

Assessment of mass variation, lipid oxidation and sensorial acceptance of microwave cooked bovine meat marinated with salt, hydrocolloids, and antioxidants

Avaliação da variação de massa, oxidação lipídica e aceitação sensorial da carne bovina cozida em micro-ondas marinada com sal, hidrocoloides e antioxidantes

DOI:10.34117/bjdv8n5-599

Recebimento dos originais: 21/03/2022

Aceitação para publicação: 29/04/2022

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ABSTRACT

This work aimed to assess the mass variation (the relative mass gain (*MG*), mass loss (*ML*), and total mass variation (*TMV*)), the lipid oxidation and the sensorial acceptance (triangular and ordering tests) of meat marinated with sodium chloride, hydrocolloids (modified starch, carrageenan, collagen, and soy protein isolate), and antioxidants (sodium erythorbate and astaxanthin), and microwave cooked. A central composite design was proposed to quantify the *MG*, *ML*, and *TMV* after the marination of the meat

for 12 hours and then, cooking in microwave. Carrageenan presented lower *ML* (8.4%) and higher *TMV* (6.45%). The lipid oxidation of the control sample was similarly reduced with astaxanthin or sodium erythorbate. The panelists did not identify the sample with carrageenan in the triangular test. In the ordering preference test, there was no significant difference by a preferred sample. Thus, meat marinated with salt, carrageenan, and astaxanthin, and microwave cooked showed promising results.

Keywords: food formulation, microwave processing, product assessment.

RESUMO

Este trabalho teve como objetivo avaliar a variação de massa (ganho de massa relativo (*MG*), perda de massa (*ML*) e variação de massa total (*TMV*)), a oxidação lipídica e a aceitação sensorial (testes triangular e de ordenação) de carnes marinadas com sódio. cloreto, hidrocoloides (amido modificado, carragena, colágeno e isolado de proteína de soja) e antioxidantes (eritorbato de sódio e astaxantina) e cozidos em micro-ondas. Um delineamento composto central foi proposto para quantificar *MG*, *ML* e *TMV* após a marinação da carne por 12 horas e, em seguida, cozimento em micro-ondas. A carragena apresentou menor *ML* (8,4%) e maior *TMV* (6,45%). A oxidação lipídica da amostra de controle foi similarmente reduzida com astaxantina ou eritorbato de sódio. Os provadores não identificaram a amostra com carragena no teste triangular. No teste de preferência de pedido, não houve diferença significativa por uma amostra preferida. Assim, carnes marinadas com sal, carragena e astaxantina e cozidas em micro-ondas mostraram resultados promissores.

Palavras-chave: formulação de alimentos, processamento por micro-ondas, avaliação de produtos.

1 INTRODUCTION

Hydrocolloids are polysaccharides, popularly known as gums, used as an ingredient for food formulation. They are responsible for a variety of technological functions, such as thickening and/or gelling aqueous solutions, altering and/or controlling the flow properties and the texture of foods, releasing of flavors, and/or modifying the deformation properties of semi-solid products (Glicksman, 1969). Hydrocolloids have a high capacity to interact with water due to their ability to perform hydrogen bonds and ionic interactions (Damodaran, Parkin, & Fennema, 2008).

The addition of salt and hydrocolloids exert an influence on water retention capacity in meat products (DeFreitas, Sebranek, Olson, & Carr, 1997). Carrageenan, soy protein isolate, collagen, and starch have a prominent position among the hydrocolloids used by the meat industry. The effectiveness of these hydrocolloids, as well as the improvement of softness and yield of meat products, depends on the nature of the raw material, muscle fiber type, saline concentration and processing conditions (Trius &

Sebranek, 1996). Myofibrillar proteins, mainly the actin and the myosin, are characterized by their solubility in saline solutions, gelation capacity and water retention influencing the texture and yield of meat products (Vega-Warner, Merkel, & Smith, 1999).

Lipid oxidation is the major non-microbiological cause of quality deterioration of meat and meat products due to the vulnerability of lipids and proteins to oxidative damage from the rapid depletion of endogenous antioxidants after slaughter (De Lima Júnior, Do Nascimento Rangel, Urbano, & Moreno, 2013). Cooking, grinding, and salting can influence on oxidation (Rocha Garcia, Yamashita, Youssef, Prudencio, & Shimokomaki, 2013). Antioxidants extend the shelf life of foods, protecting them against deterioration caused by oxidation, such as the rancidity of fats and color changes. The most commonly used primary antioxidants in food are synthetic compounds (André, Castanheira, Cruz, Paseiro, & Sanches-Silva, 2010), such as sodium erythorbate (Lee, Decker, Faustman, & Mancini, 2005). Several countries have limited the use of chemical additives which are considered harmful to health, and the consumers have required natural options as alternatives to these synthetic additives.

Astaxanthin is a red-orange carotenoid of the xanthophyll group, widely distributed in nature (Kusdiyantini, Gaudin, Goma, & Blanc, 1998; Miao, Lu, Li, & Zeng, 2006). It represents one of the main pigments of the carotenoid family that confers characteristic coloration of some crustaceans (shrimp and lobster), fish (trout and salmon) and other organisms (Johnson & An, 1991). It has been associated with the colour change observed when lobsters are cooked, its name derives from the Latin taxonomical classification for the edible lobster *Astacus gammarus*, its IUPAC name is 3,3'-dihydroxy- β,β -carotene-4,4'-dione, it contains 40 carbon atoms ($C_{40}H_{52}O_4$) and is cyclised at either end of the carbon back bone (Lagocki, 2001; Renstrøm, Borch, Skulberg, & Liaaen-Jensen, 1981). The presence of these groups in each ionone ring justifies their esterification characteristics, their high antioxidant power, and their more polar configuration compared to other carotenoids (Guerin, Huntley, & Olaizola, 2003).

