0	10.71 ^{ab,A}	10.98 ^{a,A}	10.43 ^{b,A}	10.26 ^{b,B}	0.053	< 0.01
b*	30	10.82 ^{a,A}	10.63 ^{ab,B}	10.65 ^{ab,A}	10.39 ^{b,A}	0.054	0.038
<i>P</i> value		0.391	0.013	0.202	< 0.01		
Hardness (N)	0	14.33 ^{b,A}	14.98 ^{b,B}	18.84 ^{a,A}	18.79 ^{a,B}	0.457	< 0.01
Hardness (N)	30	14.8 ^{c,A}	18.62 ^{b,A}	22.92 ^{a,A}	23.83 ^{a,A}	0.608	< 0.01
<i>P</i> value		0.522	0.002	0.051	< 0.01		
Cohesiveness	0	0.49 ^{a,A}	0.44 ^{a,A}	0.42 ^{a,A}	0.47 ^{a,A}	0.009	0.068
Cohesiveness	30	0.37 ^{a,B}	0.42 ^{a,A}	0.44 ^{a,A}	0.42 ^{a,B}	0.009	0.086
<i>P</i> value		< 0.01	0.592	0.482	0.038		
Springiness	0	0.69 ^{a,A}	0.70 ^{a,A}	0.79 ^{a,A}	0.74 ^{a,A}	0.015	0.089
Springiness	30	0.68 ^{a,A}	0.76 ^{a,A}	0.77 ^{a,A}	0.77 ^{a,A}	0.016	0.252
<i>P</i> value		0.916	0.296	0.730	0.332		
Chewiness	0	4.99 ^{b,A}	4.76 ^{b,B}	6.52 ^{a,A}	6.69 ^{a,A}	0.255	0.006
Chewiness	30	3.96 ^{c,B}	5.92 ^{b,A}	7.87 ^{a,A}	7.83 ^{a,A}	0.278	< 0.01
<i>P</i> value		0.014	0.045	0.133	0.072		

^{a-c} Mean values in the same line not followed by a common letter differ significantly ($P < .05$).

^{A-B} Mean values in the same column not followed by a common letter differ significantly ($P < .05$).

SEM: standard error of the mean. CT – Control Treatment; UsT – Ultrasound Treatment; KT – KCl Treatment; UsKT – Ultrasound and KCl Treatment. Fonte: elaborado pelo autor

For the KT and UsKT treatments, the TFR values did not differ ($P > 0.05$), however they were lower ($P < 0.05$) than the CT and UsT values for zero and thirty days of refrigerated storage, showing that the use of 0.5% potassium chloride in the restructured cooked ham formulation contributed to the reduction of TFR and was also better than the use of ultrasound alone. Salt activates proteins to increase hydration and the ability to bind to water in meat products because chloride ions bind strongly to proteins. When salt content is reduced, excess exudate is one of the first problems to appear. Consequently, low salt meat products may present problems in water retention capacity and emulsion stability (Desmond, 2006, Ruusunen & Puolanne, 2005). Potassium chloride is one of the most effective substitutions for sodium chloride due to the similarity of its molecular compositions. The chloride anion is responsible for extracting the myofibrillar protein that help in binding and contribute to stability with water (Horita et al., 2014). In the development of sausages with reduction of NaCl and partial substitutions by other chlorinated salts, Horita et al. (2014) found no difference in fluid exudation between the control treatment and the 50% NaCl replacement by KCl treatment. Similar results were also obtained by Alves et al. (2017) who reported no differences in post-cooking exudation losses for bologna-type sausages that had 50% NaCl replaced by KCl. Horita et al. (2016) found that the replacement of 50% NaCl by a mixture containing 25% KCl and 25% CaCl_2 in sausages resulted in an ionic strength equivalent to the sample without NaCl replacement. In the current study, no differences were observed for TFR between KT and UsKT, so the combination of KCl use and ultrasound technology was no better than the use of KCl alone in restructured cooked ham under these processing conditions.

