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FILMES BIODEGRADÁVEIS E ATIVOS NA CONSERVAÇÃO DE HAMBÚRGUER BOVINO COM REDUZIDO TEOR DE SÓDIO E ADITIVOS NATURAIS

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Maringá

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Tese apresentada ao programa de Pós-Graduação de Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de Doutor em Ciência de Alimentos.

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BIOGRAFIA

CAMILA BARBOSA CARVALHO nasceu em PARANAVAÍ no estado do PARANÁ. Possui graduação em NUTRIÇÃO pela UNIVERSIDADE FEDERAL DO PARANÁ. Mestre em Ciência de Alimentos pela Universidade Estadual de Maringá em 2013, na área de desenvolvimento de novos produtos. Dissertaçao: Carnes bovinas e de frango marinadas com reduzido teor de sódio: Qualidade da carne, composição química e aspectos microbiológicos. Docente do Curso de nutrição pelas faculdades, Bom Jesus Ielusc – Joinville- SC e Faculdade Ingá- Maringá-Pr de 2007 a 2011. Tem experiência nas áreas de desenvolvimento de novos produtos, controle de qualidade, técnica dietética e alimentos especiais, assim como em Boas Práticas de Fabricação e APPCC, atuando principalmente nos seguintes temas: Controle de qualidade para alimentos, análise sensorial, desenvolvimento de novos produtos e alimentos especiais.

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“*Quando caminhamos sozinhos, chegamos muito mais rápido, porém, quando caminhamos juntos, vamos muito mais longe*”.

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de três artigos científicos, descritos a seguir:

1. Camila Barbosa Carvalho, Grasiele Scaramal Madrona, Péricles Martins Reche, Fernando Zawadzki, Ana Carolina P. Vital, Ana Guerrero, Ivanor Nunes do Prado. QUALITY AND SENSORIAL EVALUATION OF BEEF HAMBURGER MADE WITH HERBS, SPICES AND REDUCED SODIUM CONTENT. **Journal of the Science of Food and Agriculture - Qualis Capes: B1 (artigo submetido).**

2. Camila Barbosa Carvalho, Grasiele Scaramal Madrona, Lucinéia A. Cestari, Ana Guerrero, Nilson Evelázio de Souza, Ivanor Nunes do Prado. SENSORY PROFILE OF BEEF BURGER WITH REDUCED SODIUM CONTENT. **Acta Scientiarum- Qualis Capes: B2 (artigo publicado).**

3. Camila Barbosa Carvalho, Grasiele Scaramal Madrona, Jane Graton Mitcha, Maribel Velandia Valero, Ana Guerrero, Mônica Regina da Silva Scapim, Fábio Yamashita, Ivanor Nunes do Prado. EFFECT OF ACTIVE PACKAGING WITH OREGANO OIL ON BEEF BURGERS WITH LOW SODIUM CONTENT. **Journal of Food Science & Technology - Qualis Capes: B1 (artigo submetido).**

RESUMO GERAL

INTRODUÇÃO

Nos últimos anos, a demanda dos consumidores por alimentos mais seguros e de maior qualidade da carne e de produtos à base de carne com níveis reduzidos de gordura, cloreto de sódio, colesterol e nitrito cresceu mundialmente. Os produtos cárneos industrializados têm expressiva contribuição nos teores de sódio consumidos pela população, porém, o uso de substitutos como o cloreto de potássio é limitado, principalmente pelo seu sabor amargo. A substituição parcial do cloreto de sódio pelo cloreto de potássio, com adição de ervas aromáticas e especiarias ao hambúrguer bovino, torna-se uma saída promissora para a redução de sódio e a manutenção da sua palatabilidade. A manutenção da vida de prateleira do hambúrguer bovino com redução de sódio ainda depende de outras variáveis tecnológicas, tais como níveis aceitáveis de oxidação lipídica e contagens microbianas. Aliado a este fato, o uso de embalagens ativas, atualmente, é uma das tecnologias mais dinâmicas utilizadas para preservar a qualidade da carne, por meio da libertação de agentes ativos como os óleos essenciais de orégano, contribuindo para a manutenção da vida de prateleira de produtos à base de carne de reduzido teor de sódio, sem alterar as suas características tecnológicas e sensoriais, eliminando o uso de aditivos sintéticos.

OBJETIVOS

Desenvolver e avaliar hambúrgueres com teor reduzido de sódio em 25 e 50% quanto a sua composição química, física, características microbiológicas, sensoriais e realizar pesquisa de mercado.

Descrição do perfil sensorial dos hambúrgueres hipossódicos utilizando a metodologia de Análise Descritiva Quantitativa (ADQ).

Avaliar a eficácia de embalagens ativas (com adição de óleo essencial) preservando a qualidade do hambúrguer bovino com 25 e 50% de redução de cloreto de sódio, armazenado por 120 dias, sob congelamento.

MATERIAL E MÉTODOS

Os hambúrgueres foram preparados substituindo o cloreto de sódio (NaCl) por cloreto de potássio (KCl) e com adição de ervas aromáticas(Alho, orégano, colorau, pimenta calabresa). As análises químicas foram efetuadas em triplicata, seguindo a metodologia da AOAC. Testes sensoriais foram realizados por consumidores não treinados saudáveis e hipertensos, utilizando escala hedônica estrutura de 9 pontos, análise de componentes principais e Análise Descritiva Quantitativa(ADQ) com doze provadores selecionados e treinados utilizando como critérios o poder discriminativo, reprodutividade e consenso dos provadores entre si. Além disso, foi realizada pesquisa de mercado com 250 potenciais consumidores on line. Foi avaliada a eficácia das embalagens biodegradáveis e ativas (produzidas por extrusão e com adição de óleo essencial de orégano) aplicadas aos hambúrgueres bovinos de baixo teor de sódio armazenados a -18ºC por 120 dias mediante diversas técnicas. Foram testados quatro tratamentos: Hambúrgueres de carne com 25% (B25) e 50% (B50) de redução de sódio em embalagem biodegradável e hambúrgueres de carne com 25% (BOEO25) e 50% (BOEO50) de redução de sódio em embalagem biodegradável com 1% de óleo essencial de orégano. As análises realizadas foram oxidação lipídica (TBARS), microbiológicas, pH nos tempos 1, 30, 60, 90 e 120 dias, análises de composição química (umidade, cinzas, proteína, lipídio) nos tempos 1 e 120 dias, análises de atividade de água (Aw), perda por cocção, textura e cor (L, a^*, b^*) nos tempos 1, 60 e 120 dias e análise sensorial nos tempos 30 e 120 dias.

RESULTADOS E DISCUSSÃO

Em relação ao artigo1, observou-se que os maiores valores de textura foram detectados nos hambúrgueres com 50% de redução de sódio, no entanto, os aspectos microbiológicos foram adequados e o índice de aceitabilidade manteve-se acima de 70% para ambos os tratamentos, os consumidores hipertensos tiveram maior aceitabilidade (92%) em relação ao hambúrguer com 50% de redução de sódio. Os resultados da pesquisa de mercado revelaram interesse dos consumidores em adquirir produtos com menor teor de sódio. A Análise Descritiva Quantitativa (ADQ) tema do artigo 2 demonstrou que os hambúrgueres hipossódicos apresentaram menor sabor de gordura e salgado, quando comparados ao tratamento controle, os quais sabor e odor de especiarias se sobresaem , este diferencial comprova que a redução de sódio no hambúrguer provoca uma maior percepção de intensidade pelos provadores em relação a presença de especiarias no produto. Em relação ao artigo 3, observou-se que a composição das embalagens durante o tempo de armazenamento dos hambúrgueres bovinos hipossódicos não influenciaram os teores de umidade, proteína bruta, gordura total, cinzas, perda por cocção e atividade de água. As embalagens ativas com óleo de orégano apresentaram maior proteção da cor em hambúrgueres durante o período de armazenamento. A textura, manteve-se estável, em todo o período de armazenamento. Em relação à análise sensorial, as amostras de hambúrguer com redução de 25% de sódio e embalagem com 1% de óleo essencial de orégano (BOEO25) tiveram melhor sabor e aroma, obtendo um índice de aceitabilidade acima de 80% para ambos os períodos (30 e 120 dias). A utilização de 1% de óleo de essencial de orégano incorporado à embalagem reduziu a oxidação lipídica dos hambúrgueres analisados em 14% quando comparado à embalagem sem a adição de óleo essencial de orégano. As análises microbiológicas mantiveram-se adequadas e estáveis durante todo o armazenamento. Assim, em geral, a utilização da embalagem ativa com óleo essencial de orégano para o armazenamento dos hambúrgueres hipossódicos mostrou-se uma alternativa viável, possibilitando sua produção e comercialização, mantendo a qualidade dos mesmos e contribuindo para sua aceitabilidade pelos consumidores.

CONCLUSÕES

As características físicas, químicas e microbiológicas dos hambúrgueres de carne bovina com baixo teor de sódio foram mantidas. Do ponto de vista sensorial, a substituição de até 25% de sódio é adequada para todos os consumidores, porém, a redução de 50% de sódio foi melhor aceita pelos consumidores hipertensos. A adição de especiarias ao produto (alho, colorau, orégano, pimenta calabresa) melhora atributos de sabor, aroma e textura dos hambúrgueres hipossódicos, mascarando o sabor de gordura. A redução de sódio em 25% e 50%, não afeta a manutenção da qualidade dos

hambúrgueres durante o período de armazenamento, bem como as suas características físicas e microbiológicas. As embalagens ativas com 1% de óleo essencial de orégano foram eficazes em controlar a oxidação lipídica dos hambúrgueres bovinos durante a sua vida útil, melhorando sua qualidade sensorial.

Palavras chaves: Cloreto de sódio. Cloreto de potássio. Controle de qualidade. Hambúrguer bovino. Embalagens ativas.

ABSTRACT

INTRODUCTION

In recent years, consumer demand for safer food and quality of meat, as well as meat products with reduced levels of fat, sodium chloride, cholesterol and nitrite increased. Processed meat products have significant contribution in levels of sodium consumed by the population, however, the use of substitutes, such as potassium chloride is limited, mainly due to its bitter taste. The partial replacement of sodium chloride by potassium chloride with the addition of herbs and spices to beef burger, it is a promising solution for decreasing sodium and maintenance palatability of meat products. The maintenance of the beef burger shelf life with sodium reduction still depends on other technological variables such as keep acceptable levels of lipid oxidation and microbial counts. The use of active packaging is currently of the most dynamic technologies used to preserve meat quality. Through the release of active agents, such as essential oils of oregano, contributing to the preservation of shelf life of low-sodium meat products, without changing its technological and sensory characteristics, eliminating the use of synthetic additives.

AIMS

The aim of the study was developed hamburgers with reduced sodium content by 25% (F25) and 50% (F50) and analyzed regarding their physical, chemical, microbiological, sensorial characteristics and market research.

Description of the sensory profile of low-sodium burgers using the methodology of Quantitative Descriptive Analysis (QDA).

The second aim was evaluate the efficacy of active packaging (with addition of essential oils), while preserving quality of beef burger with 25% and 50% of reduction of sodium chloride, stored for 120 days under freezing.

MATERIAL AND METHODS

Hamburgers were prepared in order to replace the effects of sodium chloride (NaCl) by potassium chloride (KCl), also aromatic herbs and species were added(*Allium sativum, Oreganum Vulgare, Bixa orellana, Capsicum frutescens)*. Chemical analysis were performed in triplicate, following methodology proposed by AOAC. Sensorial tests were performed on both type of consumers (healthy and hypertensive) using an hedonic scale, principal component analysis and Quantitative Descriptive Analysis (QDA) with 12 selected and trained tasters using as criteria the discriminative power, reproducibility and individual consensus among themselves. In addition, a market research with 250 consumers was done. Four treatments were evaluated in order to know efficiency of biodegradable and active packaging (produced by extrusion) applied to beef burgers with low sodium content stored at -18 ° C for 120 days through various techniques. Treatments studied were: Beef burgers with 25% (B25) and 50% (B50) of sodium reduction in biodegradable packaging and beef burger with 25% (BOEO25) and 50% (BOEO50) of sodium reduction biodegradable packaging with 1% oregano oil. It was analyzed lipid oxidation (TBARS), microbiological, pH at 1, 30, 60, 90 and 120 days of

storage, chemical composition analysis (moisture, ash, protein, lipid) at time 1 and 120 days, activity analysis water (Aw), cooking loss, texture, and color (L , a *, b *) at 1, 60 and 120 days and sensory analysis at 30 and 120 days.