The microwave processing technologies, including microwave drying, heating and sterilizing, have been extensively applied for processing meat and meat products (e.g., sardine fish, chicken steak, salmon, cod, drumettes, and beef slices) (Guo, Sun, Cheng, & Han, 2017). The cooking process in the microwave bands can deliver a high temperature in a short time resulting in nutritional and sensorial advantages (Orsat, Raghavan, & Krishnaswamy, 2016). In a recent study, grass carp meat cooked by microwave presented changes of water distribution and a compact microstructure with

more and uniform salt distribution, in which the sodium reduction effect was further confirmed by sensory analysis, besides decreasing the cooking loss and maintain a compact structure of meat in contrast with traditional water bath cooking (Wang et al., 2019). However, the total mass loss during microwave cooking is higher than other cooking methods such as grilling, frying, roasting and braising due to no crust formed during microwave cooking (Domínguez, Gómez, Fonseca, & Lorenzo, 2014). Therefore, microwave cooking has some advantages, and mass loss in such a process is a relevant topic to be considered.

The aim of this work was to assess the mass variation (the relatives mass gain, mass loss, and total mass variation), the lipid oxidation (Thiobarbituric Acid Reactive Substances methodology), and the sensorial acceptance (triangular and ordering preference tests) of meat (*M. vastus lateralis*) marinated with salt (sodium chloride), hydrocolloids (modified starch, carrageenan, collagen, and soy protein isolate), and antioxidants (sodium erythorbate and astaxanthin) and microwave cooked.

2 MATERIAL AND METHODS

2.1 OBTENTION, PREPARATION, AND CHARACTERIZATION OF THE RAW MEAT

Raw meat (*M. vastus lateralis*) was purchased from a butcher shop in Curitiba (Parana, Brazil) and taken to the laboratory, where the meat was diced manually with a knife (~25 g per cube) and packed in polypropylene packages.

The analyzes of the moisture, the protein, the lipid, the fixed mineral residue contents, and the water activity were performed to characterize the raw meat, all in triplicate. The moisture content of the samples was determined by the traditional method in an oven at 105 °C and calculated by mass difference before and after dehydration to constant weight (AOAC, 2005). The protein content of the samples was determined by the Kjeldahl method, and the nitrogen conversion was calculated with the factor 6.25 (AOAC, 2005). The lipid content was determined by Soxhlet extraction using petroleum ether and calculated by mass difference (AOAC, 2005). The fixed mineral residue content was determined by the muffle incineration at 540-550 °C and calculated by mass difference (AOAC, 2005). The water activity was determined using an Aqualab water activity meter, model CX-2 (Decagon Devices, Inc., Pullman, WA) with the controlled temperature at 25 °C (± 1 °C).

2.2 PROCEDURES TO DETERMINE MASS VARIATION AFTER MARINATION AND COOKING

Cubes of raw meat were individually weighted (initial mass of the sample, m_i), and two cubes were placed in a glass beaker. Then, 60% (m/m) of water was added followed by the addition of appropriated amounts of salt (sodium chloride) and hydrocolloid (modified starch, soy protein isolate, collagen or carrageenan, donated by Globalfood (Itatiba, São Paulo, Brazil)), according to the experimental design (presented next). The meat samples were marinated for twelve hours under refrigeration (1 °C). After that, each sample was weighed (mass of marinated sample, m_m), and the relative mass gain (MG) was quantified by Equation (1).

$$MG = 100 \left(\frac{m_m - m_i}{m_i} \right) \quad (1)$$

The marinated samples were submitted to the microwave cooking during 60 s (final temperature close to 85 °C). After that, each sample was left to cool until ambient temperature and then, weighted (cooked sample, m_c). The relative mass loss (ML) was quantified by Equation (2).

$$ML = 100 \left(\frac{m_m - m_c}{m_m} \right) \quad (2)$$

The relative total mass variation (TMV) after the two processes (marination and cooking) was quantified by Equation (3).

$$TMV = 100 \left(\frac{m_c - m_i}{m_i} \right) \quad (3)$$

The hydrocolloid with the best results (higher MG , lower ML , and higher TMV) was selected to proceed with further analyses of this research.

2.3 EXPERIMENTAL DESIGN

A Central Composite Design (CCD) of two factors and three levels (3^2) was performed as a sampling method, as shown in Table 1. The procedures (presented in section 2.2) were replicated six times for each combination of factors and levels (total of

nine combinations), resulting in 54 runs for each hydrocolloid. The experiments of the CCD were performed to all four different hydrocolloids (modified starch, soy protein isolate, collagen, or carrageenan). The responses measured in each experiment were m_i , m_m , and m_c , which were used to calculate MG , ML , and TMV .

Table 1. Factors, symbols and levels of Central Composite Design for assessment of the mass variation of meat (relative mass gain, relative mass loss, and relative total mass variation) marinated with salt (sodium chloride) and different hydrocolloids (modified starch, soy protein isolate, collagen or carrageenan) and cooked in a microwave.