3.3 Instrumental color

Thirty day of refrigerated storage time only showed an increase ($P < 0.05$) for the L^* values for UsT. Caraveo, Alarcon-Rojo, Renteria, Santellano & Paniwnyk (2015) reported a similar result in which beef was lighter in the sample treated with ultrasound for 60 and 90 minutes compared to the control sample without the use of ultrasound. No differences ($P > 0.05$) were observed for L^* between treatments at zero refrigerated storage day, as also reported by Alves et al. (2018), who found no change in lightness in Italian salami processed using ultrasound at 500 W.cm^{-2} . At 30 days of refrigerated

storage, UsT presented a higher L^* ($P < 0.05$) than UsKT. Ultrasound contributed to the increased L^* in restructured cooked ham. The incorporation of 0.5% KCl in the formulation possibly reduced L^* values. Similar results were obtained by Horita et al. (2014) when adding 0.6% of KCl, a significant reduction in the value of L^* in sausages was seen. Caraveo et al. (2015) emphasize that the increase in lightness caused by the use of ultrasound can be considered a negative effect of this technology. However, the use of KCl in the processing of low sodium restructured cooked ham with ultrasound application helped to maintain the lightness of the product.

Ultrasound application, addition of KCl and refrigerated storage time did not affect the values of a^* between treatments (Table 3). Consistent with these results, some studies suggest that ultrasound has no effect on meat color, since the heat generated is not sufficient to denature proteins and pigments (Sikes, Mawson, Stark & Warner, 2014). After 60 days of refrigerated storage of restructured cooked ham with reduced sodium, Barretto et al. (2018) observed that the use of ultrasound did not affect the value of a^* in the product. Ferrentino and Spilimbergo (2016) also reported that ultrasound and high pressure treatment in restructured cooked ham did not affect the redness of the product. Horita et al. (2014) observed that the use of KCl in meat products did not affect the redness of the product. The colour of meat products is one of the most important visual characteristics for consumers, and redness is an attribute that may interfere with its acceptability (Alarcon-Rojo, 2018). The addition of KCl and the use of ultrasound in low sodium restructured cooked ham did not affect the redness. Ultrasound application did not affect the value of b^* during the storage time studied. Some studies also reported no changes in the value of b^* in meat products submitted to ultrasound (McDonnell, Lyng & Allen, 2014; Barretto et al., 2018).

3.4 Texture profile analysis

Table 3 shows that the hardness increased during the refrigerated storage time for the UsT and UsKT treatments. Ultrasound contributed to increased hardness in low sodium restructured cooked ham after thirty days of refrigerated storage. At day 0 of refrigerated storage the hardness was higher ($P < 0.05$) for KT and UsKT and, after 30 days, the control was less hard ($P < 0.05$) than the other three treatments. So, the use of ultrasound and the addition of KCl in low sodium restructured cooked ham increase

the hardness of the product. Zou, Zhang, Kang & Zhou (2018) reported an increase in hardness in beef steaks cooked at 80 °C which had been subjected to ultrasound (1000 W.cm⁻²). Ojha et al. (2016) reported a greater diffusion of the salt when ultrasound was used in pork salting, and it increased the shear force in the product. A similar result was reported by Barretto et al. (2018) who verified an increase in the hardness of the restructured cooked ham with reduced sodium submitted to ultrasound (600 W. cm⁻²). Ultrasound increases the diffusion of salt added in meat systems (Ojha et al., 2016; González-González et al., 2017). Salt helps to solubilize myofibrillar proteins in meat systems, contributing to water retention capacity and improving texture (Desmond, 2006, Çarkcioğlu, Rosenthal and Candoğan, 2016). The replacement of 50% NaCl by KCl in sausages resulted in increased hardness values (Horita et al., 2014). Lower values for hardness were observed as the NaCl content was reduced in cooked hams (Paula et al., 2019). For Ramos & Gomides (2017), this occurs due to the greater solubility of myofibrillar proteins in saline solutions, since the texture of meat products is affected by the availability of these proteins in the gel formation process. As protein availability increases, the strength of the gel formed increases too. This, in turn, can increase the hardness of the product. The addition of KCl in KT and UsKT increased the ionic strength of the system and contributed to better extraction of myofibrillar proteins. Different results were reported by Yeung & Huang (2017) who observed a reduction in the hardness of pork loin treated with ultrasound. The authors justified this because cavitation may partially denature the proteins of the meat, increasing the softness of the product. In the current study, there was no difference ($P > 0.05$) between KT and UsKT at zero and 30 days refrigerated storage for hardness. Thus, the application of ultrasound did not increase the hardness of the product when used in association with KCl.