RESULTS AND DISCUSSION

On article 1 it was observed that the highest rates on texture were reported for hamburgers belowing to 50% sodium reduction treatment. Although microbiological aspects were adequate and acceptability of low-sodium hamburgers remained over 70%. Hypertensive consumers had great overall acceptability (92%) and only slightly detected sodium decrease. The Quantitative Descriptive Analysis (QDA) of the article 2 topic has showed that low-sodium burgers had lower taste of fat and salt when compared to the control and taste and odor of added spice, this difference shows that the reduction of sodium in the burger causes increased intensity perceived by the panelists compared the presence of spices in the product. On article 3, market research results revealed great consumers´ interest in acquiring products with reduced quantities of sodium. The composition of packaging during the time of storage on low-sodium beef burgers did not influence attributes as: moisture, crude protein, total fat, ash, cooking loss and water activity. Active packaging which contained also oregano oil showed higher color protection on burgers during the storage period. Texture showed stable on all days of storage, being stabilizate on the other periods until the end of storage (120 days) burgers from 25% sodium reduction and packaged with 1% oregano essential oil (BOEO25) had better taste and aroma, getting a acceptability index above 80% for both periods (30 and 120 days). The incorporation of 1% of oregano essential oil into the packaging reduced lipid oxidation of burgers analyzed untills 14% when it is compared with packaging without oregano adition. Microbiological analyzes were maintained adequate and stable during the complete storage time. The use of active packaging with essential oil of oregano for the storage of low-sodium burgers was proved to be viable alternative, allowing production and commercialization, maintaining their quality and contributing to its acceptability by consumers.

CONCLUSION

The microbiological, chemical and physical characteristics of low-sodium beef hamburgers were kept. From the sensorial point of view, the replacement of up to 25% is adequate for both types of consumers and 50% of sodium by potassium in beef hamburgers were better accepted for hypertensive than healthy consumers. The addition of spices (*Allium sativum, Oreganum Vulgare, Bixa orellana, Capsicum frutescens)* to the product attributes improved flavor, aroma and texture of low-sodium burgers, masking the taste of fat. The reduction of sodium in 25% and 50% did not affect the quality of the burgers during the storage time, as well as, their physical and microbiological characteristics. Active packaging with 1% of oregano essential oil, prove its feasibility to control lipid oxidation in beef burgers during its shelf life, improving the sensory quality.

Key Words: Sodium chloride. Potassium chloride. Quality control. Beef burger. Active packaging.

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Quality and sensorial evaluation of beef hamburger made with herbs, spices and reduced sodium content

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Abstract

BACKGROUND: Strategies aiming to reducing dietary sodium are being implemented based on studies which supporting that excessive consumption of sodium has been associated with negative health effects such as elevated blood pressure (hypertension). Meat and meat products significantly contribute to the intake of sodium since it is frequently used in meat processing (it affects the flavour, texture and shelf life of meat products). The objective of this study was to characterize hamburgers with reduced sodium (NaCl) content by 25% (F25) and 50% (F50). Microbiology, physico-chemical composition and sensory analysis (non-hypertensive and hypertensive consumers) were performed in hamburgers. In addition, a market survey with 250 consumers was conducted.

RESULTS: The acceptability of hyposodium hamburgers remained over 70%, hypertensive consumers had great overall acceptability and only non-hypertensive consumers detected sodium reduction (F50). The effects of reduced sodium (NaCl) content on the firmness of hamburger were evaluated and F50 presented the highest value. Microbiological aspects were adequate. Market survey showed consumers' interest in acquiring products with sodium reduction.

CONCLUSION: Replacement of sodium by potassium until 50% did not produce notable changes on microbiological, chemical or instrumental characteristics of beef burgers. The production and commercialization of reduced sodium content (up to 50%) hamburgers could be feasible.

Keywords: Sodium chloride; potassium chloride; meat products; hypertension.

INTRODUCTION

In many industrialized countries sodium ingestion exceeds nutrition recommendations. Excessive sodium intake is associated with hypertension and the occurrence of cardiovascular diseases. Sodium chloride, its main sodium source, is associated with increase in blood pressure when consumed above 6g/day/person[1].

Epidemiological studies have shown low rates of arterial hypertension cases in populations who intake less than 3g of salt/day and high rates in populations that consume more than 20g of sal/day^{-1}[2].

Research in European countries[3] shown evidences that meat and meat products contribute to 20% of sodium intake in their diet. Thus, the development of meat products with low salt rates is important not only to hypertensive part of the population. Sodium chloride play an important role in the product´s conservation and in sensorial characteristics, such as taste intensity, decrease when salt is reduced[1].

Several ingredients may be employed as salt substitutes in meat products. These include potassium chloride that presents the same characteristics of salt and is acknowledged as a safe ingredient. In fact, it may be replaced without loss of product’s functionality. However, due to its bitter taste, potassium chlorate in meat products is restricted[4].

Partial replacement of sodium chloride by potassium chloride with the addition of aromatic herbs and spices in hamburgers may be an alternative to maintaining the product’s tastiness[5, 6].

Industries are exploring new alternatives to develop products with reduced sodium content similar to the standards (related to texture and flavor), according consumers habits. The addition of aromatic herbs and spices may also contribute to the acceptability of products with sodium reduction. The objective of this study was investigate the effects of beef hamburgers made with reduced sodium content (substituting by potassium chloride at 25 and 50 %), herbs and spices on microbiological, physicochemical, texture and sensorial analyses.

MATERIALS AND METHODS

Samples

Beef hamburgers (beef + textured soy protein + spices and herbs + cold water) were produced in the meat laboratory of the Food Engineering. Hamburger meat was acquired from an abattoir in Maringá, and it was from animals finished in confinement at Iguatemi Experimental Farm of the State University of Maringá. Selected meat came from the rib section of the muscle *multifidus dorsi*, whereas herbs, spices and textured soy protein were acquired on the local market. Were used 6 kg of meat for production in total the 75 hamburgers and separate in 25 hamburgers of each treatment in a single production.

Formulations and processing

Hamburgers were prepared replacing sodium chloride (NaCl) by potassium chloride (KCl), aromatic herbs and species. Three seasonings were prepared: CON (100% sodium chloride); F25 (reduction of 25% in sodium chloride) and F50 (reduction of

50% in sodium chloride) as described in Table I. Beef hamburger formulation was made according to Carvalho, Madrona, Corradini, Reche, Pozza and Prado [7].

Hamburgers were made according to the Figure 1. During processing meat was weighed (80g for each hamburger), and molded by a hand cutter (1 cm thickness). Textured soy protein was hydrated with boiling water. After cooling, water excess was removed and soy was incorporated into the process. Beef hamburgers were packed in polyethylene bags and frozen at -18º C for fifteen days until rear analysis.

Chemical composition

Chemical analysis were performed in triplicate, moisture and ashes[8], total fat[9], crude protein[8] and carbohydrates by difference. Sodium and potassium analyses were prepared on a dry basis, at 550°C and diluted with nitric acid following methodology by AOAC [8].Samples were quantified by atomic absorption spectrophotometer AA240FS (Varian, USA), in g per kg of the products mineral, employing standard solutions and analytic curves.

Microbiological analyses

Three samples from hamburger at zero time were used to evaluate the microbiological quality. Fecal coliforms at 35 ºC and coliforms at 45 ºC, coagulase-positive staphylococcus, sulfate-reducing clostridium at 46 ºC and *Salmonella* sp. were evaluated , quantified and detected according to methodology by Silva, Junqueira and Silveira [10].

Losses by cooking

Samples were thawed at 4ºC ± 1ºC and thermally processed by conventional cooking. Samples were previously weight. Hamburgers were grilled on an electric grill Multi-Britania 127V for approximately 5 minutes up to an internal temperature 70ºC verified by a digital thermometer Incoterm (-50 and 300ºC), 145mm long with a 4mm diameter. Samples were cooled up to 25ºC and weighed. Losses by cooking were calculated by $\%\,\mathrm{CL} = (\mathrm{Thawed\ weight} - \mathrm{cooked\ weight})/(\mathrm{Thawed\ weight}))\mathrm{x}\ 100$.

Determination of texture and color

Beef hamburgers mechanical characteristics were determined by Warner – Bratzler shear force, analyzer with Stable Micro Sistemas Taxt Plus (Texture Technologies Corp., UK) and 5.00 kg charge cell according to Research Center for Meat of USDA[14].

Each sample weighing approximately 80g was wrapped in aluminum paper and grilled in an electric grill Multi Grill 2 Britânia 127V, up to 70ºC, measured by Incoterm thermometer, tube 145mm long by 4mm diameter. Six samples with 1 cm^2 were taken.

Color was determined in four points at the surface of burgers after 30 min of exposure without packaging (ambient temperature). The L^* value (brightness), a*(red/ green) and b*(yellow/blue) rates were determined by portable digital color meter Minolta ® CR10, with integration sphere and 3º angle of vision, or rather, illumination D3 and illuminating D65, following CIE [15] system.

Sensorial analysis and market research

This study was approved by the Ethics and Research Committee of the State University of Maringá, by Protocol 21879413.9.0000.0104. Participants signed a consent form on their participating in the sensorial analysis.

Two groups participated in the sensory analysis. The first group was composed by one hundred non-hypertensive consumers, 60% women and 40% men, of whom 75% were habitual hamburger consumers, ages: < 30 years= 50%; 31 – 44 years= 30%; 45 – 59 years= 17%; over 60 years= 3%. The second group was composed by hypertensive consumers, 50% women and 50% men of whom 60% were usual hamburger consumers, with < 30 years = 0; 31 – 44 years= 26%; 45 – 59 years= 16%; over 60 years = 58%. Most of the population studied in this group over 60 years is justified by hypertensive disease have a higher prevalence among the elderly.

Sensorial analysis was performed in two stages with possible consumers. The first group was comprised of 100 consumers and the second of the 30 hypertensive in order to compare the acceptability by different tasters. Tasters evaluated the acceptability of grilled 2x2cm hamburgers served immediately and randomly after being prepared at 50ºC[11]. Hamburgers were evaluated for taste, smell, texture and overall acceptance by a hedonic 9-point scale (9=I liked it very much; 1=I did not like it absolutely)[12]. Buying intention (3-point scale), age, gender, consuming habit were added to the questionnaire. Samples acceptance index was calculated by the following expression[13]:

$$IA\% = \frac{x * 100}{n}$$

Where: x = mean of each sample; N = highest score of each sample given by tasters.

Market survey with 250 participating were performed, using Google docs (online) tools as a questionnaires with alternative questions to participants in southern

Brazil of random. The interviewed were asked about their family history hypertension, consumption of light hamburger and frequency, type of intake (fried, grilled, cooked), preparation, seasoning and price paid for the product.

Costs of formulations

Cost of hamburgers (in US $) were calculated by each specific ingredient, as provided by manufacturers.

Statistical analyses

Data were assessed by analysis of variance (ANOVA), using Statistics 7.0[16]. Means and standard deviations of data were calculated. Significant differences between means were analyzed by Bonferroni at 5% significance level. Principal Component Analysis was performed used XLSTAT 7.5.3 in order to identify relationships between variables. The correlations between attributes were evaluated using Pearson correlation coefficient.