Factor	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Salt (%)	<i>S</i>	0	5	10
Hydrocolloid (%)	<i>H</i>	0	0.5	1.0

2.4 PHYSIC-CHEMICAL ANALYZES OF COOKED MEAT SAMPLES

The physic-chemical analyses were performed for two different formulations: the control sample, prepared according to the procedures to determine mass variation after marination and cooking (described in section 2.2) with salt (abbreviated by CTRL+ST), and the control sample with salt and the best hydrocolloid (abbreviated by CTRL+ST+HC).

The moisture and the fixed mineral residue contents, and the water activity analyses were performed according to the methodologies described in section 2.1.

2.5 ANTIOXIDANT ADDITION AND LIPID OXIDATION ANALYSIS

Lipid oxidation analysis were performed to samples with five different formulations: CTRL (control – without salt, best hydrocolloid and antioxidant), CTRL+ST (control with salt, without best hydrocolloid and antioxidant), CTRL+ST+HC (control with salt and best hydrocolloid, without antioxidant), CTRL+ST+HC+ERI (control with salt, best hydrocolloid and sodium erythorbate antioxidant) and CTRL+ST+HC+AST (control with salt, best hydrocolloid and astaxanthin antioxidant). All formulations were prepared according to the procedure to determine the mass variation after marination and cooking (section 2.1). The sodium erythorbate and astaxanthin (150 ppm), donated by Globalfood (Itatiba, Sao Paulo, Brazil), were added at the same step as salt and hydrocolloid (before marination and cooking).

The determination of Thiobarbituric Acid-Reactive Substances (TBARS) through the method proposed by Tarladgis et al. (1964) and modified by Torres et al. (1989) was carried out to evaluate the lipid oxidation levels in the five different formulations. The

assay started by taking 5 g of meat sample and homogenizing it in 25 mL distilled water using an Ultra turrax T25 (equipment) at 9,000 rpm for 1 min. A 3 mL aliquot of the sample homogenate was added to 3 mL of trichloroacetic acid/thiobarbituric acid stock solution (1.14 M trichloroacetic acid, 0.032 M thiobarbituric acid in 0.32 M HCl) in a glass tube and mixed in a vortex. Samples were incubated in a water bath at 94 °C for 15 min for color development, following a cooling period of 10 min on ice. Then, samples were centrifuged at 2,500 rpm for 15 min. The absorbance of the supernatant was measured at 535 nm (equipment). The results were expressed in milligram of malondialdehyde (MDA) per kilogram of meat sample using a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane.

2.6 SENSORY ANALYSES

The sensory analyses (approved by the Committee of Ethics in Research with Humans of the Sector of Health Sciences/UFPR – Technical Opinion CEP/SD-PB No. 2,923,140) were performed with 30 untrained tasters in individual booths under white light and coded containers. The triangular and the ordering preference tests were performed.

In the triangular test, the tasters received three samples each at random order. Two were equal in composition (CTRL+ST), and one was different (CTRL+ST+HC). The triangular test aimed to identify if the tasters were able to identify the presence of hydrocolloid in the sample.

In the ordering preference test, the tasters received three samples each at random order, each of them with different compositions (CTRL+ST, CTRL+ST+HC, and CTRL+ST+HC+AST). The ordering preference test aimed to identify the preference of the tasters by the samples in ascending order (from least to most liked).

2.7 STATISTICAL ANALYSES

Mean of the experimental data and standard deviation were calculated using LibreOffice Calc[®] software, version 6.2.2.2 (The Document Foundation). Analysis of variance (ANOVA) and Tukey test (with 5% of significance) were calculated using Past[®] software, version 3.25 (University of Oslo, Oslo, NOR).

The Quadratic Response Surface Model (Equation (4)) was fitted to the experimental data of each hydrocolloid with R[®] software, version 3.5.3 (R Foundation for Statistical Computing), in which different *b_{ii}* are the model coefficients estimated by

fitting, H (hydrocolloid) and S (salt) are the independent variables, ϵ is the residual associated to the experiments, and Y is the dependent variable (model responses for MG , ML , and TMV). The goodness-of-fit indices (Coefficient of determination – R^2 and Residual Standard Error – RSE) were calculated by R software.

$$Y = \beta_0 + \beta_1H + \beta_2S + \beta_{11}H^2 + \beta_{22}S^2 + \beta_{12}HS + \epsilon \quad (4)$$

3 RESULTS AND DISCUSSION

3.1 CHARACTERIZATION OF THE RAW MEAT

The results of the analyses for the characterization of the raw meat (*M. vastus lateralis*) are shown in Table 2. Similar values for this type of meat can be found in the literature (Youssef, Garcia, Yamashita, & Shimokomaki, 2007).

Table 2. Results of the physic-chemical analyzes for the characterization of the raw meat (*M. vastus lateralis*), the sample marinated with salt (CTRL+ST), and the sample marinated with salt and hydrocolloid (CTRL+ST+HC).