There were no differences ($P > 0.05$) between the treatments over storage time for cohesiveness and springiness. The use of ultrasound and/or the addition of KCl in low sodium restructured cooked ham did not influence these texture parameters. Refrigerated storage time affected chewiness for CT and UsT. In the control treatment, this parameter decreased and, the use of ultrasound increased the chewiness of the product during refrigerated storage, the same behavior as was observed for hardness. At zero and 30 days refrigerated storage, the treatments with added KCl (KT and UsKT) had the highest ($P < 0.05$) values for chewiness. In thirty days of refrigerated

storage, the use of ultrasound in UsT showed higher ($P < 0.05$) values for chewiness compared to the control treatment. Barretto et al. (2018) obtained similar results when observing that the chewiness increased in restructured cooked ham with reduction of sodium treated with ultrasound. Possibly the increase of the hardness of this product due to the ultrasound and the addition of the KCl influenced the increase in the chewiness values.

3.5 Microbiological analysis

The results for thermo-tolerant fecal coliforms showed growth < 3 MPN/g and for coagulase-positive *Staphylococci* growth was $< 10^2$ CFU/g for all treatments. The sulfite-reducing *Clostridia* was 10^1 CFU/g and *Salmonella* spp. was absent in 25 g of sample for all treatments. These results are within the limits set by Brazilian legislation for this product (Brazil, 2001). The use of ultrasound and the addition of KCl did not affect the development of these microorganisms.

3.6 Sensorial acceptance

The results for sensory acceptance are presented in Table 4. Regarding sensorial color, the treatment with KCl (KT) obtained the highest score ($P < 0.05$) compared to the control (CT). The use of KCl increased the acceptance for color in low sodium restructured cooked ham. UsT and UsKT did not differ ($P > 0.05$) from KT and CT. In other studies, Lorenzo, Cittadini, Bermúdez, Munekata & Dominguez (2015); Alves et al. (2017) and Horita et al. (2014) reported different results. They did not see any differences in sensory acceptance for color and appearance of meat products with partial replacement of NaCl by KCl. The higher protein extraction and lower TFR observed in KT compared to CT may have contributed to the increase in its color score.

The salty taste attribute presented the lowest mean score for the control treatment, CT. The use of ultrasound, in UsT, increased the mean of the scores for salty taste. A similar result was reported by Barretto et al. (2018) who observed a higher score for flavor in restructured cooked ham with sodium reduction treated with 600 W.cm^{-2} of ultrasound. The authors noted that the better diffusion of the added

sodium improved the flavor score attributed by consumers. These results are similar to those reported by Ojha et al., 2016; González-González et al., 2017 and Kang et al., 2016, who all observed that the use of ultrasound increased the diffusion of sodium added to meat products. The mean of the scores for the flavor attribute presented the same behavior as the mean for salty taste. So, the high score for the salty taste could be related to the increase in the score attributed to the flavor in low sodium restructured cooked ham. The addition of potassium chloride in KT also increased the scores for salty taste and flavor attributes compared to CT and did not differ ($P > 0.5$) from the ultrasound treatment, UsT. The combination of the use of ultrasound and the addition of potassium chloride in UsKT obtained a higher score ($P < .05$) than the ultrasound treatment alone, for both salty taste and flavor. However, it was no different from the treatment that used only the addition of potassium chloride, KT. The combination of ultrasound application and the addition of KCl obtained the same as the KT scores for salty taste and flavor attributes in low sodium restructured cooked ham.

Table 4. Sensorial acceptance of restructured cooked ham.

	Treatments				SEM	Sig.
	CT	UsT	KT	UsKT		
Sensorial acceptance						
Color	7.08 ^b	7.24 ^{ab}	7.64 ^a	7.51 ^{ab}	0.067	Sig.
Flavor	6.37 ^c	6.90 ^b	7.32 ^{ab}	7.39 ^a	0.066	Sig.
Salty taste	6.09 ^c	6.75 ^b	7.22 ^{ab}	7.29 ^a	0.073	Sig.
Texture	6.49 ^b	6.83 ^b	7.51 ^a	7.59 ^a	0.073	Sig.
Global acceptance	6.42 ^c	6.91 ^b	7.44 ^a	7.48 ^a	0.064	Sig.
Purchase intention	3.37 ^c	3.90 ^b	4.08 ^{ab}	4.21 ^a	0.044	Sig.