RESULTS

Instrumental and microbiological meat quality of beef burger

As it is shown in Table 2 the replacement of sodium by potassium did not change the chemical composition, being not significant the differences between control and hyposodium beef burgers, those products presented an average of 72.72% of moisture, 2.44% of ashes, 22.23% of crude protein and 2.25% of total fat levels.

Related to the color and cooking losses, the replacement of sodium by potassium did not have a significant effect (Table 3). L* values ranged between 28.06 - 29.56, redness (a*) between 8.93 - 11.13 and yellowness (b*) 10.06 - 11.90 and the average of cooking loss percentage was 29.55%, however when tenderness was evaluated (shearing force) differences between treatments ($P \leq 0.05$) are shown. Hamburgers with 50% of sodium reduction presented higher values than control and reduction of 25%.

Results of microbiological analyses showed coliforms rates at 45ºC and 35ºC <3 (NMP/g), coagulase-positive staphylococcus < $1x10^2$ (UFC/g), absence of *Salmonella* sp. in 25g, sulfate-reducing clostridium counts < 10 (UFC/g), according with Brazilian legislate (RDC nº12 2001).

Sensorial perception and costs of formulations

As it is shown in table 4, in non-hypertensive consumers group there were statistical differences ($P \leq 0.05$) in the acceptability of all studied attributes between the control hamburger and F50 group, except for smell,. Acceptability of hamburgers T25 did not present statistical differences compared to control group in any attribute evaluated, being also at same statistical level than F50 group for texture and overall acceptance. There were not statistical differences in any attribute evaluated by hypertensive consumers between the different hamburgers formulation. Scores given by non-hypertensive consumers were lower (respectively 6.77 and 6.22 for F25 and F50) than those of hypertensive consumers (respectively 8.30 and 8.06) when general acceptability and taste of the hamburgers were evaluated. The data shown in Table 4 indicate a perception of potassium in taste by not-hypertensive consumers (F50). In this case, the intention to buy the product decreased and differs for the control and F25.

Principal Components Analysis (PCA) (Figure 2) showed that sensorial answers of non-hypertensive and hypertensive consumers were different according to Table 4. The three different treatments evaluated by hypertensive consumers were on the right side of PC1, and closer to the acceptability of all evaluated attributes; however the three treatments evaluated by non-hypertensive were located in the left side of PC1, far from the acceptability attributes, especially when sodium reduction was at 50%. According to Pearson correlation coefficient´s obtained overall acceptability of beef burgers was more related to taste and texture attributes (r = 0.976 and 0.962 respectively) than smell (r = 0.956).

Formulations costs were very similar. Control treatment was about U$ 4.66 per kg; U$ 4.68 for F25; U$ 4.73 for F50. Prices of hamburger (80g) were U$ 0.3728, U$ 0.3744 and U$ 0.3784 respectively for Control, F25 and F50.

Market survey

Participants' profile included 68% women and 32% men; 31% between 18 and 20 years old, 62% people between 21 and 30 years old; 4% between 31 and 40 years old; 3% over 40 years old. Further, 96% alleged being non-hypertensive and 4% hypertensive. When asked about the presence of hypertension in their families, 65% answered positively and 35% negatively. The results of the market survey showed high interest of consumers in buying beef burgers with less sodium content. When it was questioned their intention of consuming beef burgers with less sodium, 95% of consumers replied positively and only 5% said they wouldn´t like the product. Frequency in hamburger intake demonstrated that 59% of consumers rarely eat hamburgers; 26% once a week; 11% twice a week and 4% did not eat it. The reasons for eating burger with less sodium were: 43% about healthiness; 25% taste; 24% quality; 5% price; 4% indication by another person. Besides, 47% of consumers would pay between U$ 0.44 and 0.87/hamburger; 34% less than U$ 0.44; 18% between U$ 0.88 and 1.32; 1% over U$ 1.32. Hamburger is more consumed with bread, totaling 90%; 10% like as meat. 48% of consumers liked it fried; 39% grilled and 13% cooked. Moreover, 60% of consumers preferred to eat it with traditional seasoning, used in current experiment; 23% with barbecue; 9% with pepper; 8% with fine herbs.

DISCUSSION

It is known that salt plays an important role in maintaining the characteristics of fresh and processed meats[17], and a modification in the content or composition could affect physical, instrumental and sensorial characteristics of the product, which could affect the consumer acceptability and purchase intention. In this study, the replacement of sodium by potassium at 25 and 50% did not change chemical composition of hamburgers prepared with beef meat. Results show (Table 2) that hamburgers were chemically health-safe when sodium decrease and fat intake were taken into consideration. According to World Health Organization[18], sodium intake should not exceed 2 g/day and a 50% sodium chloride reduction in hamburgers boils down to an approximate 3 g of sodium per 1 kg product.

Color is one of the main attributes that consumers evaluate before buy meat products. Color of hamburgers were different from beef meat *in natura* due to seasoning with urucum (*Bixa orellana)* which provided a redder color to the product, with a reduction of luminosity to 28.80. Being normal rates for beef *in natura* above 35[19]; however as it was demonstrated in other processed meat products as sausages' sometimes differences detected by instrumental techniques (CIE lab scale) are not detected by consumers by visual inspection[17]. Salt plays an important role in relation to the texture of meat processed meat because as it has been previously described by Desmond [3], it activates proteins, increase binding properties of proteins and affects to water-holder capacity. However the replacement of 25% and 50% of sodium chloride by potassium chloride did not affect cooking losses which remained within normal limits for beef burger with less than 10% fat. According to He and MacGregor [17]the substitution did not affect the cooking losses probably due to both treatments formulations had a similar ionic strength to control group. The cooking losses contributed towards the quality of the developed product which presented lower rates, compared to the ones found by Scheeder, Casutt, Roulin, Escher, Dufey and Kreuzer [20]for grilled hamburgers which varied between 30 and 33% in treatments with different fat levels and source.

On the other hand, shearing force was higher for treatment with 50% sodium reduction. Although a less tender meat, the values founded (19.79 N) were normal for this type of product without fat addition and 50% sodium reduction. Scheeder, Casutt, Roulin, Escher, Dufey and Kreuzer [20] demonstrated similar values (19.40 N) in control hamburgers. An alternative to reduce this difference in texture may be the inclusion of fat or phosphate to the formulation. Ruusunen and Puolanne [1] remarked that beef meat hamburgers may be prepared with low sodium and higher yields when phosphate was included.

Bacteriostatic characteristic of salt has been previously reported[21]; however the replacement did not affect microbiological results. All groups showed adequate results on the microbiological analyses developed. Bidlas and Lambert [22] reported that KCl may be a direct replacement for common salt related to antimicrobial control.

Studies have shown a predominant hypertension rate in Brazil around 20%, regardless of gender, with an increasing trend proportional to advanced age[23]. The results showed that hypertensive group did not found differences in acceptability for any evaluated attribute, with scores were given higher than non-hypertensive group. Probably this fact is associated to the habit of consumption of each group, usually a person with hypertension change your habits decreasing the consumption frequency of some foods and reducing salt intake, so it is possibly that hypertensive group who was habituated with low salt content did not notice the differences between control and reduce sodium treatments. However, people without medical limitations of salt consumption probably perceived the differences in taste, principally when sodium is replaced at 50%, being the product less attractive which influence in the purchase intention and index acceptability. A study with tomato juice prepared with different amounts of sodium, Bobowski, Rendahl and Vickers [24] showed that repeated exposure to a food with lower sodium content gradually reduced, may increase the acceptability of the food even in the absence of a diet low in sodium. Carraro, Machado, Espindola, Campagnol and Pollonio [25] showed that 50% KCl in bologna sausage reduced sensorial quality along with a significant reduction in purchase intent, a rejection of 28% for the product when sodium was reduced by 50% and a rejection of 18% when the product was provided with spices and herbs. In this study, formulations with herbs and spices also contributed for better results in sensorial evaluation. For smell consumers did not report any difference between treatments. This factor is intrinsically linked to the seasoning in the product, the same for all treatments (Table 1). Studies by Dijksterhuis, Boucon and Le Berre [26] report the hypothesis that small variations in taste may not be significant if the product has appearance, smell and texture similar.

In fact, Brazilian consumers have the habit of consuming foods with high sodium levels around 8g kg^{-1}, hamburgers with 50% sodium reduction provide only 3g kg^{-1}. Acceptability higher than 70% may be considered a high value by the consumers for this type of product. However, for hypertensive consumers sodium decrease was less perceptible, with no significant differences between the parameters evaluated (Table 4). Chung, Lennie, De Jong, Wu, Riegel and Moser [27] compared adhesion to medicine and dietetic treatment for arterial hypertension and concluded that hypertensive people accepted efficaciously the use of drugs but failed to reduce sodium intake. Products with sodium reduction that present positive acceptance by hypertensive consumers may contribute to a lower sodium intake and consequently a reduction in blood pressure. According to results obtained from the market survey, meat hamburgers with less sodium obtained high index of positive answers in the consumption intention, which was positive by 95% of interviewee. Products with sodium reduction attended a significant percentage of consumers who require products to an improvement or maintenance of blood pressure rates. In fact, they reduced sodium daily intake can reduce the risk of high blood pressure and cardiovascular disease. [18].

Costs of formulations only vary slightly between treatments. Impact on the final hyposodium product was low and proportional on the expectations of consumers looking for a healthy diet. Besides, the price was lower than consumers were willing to pay (market survey). It should be remembered that the highest price for one hamburger (50% sodium chloride reduction) in this study was approximately US $0.38, and thus, within consumers' expectations, 47% of consumers were willing to pay between US $0.44 and 0.87 per hamburger.

The Brazilian policy has been planned to make a gradual decrease in the sodium content of foods. New technologies and formulations were developed in order to attend this new industries requirement which were adapted to consumers' tastes. Food such as hamburgers in fast food and snacks, frequently consumed by vulnerable health groups, such as teenagers and children, are being targeted for not prejudicial their health [28]. There is a need to reduce sodium in processed products to improve public health in the countries.

CONCLUSIONS

Replacement of sodium by potassium until 50% did not produce notable changes on microbiological, chemical or instrumental characteristics of beef burgers, just presenting a higher value for texture. Sensorially, replacing until 50% of sodium by potassium in beef hamburgers with herbs and spices, is feasible, mainly for hypertensive consumers.

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Table 1. Medium values of the **c**omposition of beef burger

Ingredients (%)	Beef burger		
	CON	F25	F50
Beef meat	88.67	88.67	88.67
Textured soybean protein	4.00	4.00	4.00
Cold water	5.00	5.00	5.00
NaCl	2.00	1.50	1.00
KCl		0.50	1.00
Allium sativum	0.20	0.20	0.20
Oreganum vulgare	0.02	0.02	0.02
Bixa orellana	0.10	0.10	0.10
Capsicum frutescens	0.01	0.01	0.01

Table 2. Medium values of the chemical analysis of beef burger

Item	Beef burger		
	CON[1]	F25[2]	F50[3]
Moisture (%)	72.43^{a} ± 0.24	72.78^{a} ± 0.69	72.96^{a} ± 0.32
Ashes (%)	2.54^{a} ± 0.14	2.35^{a} ± 0.17	2.35^{a} ± 0.02
Crude protein (%)	22.17^{a} ± 0.15	22.41^{a} ± 0.02	22.13^{a} ± 0.02
Total fat (%)	2.28^{a} ± 0.07	2.22^{a} ± 0.02	2.25^{a} ± 0.03
Sodium (g kg^{-1})	6.00^{c} ± 0.10	4.45^{b} ± 0.46	3.00^{a} ±0. 55
Potassium (g kg^{-1})	1.96^{c} ± 0.11	2.44^{b} ± 0.29	3.00^{a} ± 0.59

[1]CON (20 g kg^{-1} NaCl + 2 g kg^{-1} *Alliumsativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [2]F25 (15 g kg^{-1} NaCl + 5 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum*+ 0.2 g kg^{-1} *Oreganumvulgare* + 1 g kg^{-1} *Bixaorellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [3]F50 (10 g kg^{-1} NaCl + 10 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*). Means followed by different letters on the same line for each treatment are different (P<0.05)

Table 3. Medium values of the Color (L*, a* e b*), cooking loss (LC) and texture (N) of beef burgers

Item	Beef burger		
	CON[1]	F25[2]	F50[3]
L*	28.06[a] ± 1.02	29.56[a] ± 3.38	28.80[a] ± 1.34
a*	8.93[a] ± 1.10	11.13[a] ± 1.58	9.63[a] ± 0.81
b*	10.06[a] ± 1.10	11.90[a] ± 1.12	10.33[a] ± 1.30
LC (%)	28.06[a] ± 0.19	28.67[a] ± 2.29	31.92[a] ± 2.65
Texture (N)	16.50[a] ± 0.15	17.32[a] ± 0.71	19.79[b] ± 0.47

[1]CON (20 g kg^{-1} NaCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicumf rutescens*); [2]F25 (15 g kg^{-1} NaCl + 5 g kg^{-1}KCl + 2 g kg^{-1} *Alliumsativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [3]F50 (10 g kg^{-1} NaCl + 10 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*). Means followed by different letters on the same line for each treatment are different (P<0.05).