Component	Raw meat	CTRL+ST	CTRL+ST+HC
Moisture (%)	74.6 ^a (± 0.2)	75.7 ^b (± 0.4)	76.7 ^b (± 0.9)
Protein (%)	18.3 (± 0.3)		
Lipid (%)	4.56 (± 0.40)		
Fixed mineral residue (%)	2.56 ^a (± 0.10)	3.26 ^b (± 0.09)	3.47 ^c (± 0.06)
Water activity	0.984 ^a (± 0.008)	0.965 ^b (± 0.003)	0.971 ^{a,b} (± 0.010)

a, b, or c in the same line indicates a significant difference (p<0.05)

3.2 MASS VARIATION AFTER MARINATION AND COOKING

The results of the MG , ML , and TMV of the meat marinated with salt and four different hydrocolloids (modified starch, soy protein isolate, collagen, or carrageenan) and cooked in a microwave are shown in Table 3.

Table 3. Results of the relatives' mass gain (MG), mass loss (ML), and total mass variation (TMV) of the meat marinated with salt (S : sodium chlorine) and four different hydrocolloids (H : modified starch, soy protein isolate, collagen or carrageenan) cooked in a microwave.

S	H	Hydrocolloid	MG	ML	TMV
0	0	None	6.04 (± 0.62)	40.35 (± 1.73)	-36.37 (± 1.87)
	0.5	Modified starch	5.67 (± 1.41)	34.46 (± 3.19)	-30.86 (± 4.75)
	0.5	Carrageenan	6.05 (± 0.60)	36.42 (± 2.90)	-32.59 (± 2.90)
	0.5	Collagen	4.18 (± 1.99)	40.41 (± 3.11)	-38.34 (± 3.44)
	0.5	Soy protein isolate	2.82 (± 4.83)	39.02 (± 2.29)	-37.25 (± 4.35)
	1.0	Modified starch	9.89 (± 7.16)	38.06 (± 1.74)	-36.13 (± 7.62)
	1.0	Carrageenan	10.26 (± 1.04)	31.45 (± 3.51)	-24.91 (± 4.02)
	1.0	Collagen	5.33 (± 3.39)	41.48 (± 1.82)	-39.72 (± 3.97)
	1.0	Soy protein isolate	8.51 (± 8.31)	38.95 (± 5.11)	-36.80 (± 3.76)
	5	0	None	14.49 (± 0.97)	16.59 (± 1.65)
0.5		Modified starch	11.66 (± 2.18)	12.88 (± 1.86)	-2.70 (± 2.64)

	0.5	Carrageenan	15.55 (± 1.94)	11.67 (± 1.22)	3.15 (± 1.35)
	0.5	Collagen	12.18 (± 7.53)	17.17 (± 1.12)	-11.50 (± 2.67)
	0.5	Soy protein isolate	12.19 (± 6.12)	18.69 (± 1.23)	-10.55 (± 3.51)
	1.0	Modified starch	17.50 (± 10.35)	17.91 (± 0.46)	-11.86 (± 4.76)
	1.0	Carrageenan	14.97 (± 1.18)	12.47 (± 1.66)	1.54 (± 1.05)
	1.0	Collagen	10.94 (± 8.65)	19.47 (± 1.54)	-14.04 (± 2.84)
	1.0	Soy protein isolate	12.47 (± 7.39)	18.49 (± 1.00)	-12.10 (± 3.05)
	0	None	16.24 (± 2.36)	18.06 (± 2.01)	-4.76 (± 2.31)
	0.5	Modified starch	10.96 (± 2.18)	12.96 (± 1.10)	-3.96 (± 1.64)
	0.5	Carrageenan	15.01 (± 2.11)	11.99 (± 1.99)	2.96 (± 1.02)
	0.5	Collagen	14.51 (± 4.42)	17.93 (± 1.33)	-7.19 (± 2.89)
10	0.5	Soy protein isolate	11.81 (± 9.15)	17.23 (± 1.52)	-11.33 (± 3.78)
	1.0	Carrageenan	15.30 (± 1.75)	11.43 (± 1.20)	0.64 (± 0.99)
	1.0	Modified starch	12.16 (± 5.01)	16.53 (± 0.88)	-10.19 (± 3.23)
	1.0	Collagen	12.79 (± 3.64)	15.56 (± 1.77)	-7.16 (± 3.03)
	1.0	Soy protein isolate	10.50 (± 1.47)	17.12 (± 1.29)	-8.41 (± 2.31)

The addition of salt (from 0 to 5%) in the samples resulted in a significant increase of *MG* ($p < 0.05$) and a significant decrease of *ML* and *TMV* ($p < 0.05$) for all cases, because the protein solubility increases through low saline concentrations (dependent on temperature and pH) defining the salting-in effect, stabilizing the groups with protein surface charges resulting from the action of sodium chloride on electrostatic attractions (Samejima, Lee, Ishioroshi, & Asghar, 1992). Therefore, all samples without salt had lower mass gain and higher mass loss and total mass variation than samples with 5% of salt. The results of the samples added with 5% and 10% of sodium chloride had no significant differences ($p < 0.05$) for all comparisons (without and with each different hydrocolloid).