^{a-c} Mean values in the same line not followed by a common letter differ significantly ($P < .05$). SEM: standard error of the mean. CT – Control Treatment; UsT – Ultrasound Treatment; KT – KCl Treatment; UsKT – Ultrasound and KCl Treatment. Fonte: elaborado pelo autor

The addition of 0.5% KCl in low sodium restructured cooked ham improved the sensory acceptance for the salty taste and flavor attributes. Other studies have also pointed out that the use of KCl can improve the sensorial acceptance of meat products with low sodium content. Vidal et al. (2019) observed that the 50% NaCl replacement by KCl in jerked beef provided sensory characteristics similar to the jerked beef

traditionally processed with 100% NaCl. Stanley et al. (2017) reported that the addition of 0.94% salt blend composed mainly of KCl improved the flavor and salty taste of pork sausage patties with low NaCl content. Bis et al. (2016) found that the replacement of 35% NaCl by KCl did not negatively affect the flavor of roast beef compared to the product with no reduction of NaCl.

Regarding the sensory texture, it is possible to observe that CT and UsT did not differ ($P > 0.05$), showing that the use of ultrasound alone did not contribute to the texture attribute in low sodium restructured cooked hams. KT and UsKT presented higher scores for texture and did not differ ($P > 0.05$) from each other, showing that KCl contributed significantly to the improvement of the sensorial texture. These results are in agreement with the results obtained in the instrumental analysis of TPA, in which KT and UsKT obtained higher values for hardness and chewiness of the product. As already discussed, the increased ionic strength caused by the addition of KCl increases the solubility of myofibrillar proteins, which increases the binding force of the water. This is reflected in a product with greater hardness and chewiness. Possibly, for this type of product, consumers possibly appreciate this type of product harder and chewier, rather than very soft products resulting from lower ionic strength, softness not being a characteristic of cooked ham. The following results reflect the overall acceptance of the product. UsT presented greater ($P < 0.05$) overall acceptance than the control treatment, due to the contribution of ultrasound to the flavor and salty taste scores. KT and UsKT did not differ ($P > 0.05$) from each other and obtained higher scores ($P < 0.05$) for overall acceptance, possibly due to the greater contribution that the addition of KCl provided to the texture.

4. CONCLUSION

The application of ultrasound in low sodium restructured cooked ham improved the total fluid released, the flavor and the overall acceptance of the product, confirming the effectiveness of this technology in low sodium restructured cooked ham. However, the addition of KCl was more effective in improving total fluid release, instrumental texture parameters and all sensory parameters. The combination of ultrasound and the addition of KCl showed no additional benefit, meaning that the use of KCl or ultrasound

is a technological and sensorially viable alternative for low sodium restructured cooked ham.

Chemical compounds used in this research

Methanol (PubChem CID: 887); Chloroform (PubChem CID: 6212); Sodium Carbonate (PubChem CID: 10340); Sodium hydroxide (PubChem CID: 14798); Boric acid (PubChem CID: 7628).

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CONCLUSÃO GERAL

A tecnologia do ultrassom melhorou as propriedades físico-químicas e a aceitação sensorial do presunto cozido com baixo teor de sódio. A adição de cloreto de potássio aumentou a força iônica e foi mais efetiva que o ultrassom para melhorar esses parâmetros no presunto cozido com baixo teor de sódio. A combinação de ultrassom e adição de cloreto de potássio não mostrou benefício adicional.

A aplicação do ultrassom é uma alternativa tecnológica e sensorialmente viável na produção de presunto cozido de baixo teor de sódio. A adição de cloreto de potássio melhora as propriedades sensoriais e tecnológicas de presunto cozido com baixo teor de sódio.

Há necessidade de intensificação em pesquisas sobre a aplicação da tecnologia de ultrassom em carnes e produtos cárneos, contemplando diferentes condições de aplicação em diferentes tipos de produtos, colaborando dessa forma para formulações de produtos cárneos mais saudáveis.

APÊNDICE A – Modelo de ficha utilizada para o teste de aceitação sensorial e intenção de compra de presunto cozido do artigo do capítulo III.