Table 4. Medium values of the sensory analysis by consumers for beef burger with sodium reduction

	Beef burger		
	CON[1]	F25[2]	F50[3]
Non-hypertensive consumer (n 100)			
Smell	$7.46^{a} \pm 1.57$	$7.00^{a} \pm 1.70$	$6.96^{a} \pm 1.68$
Taste	$7.55^{a} \pm 1.67$	$7.03^{a} \pm 1.69$	$6.22^{b} \pm 2.14$
Texture	$7.43^{a} \pm 1.66$	$7.28^{ab} \pm 1.47$	$6.85^{b} \pm 1.87$
Overall acceptance	$7.86^{a} \pm 1.37$	$7.29^{ab} \pm 1.46$	$6.77^{b} \pm 2.03$
Purchase intention	$2.51^{a} \pm 0.67$	$2.33^{a} \pm 0.72$	$2.01^{b} \pm 1.12$
I.A.[4]	87.33%	81.00%	75.22%
Hypertensive consumer (n 30)			
Smell	$8.10^{a} \pm 1.37$	$8.07^{a} \pm 1.31$	$8.06^{a} \pm 1.17$
Taste	$8.06^{a} \pm 1.22$	$8.33^{a} \pm 0.92$	$8.06^{a} \pm 1.20$
Texture	$8.16^{a} \pm 1.34$	$8.20^{a} \pm 1.06$	$8.30^{a} \pm 1.11$
Overall acceptance	$8.26^{a} \pm 1.04$	$8.36^{a} \pm 0.92$	$8.30^{a} \pm 1.02$
Purchase intention	$2.53^{a} \pm 0.68$	$2.76^{a} \pm 0.50$	$2.66^{a} \pm 0.60$
I.A.[4]	91.77%	92.88%	92.22%

[1]CON (20 g kg^{-1} NaCl + 2 g kg^{-1} *Allium sativum*+ 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [2]F25 (15 g kg^{-1}NaCl + 5 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [3]F50 (10 g kg^{-1}NaCl + 10 g kg^{-1}KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicumf rutescens*). Means followed by different letters on the same line for each treatment are different (P<0.05). [4]I.A. = index of product´s acceptability.

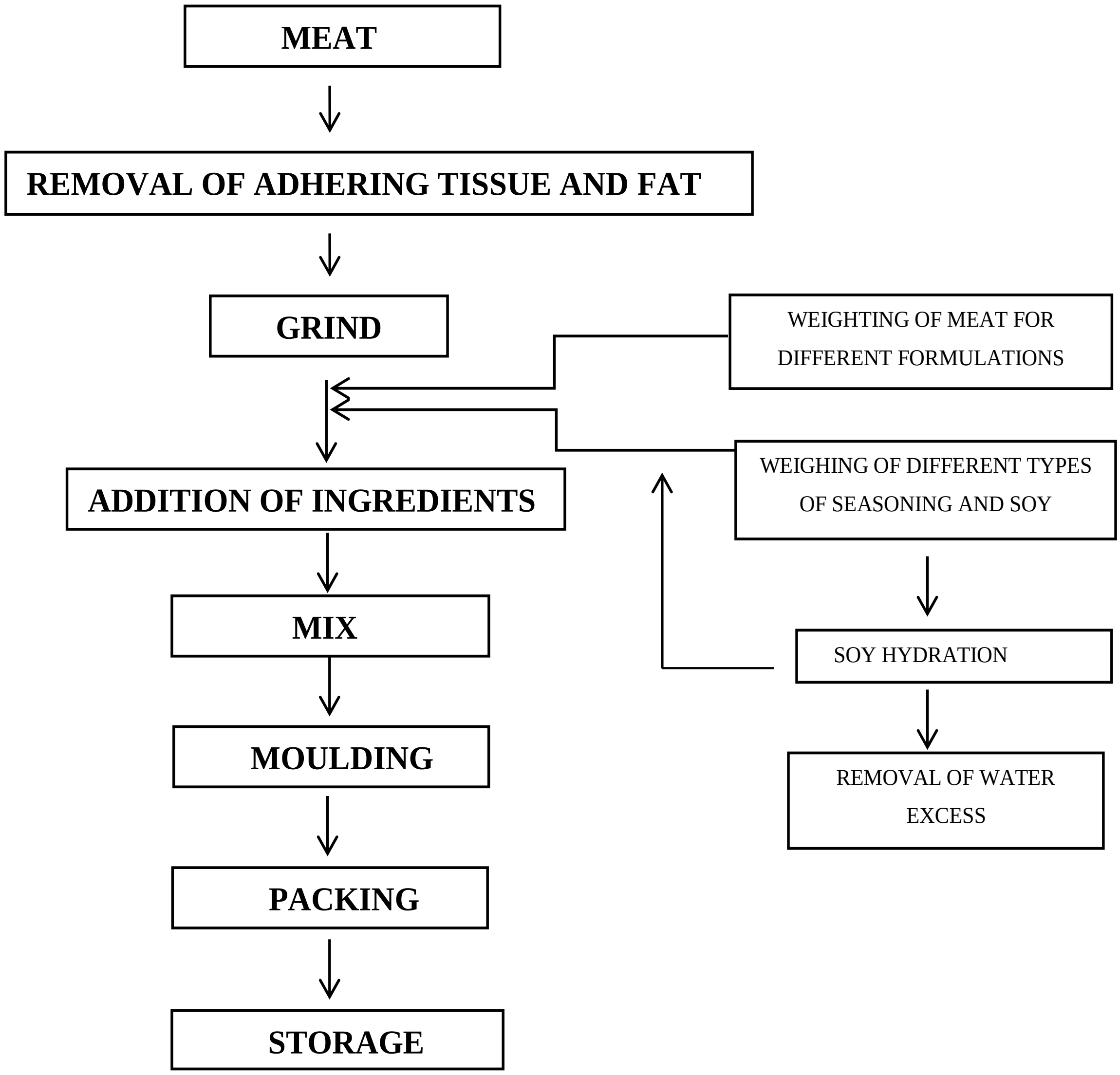

Figure 1. Flow chart in the preparation of beef burger.

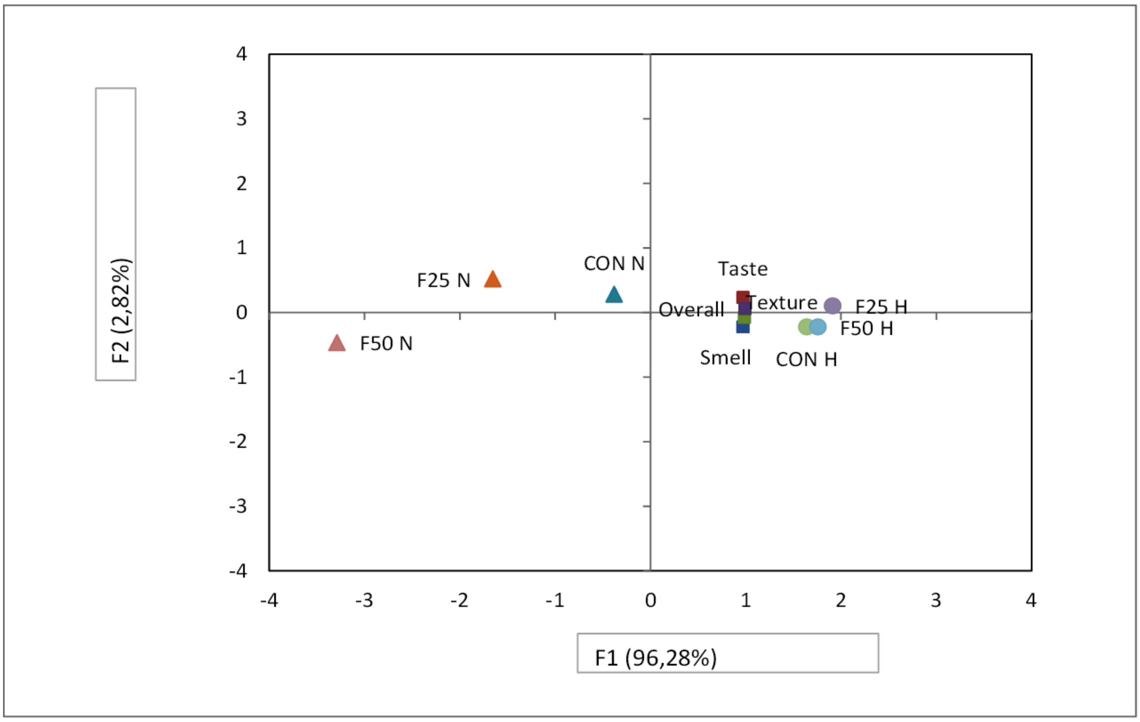

Figure 2. Principal Components Analysis of sensorial evaluations of hyposodium beef burger by non-hypertensive (N) and hypertensive (H) consumers. CON: Control, F25: 25% sodium reduction, F50: 50% sodium reduction.

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Sensory profile of beef burger with reduced sodium content

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ABSTRACT. This study determined the sensory profile of three beef burger samples, namely, CON (control), F25 (25% sodium reduction) and F50 (50% sodium reduction), based on the Quantitative Descriptive Analysis (QDA). The samples´ microbial, physical and chemical composition was evaluated. Twelve panelists were selected and trained using as criteria the panelists´ discrimination power, reproducibility and consensus. Eleven terms were generated by the method of network descriptors. The intensity of each descriptor in each sample was evaluated by unstructured scale of 9 cm. Data were analyzed by ANOVA, Duncan´s mean test and principal component analysis. The sensory profile shows that low sodium beef burgers had lower fat and salty flavor when compared to untreated control and greater flavor and spice aroma. The above proves that reducing sodium intake causes increased perception burger tasters when compared to the presence of spices in the product. Treatment with 50% sodium reduction obtained the best results for texture softness and appearance. There was no significant difference ($p < 0.05$) in the chemical composition of ash, protein and fat in all burgers. In the case of general sensory attributes, treatments with sodium reduction obtained higher intensities of the attributes evaluated, except for meat and salt flavors.

Keywords: meat products, sensory analysis, quantitative descriptive analysis, principal component analysis, sodium chloride, potassium chloride.