The fitting of the quadratic response surface model to the experimental data of *MG*, *ML* and *TMV* presented satisfactory goodness-of-fit indices ($R^2 \geq 0.965$ and $RSE \leq 2.728$), as shown in Table 4. The coefficients (*b*) estimated in the fitting of the model to the data are also shown in Table 4. The β_0 coefficient (relative to the intercept) was significant ($p < 0.05$) for all cases (as expected), which indicates that the marination with salt and/or hydrocolloid results in significant variation of *MG*, *ML* and *TMV*. The β_2 and β_{22} coefficients (relative to the salt concentration) were also significant ($p < 0.05$) for all cases, which indicates that the marination with salt has linear and quadratic effect on *MG*, *ML* and *TMV*. The β_1 and β_{11} coefficients (relative to the hydrocolloid concentration) were significant ($p < 0.05$) only for *ML* and *TMV* of modified starch, which indicates that the marination with modified starch has linear and quadratic effect on *MG*, *ML* and *TMV*. The β_{12} coefficient (relative to the interaction between salt and hydrocolloid) was

significant ($p < 0.05$) only for *MG* of modified starch and soy protein isolate, which indicates there is a significant interaction between salt and hydrocolloids resulting in *MG*.

Table 4. Coefficients (*b*) estimated by the fitting of the quadratic response surface model (Equation (4)) to the experimental data of different responses (*MG*: relative mass gain, *ML*: relative mass loss and *TMV*: relative total mass variation) for each hydrocolloid and the goodness-of-fit indices.

Hydrocolloid	Response	Coefficients						Goodness-of-fit	
		β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	R^2	<i>RSE</i>
Modified starch	<i>MG</i>	5.851*	-8.268	2.744*	13.16*	-0.176*	-0.793*	0.965	1.244
	<i>ML</i>	39.724*	-19.147*	-6.592*	17.933*	0.438*	0.076	0.996	1.087
	<i>TMV</i>	-35.413*	17.992*	8.687*	-19.287*	-0.559*	-0.567	0.995	1.711
Carrageenan	<i>MG</i>	5.760*	1.113	2.473*	2.720	-0.141*	-0.516	0.976	1.048
	<i>ML</i>	40.482*	-14.478	-6.888*	6.793	0.455*	0.227	0.991	1.766
	<i>TMV</i>	-37.513*	21.090	9.733*	-10.373	-0.634*	-0.606	0.989	2.728
Collagen	<i>MG</i>	6.011*	-3.927	2.145*	2.727	-0.108*	-0.274	0.991	0.705
	<i>ML</i>	39.615*	1.992	-6.664*	0.327	0.449*	-0.363	0.995	1.381
	<i>TMV</i>	-34.976*	-10.418	8.006*	4.900	-0.488*	0.095	0.992	2.255
Soy protein isolate	<i>MG</i>	5.431*	-7.398	2.609*	9.740	-0.149*	-0.821*	0.970	1.177
	<i>ML</i>	39.611*	-0.163	-6.433*	-0.213	0.421*	0.046	0.995	1.207
	<i>TMV</i>	-34.849*	-12.337	8.378*	10.107	-0.535*	-0.322	0.990	2.265

*($p < 0.05$).

Table 5 shows all optimal values of *MG* (higher), *ML* (lower), and *TMV* (higher) calculated by the derivative of the quadratic surface response model.

Figure 1 shows that the four hydrocolloids presented similar behavior of *MG*. The optimal (higher) values of *MG* (close to 16%) were observed with 0% (lower level) of hydrocolloids and with more than 5% of salt. Similar optimal values of *MG* were observed with 1% (higher level) of modified starch or carrageenan, and with more than 5% of salt (Table 5).

Table 5. Optimum values of *MG* (highest values), *ML* (lowest values) and *TMV* (highest values) predicted by quadratic surface response model as result of the combinations of different hydrocolloids and salt.

Hydrocolloid	H_{opt} (%)	S_{opt} (%)	<i>MG</i>	<i>ML</i>	<i>TMV</i>
Modified starch	0.00*	7.82	16.57		
	1.00*	5.56	16.16		
	0.52	7.49		10.09	
	0.36	7.59			0.75
Carrageenan	0.00*	8.78	16.62		
	1.00*	6.95	16.39		
	0.94	7.34		8.40	
	0.80	7.29			6.45
Collagen	0.00*	10.00*	16.71		
	1.00*	7.99		14.40	
	0.00*	8.21			-2.11
Soy protein isolate	0.00*	8.74	16.83		

	1.00*	5.99	13.13	
	0.44	7.61		15.10
	0.00*	7.83		-2.07

*values limited by upper and lower levels (without extrapolation).

Figure 2 shows that the four hydrocolloids presented similar behavior of *ML*, although modified starch and carrageenan presented higher effect (and interaction with salt) than collagen and soy protein isolate on *ML*. The optimal (lower) values of *ML* were observed with close to 7.5% of salt and with different concentrations of the hydrocolloids (0.52% of modified starch, 0.94% of carrageenan, 1.00% of collagen, and 0.44% of soy protein isolate). The lowest *ML* (8.40%) was obtained with 0.94% of carrageenan and with 7.34% of salt (Table 5).

Figure 3 shows that the four hydrocolloids presented similar behavior of *TMV*, although modified starch and carrageenan presented clear optimal (higher) *TMV* from the interaction of the salt and the hydrocolloid. The optimal (higher) values of *TMV* were observed with close to 7.5% of salt and with different concentrations of the hydrocolloids (0.36% of modified starch, 0.80% of carrageenan, 0% of collagen or soy protein isolate). The highest *TMV* (6.45%) was obtained with 0.80% of carrageenan and with 7.29% of salt (Table 5).

Figure 1. Surface (left) and contour (right) plots of the predicted responses of relative mass gain (MG) as a function of salt and hydrocolloid concentrations ((a) modified starch, (b) carrageenan, (c) collagen, and (d) soy protein isolate).