Nome: _____ Idade: _____ Data: ____/____/____

1. Você esta recebendo uma amostra de Presunto Cozido. Por favor, prove-a e avalie cada item de acordo com a escala abaixo.

Código da amostra: _____

9 – Gostei extremamente
 8 – Gostei muitíssimo
 7 – Gostei moderadamente
 6 – Gostei levemente
 5 – Não gostei nem desgostei
 4 – Desgostei levemente
 3 – Desgostei moderadamente
 2 – Desgostei muitíssimo
 1 – Desgostei extremamente

Cor	
Sabor	
Textura	
Aceitação global	

2. Assinale, para esta amostra, qual seria sua intenção de compra.

() Eu certamente compraria esta amostra
 () Eu provavelmente compraria esta amostra
 () Tenho dúvidas se compraria ou não esta amostra
 () Eu provavelmente não compraria esta amostra
 () Eu certamente não compraria esta amostra

Comentários: _____

Fonte: elaborado pelo autor

APÊNDICE B - Modelo de ficha utilizada para o teste de aceitação sensorial e intenção de compra de presunto cozido do artigo do capítulo IV.

Nome: _____ Idade: _____ Data: ____/____/____

1. Você esta recebendo uma amostra de Presunto Cozido. Por favor, prove-a e avalie cada item de acordo com a escala abaixo.

Código da amostra: _____

9 – Gostei extremamente	
8 – Gostei muitíssimo	
7 – Gostei moderadamente	
6 – Gostei levemente	
5 – Não gostei nem desgostei	
4 – Desgostei levemente	
3 – Desgostei moderadamente	
2 – Desgostei muitíssimo	
1 – Desgostei extremamente	

Cor	
Sabor	
Gosto salgado	
Textura	
Aceitação global	

2. Assinale, para esta amostra, qual seria sua intenção de compra.

() Eu certamente compraria esta amostra
 () Eu provavelmente compraria esta amostra
 () Tenho dúvidas se compraria ou não esta amostra
 () Eu provavelmente não compraria esta amostra
 () Eu certamente não compraria esta amostra

Comentários: _____

Fonte: elaborado pelo autor

ANEXO A - Direitos de uso de artigo em tese.



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Title: Improving sensory acceptance and physicochemical properties by ultrasound application to restructured cooked ham with salt (NaCl) reduction

Author: Tiago Luis Barretto, Marise Aparecida Rodrigues Pollonio, Javier Telis-Romero, Andrea Carla da Silva Barretto

Publication: Meat Science

Publisher: Elsevier

Date: November 2018

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ANEXO B - Parecer do Comitê de Ética em Pesquisa do Ibilce-Unesp - número 58925516.9.0000.5466.

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Continuação do Parecer: 1.733.288

sensorial a fim de assegurar a qualidade dos produtos.

Em relação aos benefícios, este trabalho contribuirá com o desenvolvimento de presuntos com a redução de sódio e uso de tecnologias não convencionais como ultrassom. Os resultados desta pesquisa serão divulgados na comunidade científica e também em indústrias processadoras de presunto que visam à produção de produtos com redução de sódio nos produtos.

Comentários e Considerações sobre a Pesquisa:

A pesquisa é relevante para a área de tecnologia de carnes e derivados, pois se pretende desenvolver presunto cozido com reduzido teor de sódio e uso de tecnologia de ultrassom. O brasileiro consome, em média, 12 gramas de sódio por dia, o que representa mais que o dobro do recomendado (5 gramas) pela Organização Mundial da Saúde. A ingestão excessiva de sódio está associada ao desenvolvimento de doenças crônicas não transmissíveis, como hipertensão e doenças cardiovasculares. De acordo com as informações nutricionais das embalagens de presuntos cozidos disponíveis no mercado brasileiro, verifica-se até 1600 mg de sódio a cada 100 g do produto, o que pode contribuir com o excesso desse nutriente na dieta, já que há um aumento do consumo do produto entre os brasileiros.