Perfil sensorial de hambúrguer bovino com reduzido teor de sódio

RESUMO. Este trabalho determinou o perfil sensorial de três amostras de hambúrguer, controle e com redução de 25 e 50% de sódio, baseado na Análise Descritiva Quantitativa (ADQ). Realizaram-se análises físico-química e microbiológica. Doze provadores foram selecionados e treinados utilizando como critérios o poder discriminativo, reprodutividade e consenso dos provadores entre si. Foram gerados 11 termos descritores pelo método de rede. A intensidade de cada descritor foi avaliada em cada amostra por escala não estruturada de 9 cm. Os dados foram analisados por ANOVA, teste de Duncan e análise de componentes principais. Hambúrgueres hipossódicos apresentaram menor sabor de gordura e salgado, quando comparados ao tratamento controle e sabor e odor de especiarias maior. Este diferencial comprova que a redução de sódio no hambúrguer provoca uma maior percepção de intensidade pelos provadores em relação à presença de especiarias no produto. Para aparência o tratamento com 50% de redução de sódio obteve o melhor resultado e também para textura no atributo maciez. Na composição química dos hambúrgueres não houve diferença significativa para cinzas, proteína e gordura ($p < 0.05$). Nos atributos sensoriais de modo geral, os tratamentos com redução de sódio obtiveram maiores intensidades nos atributos avaliados, exceto para sabor salgado e carne.

Palavras-chave: produtos cárneos, análise sensorial, análise descritiva quantitativa, análise de componentes principais, cloreto de sódio, cloreto de potássio.

Introduction

Significant portion of the sodium in diets comes from processed foods, of which the most important are derived from meat, such as beef burgers. According to Brazilian legislation, it is the industrialized meat product obtained from minced meat, added or not to adipose tissue and ingredients, and molded to the appropriate technological processes (BRASIL, 2000). In addition to palatability, sodium chloride in the product is responsible for the functional development of the properties and decisively influence their stability and conservation (DESMOND, 2006)

The sodium intake by Brazilian reaches approximately 4.5 grams daily, twice the amount recommended by the World Health Organization (IBGE, 2010). The daily consumption of 50 g of meat products, such as sausages and others, may be associated with increased risk in cardiovascular diseases (42%) and diabetes (19% in the general population) according to the American Heart Association (MICHA et al., 2010).

Consumers´ concern underpinning health effects, associated to excessive sodium consumption, requires that the food industry reduces the use of salt in foods, including those derived from meat while keeping their sensory characteristics. The acceptability of products with reduced sodium by the consumer is also demanded. Sodium reduction in meat products may be achieved by the replacement of NaCl by other non-sodium salts, among which potassium chloride is the most widely used (GARCIA et al., 2013).

One of the problems in decreasing sodium percentage in food is the maintenance of the characteristics of the traditional product, i.e. similarly to the product manufactured with NaCl. Sensory analysis of the products developed is very important to guarantee to the consumer a product with reduced sodium and with sensory characteristics close to those of the traditional product.

Consequently, the sensory profile may be developed by the quantitative descriptive analysis (QDA) method that evaluates all the sensory attributes in the food product, such as appearance, flavor, aroma and texture. Their formulation is adjusted in a specific manner in relation to the analyzed attribute (STONE et al., 1974).

Current assay describes the sensory profile of beef burgers produced with full-sodium ingredients, herbs and spices replacing sodium chloride by potassium chloride at 25 and 50% percentages, using the QDA methodology.

Material and methods

The research was approved by the Committee of Ethics in Research of the State University of Maringá, Maringá, Paraná State, Brazil, under protocol CAAE 21879413.9.0000.0104. Participants signed a consent form agreeing to participate voluntarily in the sensory analysis.

The beef burgers were produced with raw beef, textured soy protein, spices and herbs and cold water in the Meat Technology Laboratory of Food Engineering, State University of Maringá. The meat from slaughtered males, comprising 22 g protein (29% DV) and 5 g total fat (9% DV) per 100 g of meat, was purchased from the same lot from Marfrig Alimentos SA, a Brazilian slaughterhouse industry in Promissão, São Paulo State, Brazil.

The meat cut consisted of the sirloin cap (m.Multifididorsi). The herbs, spices and other ingredients were bought locally in Maringá, Paraná State, Brazil.

The beef burgers were prepared to determine the influence of the substitution of sodium chloride (NaCl) by potassium chloride (KCl), associated with herbs and spices. Three ingredients were formulated: CON (100% NaCl); F25 (25% reduction in the concentration of NaCl); and F50 (50% reduction in the concentration of NaCl) (CARVALHO et al., 2013) as described in Table 1.

Table 1. Composition of the beef burgers.

Ingredients	(%)		
	CON	F25	F50
Meat	88.67	88.67	88.67
TSP[1]	4.00	4.00	4.00
Water	5.00	5.00	5.00
NaCl	2.00	1.50	1.00
KCl	-	0.50	1.00
Allium sativum	0.20	0.20	0.20
Oreganum vulgare	0.02	0.02	0.02
Bixa orellana	0.10	0.10	0.10
Capsicum frutescens	0.01	0.01	0.01

[1]TSP (textured soy protein).

The beef were ground with an electric meat grinder MCR 10 (NR12) - G. Paniz, hand mixed with other ingredients according to GMP (Good Manufacturing Practices) and molded with a manual molder cylinder (10 cm diameter) weighed into 80±0.5 g and thickness 1 cm. After processing, the burgers were identified, packed in polyethylene bags and kept frozen at -18°C for later analysis.

Three samples of each batch of beef burgers were used to assess the microbiological quality of treatments immediately after manufacturing in triplicate. According to legislation Fecal Coliform at 45°C, *staphylococcus* coagulase positive, sulfite reducing *clostridium* at 46°C and Salmonella sp. were evaluated following methodology described by (SILVA et al., 1997).

Ash, crude protein, sodium and potassium content were determined according to (AOAC, 2012) method. Fat content was quantified as described by (BLIGH; DYER, 1959). The samples were quantified in AA240FS atomic absorption spectrophotometer (Varian, USA) in mg per 100 g of product for sodium and potassium. Analyses were performed in triplicate.

For the qualitative descriptive analysis (QDA), selection of panelists was based on their interest and availability to participate in current research. Panelists who showed interest had to identify the odor of basic tastes and ten triangular tests were applied to see whether panelists were able to notice simple differences between samples. The panelist who obtained more than 75% correct scores on the triangular test initiated the development of descriptive terminology for network method described by (MOSKOWITZ, 1983).

The list of terms that comprised the evaluation form samples after panelist consensus was defined. Panelists´ selection and training were performed with the products to be evaluated and with reference materials, according to Table 2. A table of references with all the attributes, based on the terms set, was elaborated, so that the panelists based the extremes of the scales 1-9 for the analysis of three samples. Sensory tests were applied in individual booths using 9 cm unstructured scale generated for each attribute.

Individual results for each panelist were analyzed statistically by the analysis of variance (ANOVA), taking the samples as sources of variation and replications. Those who showed discriminatory ability ($P_{sample} \leq 0.05$), reproducibility ($P_{replications} \geq 0.05$) and consensus with sensory panelists for most of the attributes evaluated were selected for the descriptive analysis. Data Quantitative Descriptive Analyses were analyzed by analysis of variance (ANOVA) for three variation sources (sample, panelists and the interaction between them) as well as by Duncan´s mean test. Principal Component Analysis (PCA) was applied with XLSTAT statistical software (SAS, 2004).

Results and discussion

In all samples, coliform counts at 45°C were less than 3 MPN g^{-1}, *Staphylococcus* spp. coagulate positive was less than 102 CFU g^{-1}, *Clostridium* sulfite reducer less than 10 CFU g^{-1} and *Salmonella* spp. was absent in 25 g. These results comply with Brazilian legislation (BRASIL, 2001). NaCl has antimicrobial activity although some studies report that KCl may be a direct replacement for common salt with regard to the control of microbial growth (BIDLAS; LAMBERT, 2008).

The replacement of sodium chloride with potassium chloride did not influence significantly the content of crude protein, total lipids and ash ($p > 0.05$) according to Table 3. Moisture content was different between samples ($p < 0.05$). The replacement of sodium by potassium may have generated a higher fluid loss in treatments F25 and F50, probably due to the fact that sodium increases the water-binding of meat (RUUSUNEN; PUOLANNE, 2005).

Table 3. Proximate analysis, sodium and potassium content.

	Beef burger			
	CON[1]	F25[2]	F50[3]	p < value
Moisture (%)	$67.16^a \pm 0.05$	$66.35^b \pm 0.21$	$66.70^b \pm 0.05$	0.017
Ash (%)	$2.56^a \pm 0.27$	$2.66^a \pm 0.11$	$2.54^a \pm 0.04$	0.779
Crude protein (%)	$22.27^a \pm 0.21$	$22.63^a \pm 0.46$	$22.26^a \pm 0.25$	0.517
Total lipids (%)	$9.58^a \pm 0.16$	$9.43^a \pm 0.18$	$9.31^a \pm 0.28$	0.510
Sodium (mg 100 g^{-1})	$600.14^c \pm 10.89$	$445.41^b \pm 4.68$	$300.51^a \pm 5.56$	0.010
Potassium (mg 100 g^{-1})	$196.33^c \pm 1.08$	$244.14^b \pm 2.95$	$300.07^a \pm 5.99$	0.020

[1]CON (20 g kg^{-1} NaCl + 2 g kg^{-1} *Alliumsativum* + 0.2 g kg^{-1} *Oreganumvulgare* + 1 g kg^{-1} *Bixaorellana* + 0.2 g kg^{-1} *Capsicumfrutescens*); [2]F25 (15 g kg^{-1} NaCl + 5 g kg^{-1} KCl + 2 g kg^{-1} *Alliumsativum* + 0.2 g kg^{-1} *Oreganumvulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicumfrutescens*); [3]F50 (10 g kg^{-1} NaCl + 10 g kg^{-1} KCl + 2 g kg^{-1} *Alliumsativum* + 0.2 g kg^{-1} *Oreganumvulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicumfrutescens*).

Results from qualitative descriptive analysis (QDA) show a significant difference ($p \leq 0.001$) for all attributes (Table 4), with slightly lower differences related to aroma meat ($p \leq 0.01$). In the case of products with reduced sodium, mainly related to aroma and flavor attributes, the treatments with sodium reduction (F25 and F50) had mean aroma and spices taste attributes ranging between 1.58 and 1.78 times higher than control treatment.

Table 2. Terms generated, defining descriptors and references used for the low-sodium beef burger in Quantitative Descriptive Analysis (QDA).

Descriptors	Defining	Intensity	References
Appearance			
Color	Brown color intensity in meat	Low strong	Beef burger grilled to 72°C wrapped in aluminum foil or not
Brightness	Brightness intensity on the surface of meat	Low strong	Beef burger grilled with and without oil
Aroma			
Meat	Aroma intensity associated with roast beef	Low strong	Beef burger grilled and boiled in water
Spices	Aroma intensity associated with spices	Low strong	Commercial beef burger with 1-3% spices
Fat	Aroma intensity associated with fat	Low strong	Commercial beef burger against low sodium beef burger
Flavor			
Saltiness	Saltiness intensity associated to sodium chloride	Low strong	Commercial beef burger vs low sodium beef burger
Fat	Fat intensity associated to fat in meat	Low strong	Commercial beef burger vs control low sodium beef burger
Meat	Flavor intensity associated to beef burger grilled	Low strong	Beef burger grilled and boiled in water
Spices	Flavor intensity associated to spices	Low strong	Commercial beef burger with 1-3% spices
Texture			
Tenderness	Force required for compression	Low strong	Beef burger grilled at different temperatures (70 and 80°C)
Juiciness	Given the presence of moisture in the meat juices	Low strong	Beef burger grilled at different temperatures (70 and 80°C)

The saltiness flavor and fat flavor excelled in control treatment (CON). When the sodium content is reduced, the herbs´ and spices´ flavor and aroma added to treatments are enhanced and become noticeable to the panelist. They mask the flavor of meat, fat and flavor of fat, which inversely stand out in the control treatment. In a study by (MARANGONI; MOURA, 2011) with Italian salami, the addition of essential coriander oil to the formulation improved the sensory attributes of taste and aroma.