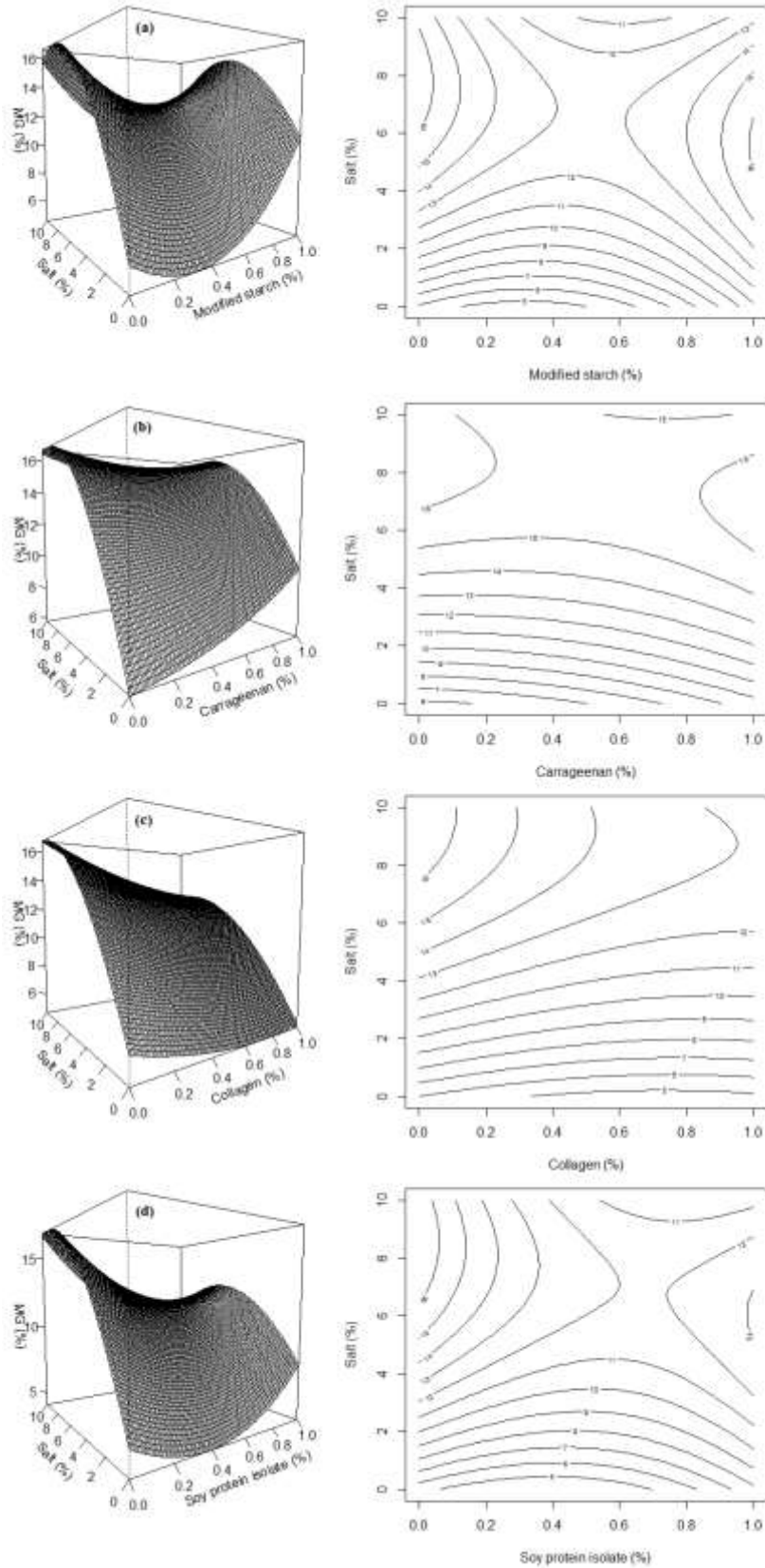


Figure 2. Surface (left) and contour (right) plots of the predicted responses of relative mass loss (*ML*) as a function of salt and hydrocolloid concentrations ((a) modified starch, (b) carrageenan, (c) collagen, and (d) soy protein isolate).