O presunto cozido é um dos produtos cárneos processados mais populares entre os consumidores. No processamento tradicional de presunto cozido, o cloreto de sódio é comumente adicionado, na forma de salmoura, que pode ser injetada ou incorporada à carne suína sob massageamento seguido de cocção e resfriamento adequados, a fim de melhorar as características tecnológicas e sensoriais do produto. Tecnologias alternativas, como o ultrassom, vêm sendo estudadas com a finalidade de melhorar as propriedades tecnológicas de produtos alimentares, como a difusão do sódio durante o processamento do presunto cozido e a capacidade de retenção de água, o que pode acarretar melhor aceitação sensorial do produto. Neste projeto, os presuntos elaborados com as quatro formulações (controle, sem redução de cloreto de sódio; com redução de 25% de cloreto de sódio, com redução de 50% de cloreto de sódio e com redução de 50% de cloreto de sódio e submetido ao tratamento com ultrassom) serão avaliados por teste de aceitação usando escala hedônica estruturada de 9 pontos para a avaliação da cor, sabor, textura e aceitação global, além da intenção de compra. A análise sensorial será realizada por 120 potenciais consumidores do produto, que receberão as amostras de presunto codificadas com números de três dígitos e de forma monádica. O teste sensorial será realizado no 15º dia após o processamento e após os resultados microbiológicos para assegurar a qualidade dos produtos. Os presuntos serão avaliados por meio de pH e análise microbiológica (coliformes a 45 oC, Estafilococos coagulase positiva, contagem de anaeróbicos, sulfito redutores a 46 oC e Salmonella

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Continuação do Parecer: 1.733.288

sp em 25 g de amostra) durante o período de armazenamento (0, 15 – antes da análise sensorial- , 30 e 60 dias), sob refrigeração. Também será determinada a composição centesimal (umidade, proteína, lipídios, cinzas e carboidratos) dos produtos. A pesquisa esta bem referendada pela literatura. Há infraestrutura necessária da instituição proponente para execução do projeto. O projeto terá o custo de R\$ 275,00 que será custeado com reserva própria.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos de apresentação obrigatória foram apresentados. O TCLE é apresentado de forma adequada e contempla os itens obrigatórios, como a pesquisa apresentar risco mínimo. No projeto foi incluído que duas vias do TCLE será entregue para o participante, antes das análises sensoriais, e que uma via assinada ficará com o participante e outra com o responsável pelo projeto que armazenará por 5 anos. Participação do teste sensorial indivíduos maiores de 18 anos, recrutados entre alunos e funcionários do IBILCE. Serão excluídos indivíduos menores de 18 anos e com patologias relacionadas à ingestão de alimentos como diabéticos, hipertensos, com intolerância à lactose e ao glúten.

Recomendações:

Nada a declarar.

Conclusões ou Pendências e Lista de Inadequações:

Nada a declarar.

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa, em reunião ordinária de 14 de setembro de 2016, deliberou, por unanimidade, pela aprovação do presente projeto de pesquisa. Os relatórios parciais devem ser encaminhados semestralmente, a contar desta data.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_774281.pdf	18/08/2016 15:34:20		Aceito
Projeto Detalhado / Brochura Investigador	Pesquisa.pdf	18/08/2016 15:32:48	Tiago Luis Barretto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	18/08/2016 15:31:28	Tiago Luis Barretto	Aceito

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Continuação do Parecer: 1.733.288

Folha de Rosto	folha_de_Rosto.pdf	18/08/2016 15:27:18	Tiago Luis Barretto	Aceito
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Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO JOSE DO RIO PRETO, 19 de Setembro de 2016

Assinado por:

Monica Abrantes Galindo de Oliveira
(Coordenador)

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ANEXO C - Parecer do Comitê de Ética em Pesquisa do Ibilce-Unesp - número 83944018.5.0000.5466.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: USO DO ULTRASSOM SOBRE AS PROPRIEDADES FÍSICO-QUÍMICAS E SENSORIAIS EM PRESUNTO COZIDO COM SUBSTITUIÇÃO PARCIAL DE NaCl

Pesquisador: Tiago Luis Barretto

Área Temática:

Versão: 1

CAAE: 83944018.5.0000.5466

Instituição Proponente: Instituto de Biociências Letras e Ciências Exatas/ Campus de São José do

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.573.067

Apresentação do Projeto:

Trata-se da apresentação de um projeto de pesquisa abordando a problemática do alto consumo de sódio pela população, visto que o brasileiro consome, em média, 12 gramas de sódio por dia, considerando o sal de mesa e o sódio obtido dos alimentos, o que representa mais que o dobro do que os 5 gramas recomendados pela Organização Mundial da Saúde. Uma preocupação para este consumo excessivo de sódio está no risco de desenvolvimento de algumas doenças, como hipertensão e doenças cardiovasculares, sendo, assim uma preocupação para a saúde pública. O projeto propõe avaliar a influência da redução de cloreto de sódio (NaCl) e sua substituição parcial por cloreto de potássio (KCl) por meio de ultrassom sobre as características físico-químicas e sobre a aceitação sensorial de presuntos cozidos. Para tanto, serão produzidos quatro tratamentos para os presuntos cozidos:

T1 – Controle: redução de 66,7% de NaCl;

T2 – Redução de 66,7% de NaCl com ultrassom;

T3 – Redução de 66,7% de NaCl e adição de 0,5% de KCl;

T4 – Redução de 66,7% de NaCl e adição de 0,5% de KCl com ultrassom.

Para todos os tratamentos serão feitas a análise de pH durante o período de armazenamento (0 e 30 dias), determinação da composição centesimal do produto: umidade, proteína, lipídios, cinzas e carboidratos, além de análises microbiológicas de coliformes a 45°C, Estafilococos coagulase positiva, contagem de anaeróbicos, sulfito redutores a 46°C e Salmonella sp em 25g de

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Continuação do Parecer: 2.573.067

amostra. As análises microbiológicas serão realizadas no tempo zero de estocagem, anterior a realização do teste sensorial. Em seguida, será feita a análise sensorial com 120 consumidores do produto que irão avaliar sua aceitação referente aos atributos de cor, sabor, textura e aceitação global e opinar sobre a intenção de compra do produto. Neste projeto serão excluídos indivíduos menores de 18 anos, além de indivíduos com patologias relacionadas à ingestão de alimentos, como diabéticos, hipertensos, com intolerância à lactose e ao glúten, entre outros.

Objetivo da Pesquisa:

Avaliar a influência da redução de cloreto de sódio (NaCl) e sua substituição parcial por cloreto de potássio (KCl) com o uso do ultrassom, sobre as características físico-químicas e sobre a aceitação sensorial de presuntos cozidos.

Avaliação dos Riscos e Benefícios:

O pesquisador relata que os riscos à saúde são mínimos, pois os produtos serão elaborados seguindo as Boas Práticas de Fabricação e serão liberados apenas para análise sensorial mediante os resultados microbiológicos em conformidade com a legislação brasileira. O pesquisador ainda relata que de acordo com os resultados obtidos com este estudo, seja possível produzir produtos cárneos mais saudáveis e com uma boa aceitação sensorial.

Comentários e Considerações sobre a Pesquisa:

O projeto está bem redigido e aborda uma preocupação de saúde pública no que diz respeito ao consumo excessivo diário de sal, que pode conferir risco aumentado para o surgimento de doenças cardiovasculares e hipertensão. Para tentar resolver essa problemática, o projeto propõe a utilização da tecnologia baseada no ultrassom para a redução parcial do cloreto de sódio ou sua substituição parcial por cloreto de potássio em presuntos cozidos com o intuito de produzir um produto mais saudável com redução de sódio e que seja de sabor agradável e que os consumidores tenham interesse em comprar. Assim, um projeto bastante relevante.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos de apresentação obrigatória foram apresentados corretamente.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

De acordo com o que foi apresentado, considero que o projeto está de acordo com as normas éticas para que possa ser executado normalmente.

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Continuação do Parecer: 2.573.067

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa, em reunião ordinária de 23 de março de 2018, deliberou, com uma abstenção, pela aprovação do presente Projeto de Pesquisa. Os relatórios parciais deverão ser encaminhados semestralmente, contando a partir desta data, conforme modelo em nossa página: <http://www.ibilce.unesp.br/#/comite/etica-em-pesquisa/relatorio-projeto>.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1083894.pdf	28/02/2018 14:46:54		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	28/02/2018 14:44:45	Tiago Luis Barretto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	28/02/2018 14:44:23	Tiago Luis Barretto	Aceito
Folha de Rosto	Folha_de_rosto_.pdf	28/02/2018 14:43:19	Tiago Luis Barretto	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO JOSE DO RIO PRETO, 01 de Abril de 2018

Assinado por:
Monica Abrantes Galindo de Oliveira
(Coordenador)

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