In the case of appearance and texture attributes, the treatment F50 (with 50% sodium reduction) averaged a higher intensity when compared to the control sample (CON) and F25, indicating that sodium reduction in this percentage (50%) did not negatively affectthese attributes. A study conducted by (CLAUDINO; BERTOLONI, 2013) with beef burgersplus different percentages of fat and plasma showed a significant reduction ($p < 0.05$) in hardness when compared to formulations containing 10 and 5% of fat content. Percentage is similar to formulations employed in current study.

Table 4. Qualitative Descriptive Analysis (QDA) of beef burger.

Attributes	Beef burger			
	CON[1]	F25[2]	F50[3]	p value
Appearance color	6.26^{c} ± 0.53	7.15^{b} ± 0.29	7.75^{a} ± 0.53	0.000
Appearance brightness	5.92^{b} ± 0.33	5.17^{c} ± 0.20	7.51^{a} ± 0.33	0.000
Aroma meat	7.12^{b} ± 0.45	7.63^{a} ± 0.33	7.46^{a} ± 0.45	0.005
Aroma spices	4.70^{b} ± 0.89	7.44^{a} ± 0.27	7.58^{a} ± 0.89	0.000
Aroma fat	6.37^{a} ± 0.17	5.35^{b} ± 0.29	4.80^{c} ± 0.17	0.000
Flavor saltiness	7.51^{a} ± 0.30	6.33^{b} ± 0.32	5.95^{c} ± 0.30	0.000
Flavor fat	7.25^{a} ± 0.30	5.55^{b} ± 0.33	4.80^{c} ± 0.30	0.000
Flavor meat	7.60^{a} ± 0.27	7.10^{b} ± 0.32	7.29^{b} ± 0.27	0.000
Flavor spices	4.65^{c} ± 0.33	7.38^{b} ± 0.27	8.28^{a} ± 0.33	0.000
Texture tenderness	7.27^{b}± 0.28	7.40^{b} ± 0.27	7.86 ^{a}± 0.28	0.000
Texture juiciness	7.17^{b} ± 0.32	7.84^{a} ± 0.37	7.57^{a} ± 0.32	0.000

[1]CON (20 g kg^{-1} NaCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [2]F25 (15 g kg^{-1} NaCl + 5 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*); [3]F50 (10 g kg^{-1} NaCl + 10 g kg^{-1} KCl + 2 g kg^{-1} *Allium sativum* + 0.2 g kg^{-1} *Oreganum vulgare* + 1 g kg^{-1} *Bixa orellana* + 0.2 g kg^{-1} *Capsicum frutescens*).

The principal component analysis (PCA) showed that the three treatments differed from each other and in the different quadrants. The attributes meat flavor, salt and fat are similar to the control treatment, while the spices´ flavor attribute is close to the treatments with 25 and 50% sodium reduction (F25 and F50); texture tenderness, appearance color and brightness are close to the F50 treatment, while aroma spices, aroma meat and texture juiciness are found in the same quadrant as treatment F25, inversely related to the attributes of flavor meat and saltiness (Figure 1).

The highest numbers of desirable attributes (aroma and flavor spices) are close to the beef burgers with low-sodium treatments. Natural ingredients, including herbs and spices have been studied as potential co-adjuvants in the sodium reduction of meat products, masking the bitter aftertaste left by potassium chloride (CARRARO et al., 2012).

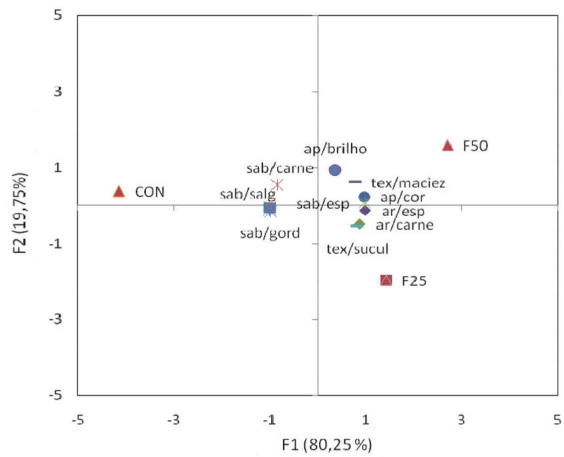

Figure 1. Principal component analysis from the sensory evaluation of low-sodium beef burgers with trained panel.

Conclusion

NaCl reduction and its partial substitution by KCl did not change the chemical composition of beef burgers (except moisture) and did not interfere with microbiological results. The added spices in this product improved sensory attributes of flavor, aroma and texture of beef burgers and masked the flavor fat that was detected with higher intensity in the control treatment.

The reduction of sodium content in percentages of 25 and 50% obtained good intensity of desirable attributes. With the addition of spices and herbs, it could be applied in meat products when its quantitative sensory aspects are observed.

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Effect of active packaging with oregano oil on beef burgers with low sodium content

Active packaging on low sodium beef burgers

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Abstract

This study was performed to evaluate active biodegradable packaging applied to low-sodium beef burgers for reducing lipid oxidation and preserving its quality . Four treatments were tested: beef burgers with a 25% (B25) and 50% (B50) sodium reduction and biodegradable packaging and burgers packed in a biodegradable packaging with 1% of oregano essential oil and a 25% (BOEO25) and 50% (BOEO50) reduction in sodium. The composition of the packaging did not influence the moisture, crude protein, total fat, ash, cooking loss or water activity. The active oregano oil packaging showed greater color protection in burgers during the storage period. The texture not showed variation, remaining stable until the end of storage. The burger samples with a 25% reduction in sodium and packaging with 1% of oregano essential oil (BOEO25) had a better taste and aroma, obtaining an acceptability index above 80% for both periods (30 and 120 days). The use of 1% of oregano essential oil incorporated into the packaging reduced the lipid oxidation of burgers analyzed by 14% when compared with packaging without adding oregano. Microbiological analyses remained stable throughout the storage. Thus, active packaging with oregano essential oil can maintain the quality of beef burgers without interfering with their physical and chemical characteristics and improve their sensory attributes during 120 days of storage.

Keywords: Low sodium; oregano oil; meat products; lipid oxidation, consumer test.

Introduction

Salt is an important ingredient in food. In meat products, salt has an important role, and affects shelf life and palatability (Mattes 1997). Furthermore, salt and fat jointly contribute to the sensory traits in meat products (Desmond 2006; Ruusunen and Puolanne 2005).However, over the past several decades, some studies have reported that a high level of sodium consumption may be linked to a risein blood pressure that affects the risk of mortality from cardiovascular disease (Armenteros et al. 2009; He and MacGregor 2007), and is also linked to stomach cancer and kidney disease (He and MacGregor 2007; He and MacGregor 2010). Thus, the reduction of sodium in meat products is necessary for the food industry around the world (Asaria et al. 2007; Dötsch et al. 2009). With the objective ofreducing chronic degenerative diseases caused by an excessive salt intake, in most of the developed countries the intake of sodium chloride by adults varies from 8 to 13 g per day, much higher than the 5g recommended by the World Health Organization (WHO 2003). However, to achieve a significant reduction of sodium in meat products it is necessary to solve technological barriers, especially those related to conservation and shelf life.

Potassium chloride is the most common substitute for salt in food (He and MacGregor 2010); however, its complete replacement is not possible, and substitution is limited to 50%, because above this level the bitter taste is intensified along with low salinity. A strategy to reduce these factors is the use of taste enhancers and masking agents such as herbs, spices and artificial flavors (Slobodan and Vesna 2011; Carvalho et al. 2013).

Beef burgers with low sodium content are more prone to lipid oxidation, which, together with microbial growth, is a major cause of deterioration of meat products (Bidlas and Lambert 2008). A new alternative for an efficient preservation of these products is active antioxidant packaging, whose main advantage is the release of antioxidants during storage, blocking the lipid oxidation process (Gómez-Estaca et al. 2014).

Oregano essential oil has been suggested for use in meat and packaging due to its effective antimicrobial potential, which can be attributed to the presence of phenolic compounds in its composition such as thymol and carvacrol (Emiroğlu et al. 2010).

Active packaging is currently one of the most dynamic technologies used to preserve food quality, throughthe release of active agents, maintaining or increasing the quality and shelf life of beef, without the direct addition of other substances (Pereira et al. 2014; Cestari et al. 2015).

The use of biodegradable and active packaging with essential oils could contribute to maintaining the shelf life of low-sodium meat products without altering its their technological and sensory characteristics, decreasing the use of synthetic additives.

This study was conducted to evaluate the efficacy of active packaging (oregano essential oil) for reducing lipid oxidation and preserving the quality of beef burgers with a 25 and 50% sodium chloride reduction storage in a freezer for 120 days.

Materials and methods

Beef burger processing and storage

Beef burger samples (beef + soy bean textured protein + spices and herbs + cold water) were produced in the meat laboratory atthe Food Engineering Section atthe State University of Maringá, Paraná, Brazil. The meat used for the preparation was purchased from the same lot of company Marfrig Alimentos SA, a Brazilian slaughterhouse industry in Promissão-SP. The meat was selected from the 12^{th}rib section of the m*ultifidus dorsi* muscle. The herbs, spices and soybean textured protein were acquired from the local market.

The beef burgers were prepared so that the effects of replacing sodium chloride (NaCl) with potassium chloride (KCl) and the addition of aromatic herbs and spices could be evaluated. Two seasonings were prepared: a 25% and a 50% decrease in the amount of sodium chloride, following the methodology described by Carvalho et al. (2013). The compositions of both seasonings are shown in Table1. During processing, the beef burgers were weighed into 80 ±0.5 g portions, with a thickness of 1 cm, and molded by a hand cutter. Textured soybean protein was hydrated with boiling water. After cooling, water excess was removed to be incorporated into the process.

The beef burgers were packed in active biodegradable bags measuring 10 x 10cm content ecoflex 40%, glycerol 13%, cassava starch 47% and biodegradable active with or without 1% oregano oil. The packages were sealed.

Extrusion employing hight pressures and temperature was used to develop packaging techniques, films were produced in the Laboratory of Food Science and Technology Department at the State University of Londrina using Starch (Indemil, Brazil) and glycerol (Synth P.A) to obtain thermoplastic starch (TPS), poly (butylene adipate- coterephthalate) (PBAT- BASF, Germany) with name trade Ecoflex S BX 7025 as described by Cestari et al. (2015) and oregano oil (Ferquima, Brazil).

The treatments consisted of: 1) beef burgers witha 25% (B25) sodium reduction and biodegradable packaging; 2) beef burgers with a 50% (B50) sodium reduction and biodegradable packaging; 3) beef burgers witha 25% (BO25) sodium reduction and biodegradable packaging with 1% oregano essential oil added films; 4)beef burgers with a 50% sodium reduction and biodegradable packaging with 1% oregano essential oil added films. All treatments were frozen (-18ºC) and stored for 120 days.Synthetic preservatives were not used during the production process and storage period.

Chemical composition

The beef burger samples were thawed at 4 ± 1°C, minced, homogenized and analyzed in triplicate on days 1 and 120. The beef burger moisture and ash content were determined according to ISO-R-1442 (1997) and ISO-R-936 (1998). The crude protein content was obtained through ISO-R-937 (1978). The total fat content was quantified as described by Bligh and Dyer (1959).

Cooking loss and water activity (aw)

The samples were thawed at 4±1ºC and thermally processed by conventional dry cooking so that losses due to cooking could be determined. The samples were weighed one by one on an electronic analytic scale. The beef burgers were grilled on an electric 127V Multi Britânia grill for approximately 5 minutes up to an internal temperature of 70ºC, verified by a digital Incoterm thermometer. The samples were cooled to 25ºC and weighed again. Cooking losses were calculated as follows:

$$\% \, CL = \left(\frac{Thawed\ weight - cooked\ weight}{Thawed\ weight}\right) x\ \mathbf{100}$$

The determination of the water activity of the beef burger sat 5°C was performed in triplicate using an Agua Lab Model Cx2T device at an operating temperature of 25.0 ± 0.3°C.