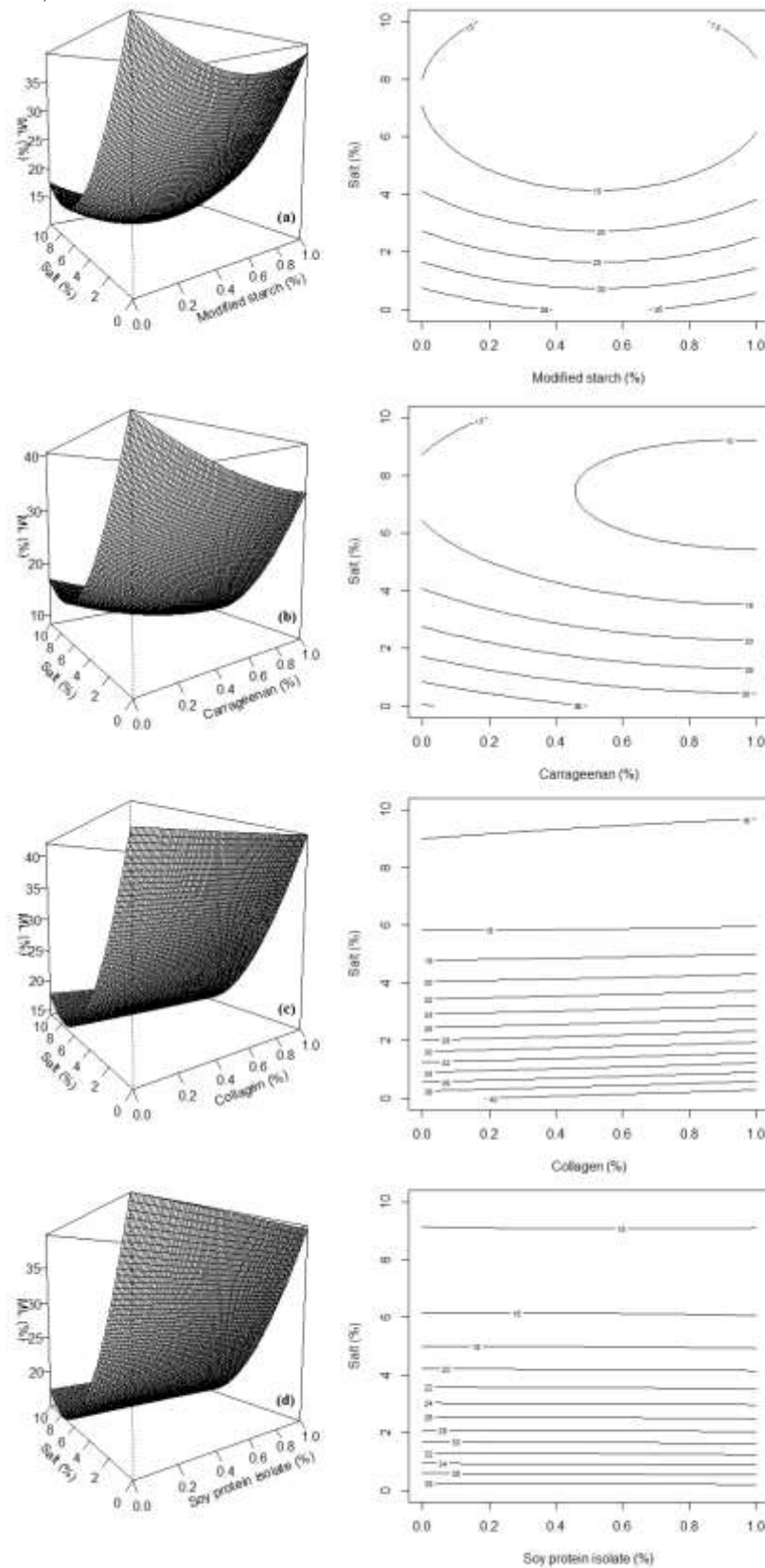
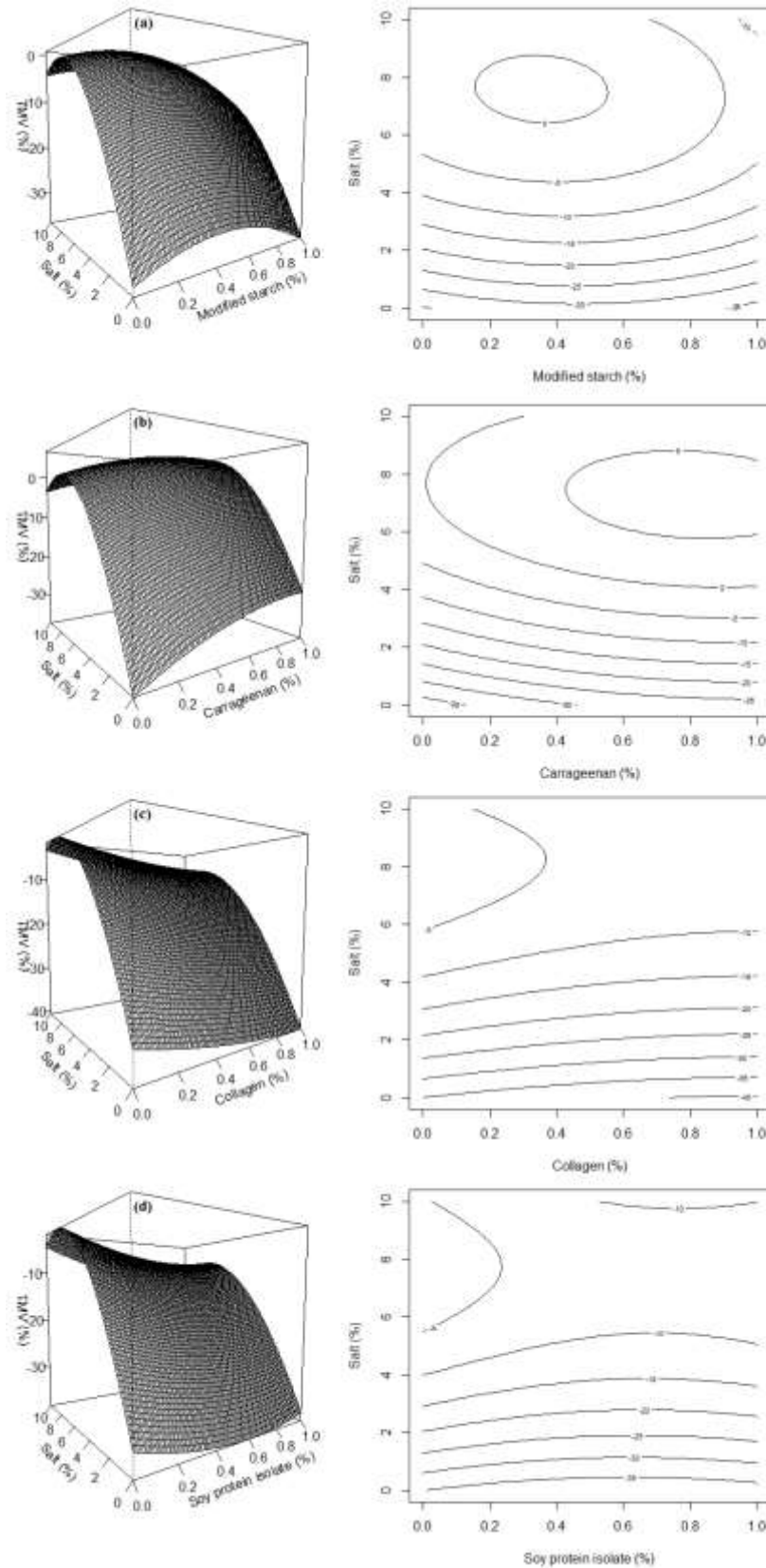