Texture and color

The meat's mechanical characteristics were determined by a Stable Micro Systems TA.XT Plus (Texture Technologies Corp., UK) texture analyzer and 25.0 kg charge cell. Analysis followed the methodology of the Research Center for Meat of the USDA (Honikel 1998). The analyses were performed at 1, 60 and 120 days of storage.Each sample was wrapped in aluminum paper and grilled on an electric grill (Multi Grill 2 Britânia 127V), up to 70ºC, measured by an Incoterm thermometer. Six 1 cm^2 (transversal square section) samples, were taken per treatment.

Color was determined by a portable Minolta® CR-10colorimeter, with an integration sphere and 3º angle of vision, or rather illumination D3 and illuminating D65, according to the CIE (1986) system. L* (brightness), a*(red) and b*(yellow) were determined in triplicate on the burger surface at 5°C after 1, 60 and 120 days of storage.

Thiobarbituric acid-reactive substance analysis (TBARS) and pH

Lipid oxidation was measured by TBARS formation according to the method of Pfalzgraf et al. (1995). The meat samples (10g) were mixed with 20 ml 10% (w/v) trichloroacetic acid, centrifuged at 4000 rpm for 20 min at 4°C, and the supernatants were filtered through filter paper. In total, 2 mL 20 mM 2- TBA was added to 2 mL of filtrate. The mixture was homogenized, placed in a boiling water bath for 20 min, and subsequently cooled. Absorbance was measured at 532 nm in a spectrophotometer (Evolution 201, UV–Visible, Thermo Scientific) and the sample concentrations were calculated using a calibration curve. TBARS values were also calculated using a calibration curve. TBARS values were expressed as mg malonaldeyde MDA/kg of meat. The analyses in triplicate were performed at 1, 30, 60,90 and 120 days of storage.

The pH measurements were performed in triplicate on all samples after thawing the beef burger meat using a portable CRISSON 503 pHmeter equipped with a penetrating electrode probe for 5 minutes (Young et al. 2004). The pH measurements were performed on days 1, 30, 60, 90 and 120.

Microbiological analyses

Four samples from each treatment were used to evaluate the treatments' microbiological quality at 1, 30, 60, 90 and 120 days of storage.

Counts of coliforms at 35°C and 45°C, coagulase-positive *Staphylococci* and *Sulfite*-reducing clostridia were evaluated according to the methodology described by Downes and Ito (2001). Coliforms were determined by the Most Probable Number (MPN) technique using a series of three tubes of Lauryl Sulfate Tryptose broth (Difco) that were incubated at 35°C for 48 h. The tubes that presented gas production were transferred to Green Bile Lactose broth (Difco) and incubated at 35°C for 48 h, and to EC broth (Difco) and incubated at 45°C for 48 h. The results are expressed as MPN/g.

Coagulase-positive *Staphylococci* counts were performed using the spread-plating technique in Baird Parker agar (Difco), and the plates were incubated at 35–37°C for 48 h. Suspect colonies were submitted to a coagulase test. The results are expressed as log CFU/g.

Sulfite-reducing clostridia were enumerated by pour-plating technique in Tryptose Sulfite Cycloserine agar (Merck), and the plates were incubated anaerobically at 46°C for 24 h. Presumptive colonies were identified by biochemical tests. The results are expressed as log CFU/g.

Salmonella spp. was determined according to Downes and Ito (2001). Briefly, 25g of each sample was homogenized with 225 mL of lactose broth and incubated at 35°C for 18 to 24h, followed by selective enrichment in Selenite Cystine (Difco) and Rappaport Vassiliadis(Difco) broth. Both cultures were plated on Hektoen Enteric agar (Difco) and incubated at 35°C for 18 to 24h. Presumptive colonies were identified by biochemical and serological tests.

Consumer test

The current investigation was approved by the Ethics and Research Committee of the State University of Maringá (Protocol 21879413.9.0000.0104). Participants signed a consent form indicating their agreement to participate in the consumer analysis.

A consumer test was developed under standardized conditions in the sensory laboratory of the Food Engineering Department. Sensorial analysis was performed on two different days. In the first consumer test, beef burgers were evaluated after 30 days of storage. Eighty consumers (40 men and 40 women, from 18 to 60 years old) were involved. In the secondconsumer test, beef burgers were evaluated after 120 days of storage, with 80 consumers(45 men and 35 women, from 18 to 60 years old) participating, of whom 90% were regular consumers of beef burgers.

The beef burgers were cooked on a double-plate grill at 200ºC until reaching an internal temperature of 70ºC, monitored by a thermometer. The beef burgers were divided into 2 x 2 cm portions, wrapped individually in aluminum foil and labeled with a unique three-digit code. The sample beef burgers were served immediately following a randomized design in order to avoid carry over (Macfie et al. 1989). Each consumer scored four samples, one for each treatment, evaluating the acceptability of the burgers in terms of taste, smell, texture and overall acceptance usinga hedonic nine-point scale (9=I liked it very much; 1=I did not like it at all) (Dutcosky 2011). Afterwards, a sample acceptance index was calculated usingthe following expression described by Dick et al. (2011):

$$IA\% = \frac{x * 100}{n}$$

where: x = mean of each sample; n = highest score of each sample given by tasters.

Statistical analyses

Data are represented as means ± standard error of mean. Analyses were performed using the statistical package SPSS (2005) (v.15.0) for Windows. An analysis of variance using a GLM procedure in which treatment and days of storage were considered fixed effects was applied. Differences between group means were assessed using the Tukey test (P ≤ 0.05%).

Results and discussion

Chemical composition

As shown in Table 2, the time of storage (0 or 120 days) did not change the chemical composition insidethe groups, and there were no differences (*P>0.05*) between treatments. The use of biodegradable packaging or biodegradable active packaging with essential oils on beef burgers with a reduction insodium did not affect the chemical composition, with those products presenting an average of 61% of moisture, 2.5% of ash, 19% of crude protein and 9% of total fat levels at the start and end of storage. A review article on meat products with reduced sodium by Oliveira et al. (2013) reported that the partial replacement of NaCl by KCl (25%) did not interfere with the water retention capacity, salty taste or stability of the sausage emulsion.

Cooking loss and water activity

By relating to cooking loss and water activity between treatments and storage times, did not have an effect on those variables as is shown in Table 3; keeping up within normal values of 0.96–0.98 for water activity and 24–28% for cooking loss.

Texture and color

With regard to texture, treatments not showed significant differences ,with the storage time (120 days) the texture values bec similar (*P>0.05*), showing no difference between the treatments and time of storage.Values for texture observed were between 19 and 21 N, considered normal for this type of product and similar to rates (19.40 N) described by Scheeder et al. (2001).

There were statistical differences between treatmentson luminosity (L*) when comparing the four treatments in their final storage time (120 days).The color of the meat can change during freezing, ranging from pinkish to a darker tone. Burgers with active packaging (BO25 and BO50 treatments) were clearer than treatments without essential oils; this difference must be due to greater protection conferred by active packaging to the product, in terms of browning and/or oxidation. L* values were comprised between 40 and 46, redness of meat (a*) between 11 and 16 and yellowness (b*) 11 and 16. In a study performed by La Storia et al. (2012), active packaging also showed increased protection in the color change of the meat surface during storage. Normal rates for beef *in natura* are above 35 (Page et al. 2001). The luminosity of over 40 given to the burger is due to the textured soy protein and 9% fat in its composition conferred to the same lighter coloration different from rates *in natura* beef meat.

Color is one of the main attributes that consumers evaluate before buying meat products and its protection is of fundamental importance for the acceptability of the product by the consumer. Active packaging (BOEO25 and BOEO50) kept the same color of the product when comparing the initial and final time, which is important factor in the acceptability of the product by the consumer.

Lipid oxidation activity (TBARS) and pH

With respect to lipid oxidation as is shown in Table 4, all treatments showed adequate control of the oxidative process during the product storage time (120 days) under freezing conditions, with the values obtained being lower than 0.44 mg MDA/kg of meat on all analyzed products. Values below those were found in similar studies with frozen burger with 30% sodium reduction conducted by (Baker et al. 2013), where TBA levels were equal to or above 1 mg MDA/kg of meat, for treatments with rosemary and ginger extract in their composition with 120 days of storage at –18ºC. Mohamed and Mansour (2012) studied chicken burger frozen for 3 months and indicated the potential use of natural herbs and essential oils to protect the burger against lipid oxidation. The TBA values found may also have been influenced by the seasoning composition containing oregano leaves (*Oreganum vulgare*),which evidenced antioxidant potential (Carvacrol and Timol) as seen in a study performed by Boroski et al. (2011) and by the matte packaging, preventing the incidence of light to the product.

Treatments with active biodegradable packaging with essential oils added (BO25 and BO50) had the highest antioxidant capacity, differing statistically from the only biodegradable packaging B25 and B50 treatments for oxidation during storage. Treatment with active packaging with essential oil of oregano (BOEO25) achieved a better performance in the lipid oxidation process, protecting the product and stabilizing its oxidation from 60 days of storage to the end of storage time (Figure 1).

A study with restructured chicken steaks packaged with active film containing 1% oregano showed it to be effective as an antioxidant during 150 days of storage in a freezer (Cestari et al. 2015), confirming the antioxidant potential of active packaging with 1% essential oil of oregano.

Values pH during the storage period showed a slight tendency to increase for all treatments, starting with more acid pH (from 5.47 to 5.52) and ending the storage period with more basic pH (from 5.76 to 5.80), but this variation was within pH values considered normal (from 5.5 to 5.8) according to Savell et al. (2005). In a study performed by Emiroğlu et al. (2010) with ground beef, the results were similar to those of our study,with pH varying from 5.43 to 6.09 during a storage period of 90 days.

Microbiological analyses

Beef burgers with low sodium due to its susceptibility to microbial contamination, were monitored monthly.

Most Probable Numbers (MPN) of coliforms at 35°C ranged from 15 to 1100 MPN/g among treatments and days of storage.The counts of coliforms at 45°C were< 3 MPN/g on all treatments and all days of storage, while coagulase positive Staphylococci and Sulfite-reducing clostridia, the CFU counts were < 10^1 CFU/g and < 10 CFU/g, respectively. *Salmonella* spp. was absent in 25g,were not detected in beef burger in any of the treatments during the period of storage .

The study performed by Emiroğlu et al. (2010) with ground beef patties and active packaging, showed inhibition zone diameters yielded by soy protein-based edible film disks against all test organisms (*Staphylococcus aureos* (27.50mm),*Escherichia coli* (32 mm), *Pseudomonas*(35.50 mm), *Lactobacillus* (22.50 mm)with even minimum concentrations of oregano oil (1%) applied into the film formulation.

The replacement of sodium chloride by potassium chloride did not affect the microbial counts between treatments. Where salt is used to help preserve the product and antimicrobial action, partial or complete replacement by KCl is possible (Bidlas and Lambert 2008).

Sensorial perception

As shown in Table 5, two groups of consumers indicated their acceptability scores in the different times of storage of the beef burgers. On 30 days' storage, treatments with 25% sodium reduction had a better acceptability compared to treatments with 50% sodium reduction, especially for the taste attribute; in all attributes BOEO25 treatment obtained a better result, with 80.88% acceptability on A.I (Acceptability Index). In work performed by Mohamed and Mansour (2012), the addition of rosemary and marjoram essential oils improved the sensory scores of beef patties during the frozen storage period.

On 120 days storage all treatments improved their acceptability indices with the exception of the B25 treatment,despite being evaluated by different consumers. Treatments with active biodegradable packaging with essential oil of oregano (BOEO25 and BOEO50) showed the best results, with an acceptability index equal to or above 80%, and obtained the highest scores for taste and odor probably due to the exposure time of the product in the packaging which are pleasing to the consumer. The use of herbs such as oregano in the composition of meat derivatives, let reduce the addition of salt meat products, such as burgers and others, maintaining consumer acceptability, as long as the quantity of added herbs does exceed the limit of the consumers' tolerance (Wang et al. 2014). According to Table 6, with 120 days of storage ,the index of acceptability of the BOEO25 treatment compared to the B25 treatment was 11.88% higher, proving the positive influence of essential oregano oil.

The use of biodegradable polymers from renewable sources such as cassava starch is a promising alternative to commercial applications, due to its low cost and availability. Cassava starch combined with a biodegradable synthetic polymer retains its mechanical and barrier properties suitable for use in meat products, as by the results obtained in this study, the combined use of biodegradable packaging with essential oils can further facilitate their use due to increased microbiological control and lipid oxidation while simultaneously improving the acceptability of the product.

Conclusions

Biodegradable packaging with 1% of oregano essential oil showed the best potential among treatments tested for the reduction and stabilization of lipid oxidation in beef burgers during 120 days of frozen storage, being effective it he characteristics of the quality of the products such as color or and sensorial proprieties. The reduction of sodium by 25% and 50% did not affect the maintenance of the quality of the burgers during the storage period, nor their physical and microbiological characteristics. Active packaging with 1% of oregano essential oil proves, through this study, its feasibility to control lipid oxidation in burgers during their shelf life, improving their sensory quality.The best result was obtained with the BOEO25 treatment, with 25% sodium reduction in active packaging containing essential oregano oil.

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Table 1. Composition of the beef burgers with 25% or 50% reduction of sodium

Ingredients (%)	F25	F50
Meat	88.67	88.67
TSP[1]	4.00	4.00
Water	5.00	5.00
NaCl	1.50	1.00
KCl	0.50	1.00
Allium sativum	0.20	0.20
Oreganumvulgare	0.02	0.02
Bixaorellana	0.10	0.10
Capsicum frutescens	0.01	0.01

[1]TSP (textured soy protein).

Table 2. Medium values of the chemical analysis of beef burger packaged with active films

Item	Beef burger				
	Storage Day	B25	B50	BOEO 25	BOEO 50
Moisture (%)	1	61.22 ± 0.45	61.15 ± 0.44	60.85 ± 0.04	60.85 ± 0.38
	120	61.49± 0.28	61.70± 0.42	60.71± 0.44	60.90± 0.72
Ashes (%)	1	2.40 ± 0.04	2.52 ± 0.07	2.48 ± 0.04	2.52 ± 0.03
	120	2.36 ± 0.01	2.55± 0.07	2.47 ± 0.14	2.51 ± 0.05
Crude protein (%)	1	19.73 ± 0.04	19.74 ± 0.03	19.72 ± 0.01	19.72 ± 0.02
	120	19.71 ± 0.01	19.72 ± 0.02	19.73 ± 0.04	19.71 ± 0.02
Total fat (%)	1	9.13 ± 0.00	9.15 ± 0.01	9.16 ± 0.03	9.14 ± 0.02
	120	9.15 ± 0.01	9.14 ± 0.01	9.15 ± 0.04	9.13 ± 0.01

B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BOEO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BOEO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

Table 3. Medium values of the colour, texture, AW and cooking loss analysis of beef burger packaged with active films

Item	Beef burger				
	Storage Day	**B25**	**B50**	**BOEO 25**	**BOEO 50**
AW	1	0.97 ± 0.01	0.97± 0.01	0.96 ± 0.01	0.97 ± 0.01
	60	0.97± 0.01	0.97± 0.01	0.97 ± 0.01	0.98 ± 0.01
	120	0.97± 0.01	0.97± 0.01	0.97 ± 0.01	0.98 ± 0.01
PC (%)	1	27.66 ± 3.01	27.72± 4.28	25.48 ± 1.07	26.53 ± 0.85
	60	24.84± 0.96	23.95± 1.65	24.17 ± 1.35	25.58 ± 1.88
	120	28.55± 4.39	27.85± 2.30	25.83 ± 3.15	25.32 ± 1.03
Texture (N)	1	19.90 ± 0.39	19.51± 0.91	19.33 ± 1.23	19.66 ± 1.16
	60	19.41± 1.39	19.49± 0.78	19.81 ± 0.21	19.35 ± 2.39
	120	20.40± 1.06	20.08± 0.80	19.34 ± 1.41	19.94 ± 0.70
L*	1	44.75 ± 1.99	42.80± 1.97	47.52 ± 1.88^{A}	44.39 ± 2.66^{AB}
	60	41.46± 1.10	40.75± 2.24	42.27 ± 0.21^{B}	41.17 ± 0.90^{B}
	120	41.40± 0.78^{b}	42.65± 0.34^{b}	45.00 ± 1.21^{aAB}	46.22 ± 0.28^{aA}
a*	1	15.44 ± 0.80^{aA}	13.08± 0.87^{bAB}	12.85 ± 0.69^{bAB}	14.37 ± 1.12^{ab}
	60	11.87± 0.79^{abB}	11.05± 0.89^{bB}	10.66 ± 0.70^{bB}	13.30 ± 0.19^{a}
	120	16.50± 0.39^{A}	13.86± 1.15^{A}	13.25 ± 1.47^{A}	15.01 ± 2.00
b*	1	14.01 ± 0.79^{A}	13.26± 0.08^{B}	13.64 ± 1.52	15.46 ± 2.20^{AB}
	60	11.58± 0.35^{B}	10.03± 0.35^{C}	11.65 ± 1.42	11.67 ± 0.47^{B}
	120	15.80± 1.05^{A}	14.89± 0.47^{A}	16.14 ± 2.71	16.48 ± 2.21^{A}

a,b,c: Means in the line with different letters represent significant differences ($p<0.05$, Tukey's test) between samples.
A,B Means in the columns with different letters represent significant differences ($p<0.05$, Tukey's test) between days of storage.
B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BOEO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BOEO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

Table 4. Medium values of TBARS and pH analysis of beef burger packaged with active films

Item	Storage Day	B25	B50	BOEO 25	BOEO 50
		Beef burger			
TBARS (mg malonaldeyde/ Kg)	1	0.323 ± 0.02 ^{abC}	0.363 ± 0.04$^{a\ C}$	0.269 ± 0.01^{cB}	0.289 ± 0.01^{bcC}
	30	0.329± 0.01^{abC}	0.367± 0.04 ^{aC}	0.270± 0.02^{cB}	0.293± 0.01^{bcC}
	60	0.379 ± 0.02 $^{ab\ B}$	0.399± 0.02 $^{a\ BC}$	0.344± 0.02^{bA}	0.340 ± 0.02^{bB}
	90	0.392± 0.02 $^{ab\ AB}$	0.422± 0.02^{aAB}	0.358± 0.02^{bA}	0.372± 0.02^{bAB}
	120	0.429± 0.02 $^{ab\ A}$	0.443± 0.02^{aA}	0.369± 0.02^{cA}	0.392± 0.02^{bcA}
pH	1	5.47 ± 0.02 ^{B}	5.51 ± 0.01 ^{CD}	5.43 ± 0.02^{C}	5.52 ± 0.03^{C}
	30	5.66 ± 0.03^{aA}	5.42 ± 0.03 $^{b\ D}$	5.51 ± 0.01 $^{b\ C}$	5.41 ± 0.02 $^{b\ C}$
	60	5.64 ± 0.06 ^{A}	5.61 ± 0.01 ^{BC}	5.63 ± 0.04^{B}	5.67 ± 0.00^{B}
	90	5.63 ± 0.04 ^{A}	5.63 ± 0.03 ^{B}	5.64 ± 0.01^{B}	5.66 ± 0.05^{B}
	120	5.76 ± 0.07 ^{bA}	5.82± 0.07 ^{abA}	5.77 ± 0.07 $^{b\ A}$	5.86 ± 0.00 $^{a\ A}$

a,b,c: Means in the line with different letters represent significant differences ($p<0.05$, Tukey's test) between samples.

A,B Means in the columns with different letters represent significant differences ($p<0.05$, Tukey's test) between days of storage.

B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BOEO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BOEO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

Table 5. Medium values of the sensory analysis by consumers for beef burger with sodium reduction

	Beef burger			
Attribute	B25	B50	BOEO 25	BOEO 50
Time 30				
Smell	6.84 ± 1.45^{ab}	6.59 ± 1.40^{ab}	7.05 ± 1.57^{a}	6.50 ± 1.50^{b}
Taste	7.00 ± 1.45^{a}	6.66 ± 1.64^{ab}	7.16 ± 1.84^{a}	6.45 ± 1.63^{b}
Texture	6.92 ± 1.67^{ab}	6.80 ± 1.77^{b}	7.30 ± 1.73^{a}	6.83 ± 1.94^{ab}
Overall acceptance	7.10 ± 1.37^{ab}	6.81 ± 1.61^{ab}	7.28 ± 1.71^{a}	6.59 ± 1.80^{b}
I.A.	78.88%	75.66%	80.88%	73.22%
Time 120				
Smell	6.23 ± 1.77^{c}	6.51 ± 1.40^{bc}	7.35 ± 1.41^{a}	6.89 ± 1.55^{ab}
Taste	6.11 ± 1.84^{c}	6.70 ± 1.53^{b}	7.43 ± 1.46^{a}	6.98 ± 1.56^{ab}
Texture	6.60 ± 1.71^{b}	6.77 ± 1.59^{ab}	7.19 ± 1.49^{a}	6.79 ± 1.58^{ab}
Overall acceptance	6.39 ± 1.71^{c}	6.85 ± 1.41^{b}	7.46 ± 1.33^{a}	7.20 ± 1.43^{ab}
I.A.	71.00%	76.11%	82.88%	80.00%

a,b,c: Means in the line with different letters represent significant differences ($p<0.05$, Tukey's test) between samples.

B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BOEO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BOEO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

I.A. = Index of product's acceptability.

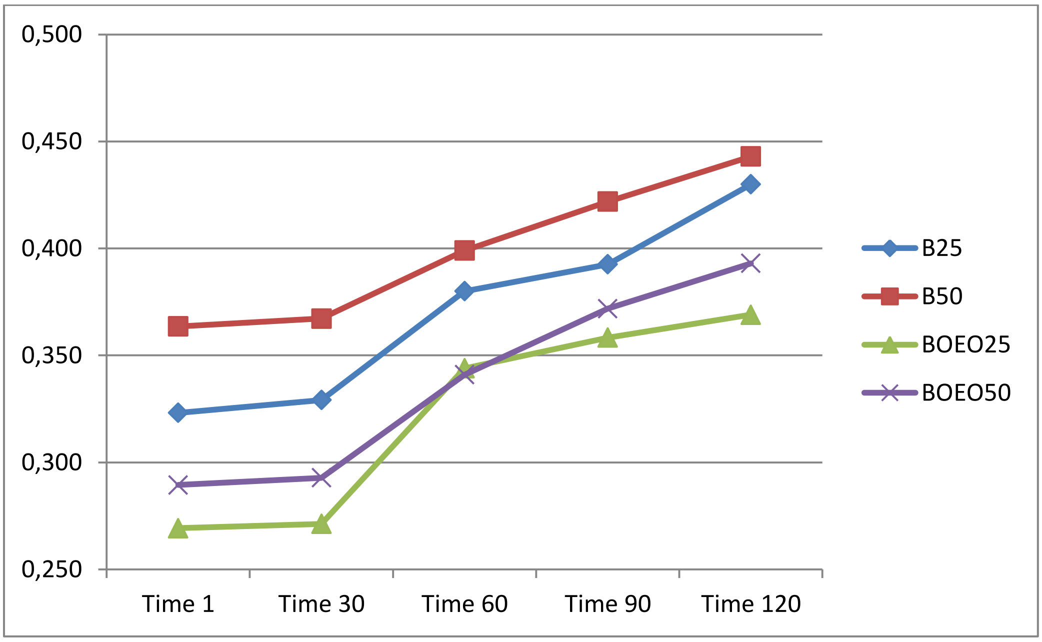

Figure 1 – TBARS (mg malonaldeyde/ Kg) beef burger packaged with active films.

B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BOEO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BOEO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.