Figure 3. Surface (left) and contour (right) plots of the predicted responses of relative total mass variation (TMV) as a function of salt and hydrocolloid concentrations ((a) modified starch, (b) carrageenan, (c) collagen, and (d) soy protein isolate).



A high capacity of natural collagen fibers to retain water was reached in the meat cooking from 50 °C to 60 °C (Bueno, 2008). The collagen undergoes gelatinization when exposed to heat (temperature close to 60 °C), its fibers tend to retract from one-third to one-quarter of the initial length and influence its action in the retention of water in the meat product (Bueno, 2008). The incorporation of native soy protein isolates lowered the onset temperature of the diminishment of myosin heavy chain and actin to 50 °C, and their disappearance became gradual rather than abrupt (Feng & Xiong, 2003). This fact was attributed to the interaction of myosin with b-conglycinin and with soybean peptides derived from enzymatic hydrolysis, enabling the formation of insoluble protein aggregates (Feng & Xiong, 2002). Therefore, the cooking in microwave (~85 °C) applied to the meat with soy protein isolate impaired the performance of the hydrocolloid. The application of heat-moisture to the starch granules caused their agglomeration due to partial surface gelatinization and adhesion of the granules (Genccelep, Saricaoglu, Anil, Agar, & Turhan, 2015). It resulted in the creation of exceptionally large particle sizes about hundreds of micrometers. Carrageenan has reversible thermal gelatinization, i.e., the ability to fully dissolve in the meat product and gelatinize when cooled, increasing its water retention, texture, and consistency of meat products (Pedroso & Demiate, 2008).

Therefore, the meat marinated with the combination of salt and carrageenan showed the lowest *ML* and the highest *TMV*. As shown in Table 3, the addition of 0.5% or 1.0% of carrageenan resulted in closer *TMV* (statistically equal, $p < 0.05$), as well as the addition of 5% or 10% of salt. Thus, the best combination assumed was the meat marinated with 0.5% of carrageenan and 5% of salt, and further analyses (lipid oxidation and sensory) were performed with that combination.

3.3 PHYSIC-CHEMICAL RESULTS OF COOKED MEAT SAMPLES

The results of the analyses of moisture and the fixed mineral residue contents, as well as the water activity performed for two different formulations (CTRL+ST and CTRL+ST+HC) are shown in Table 2. In such context, the meat was marinated with 5% of salt, and 5% of salt and 0.5% of carrageenan, respectively, as justified before.

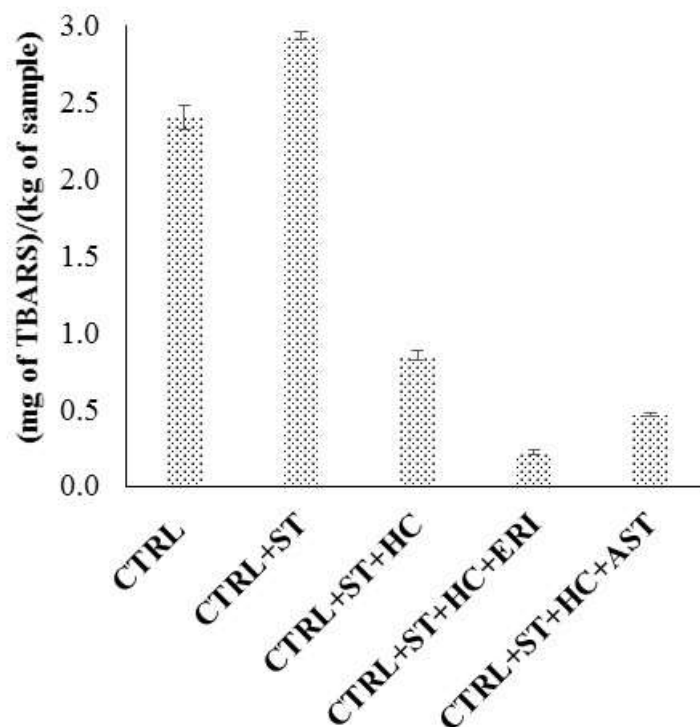
The fixed mineral residue in CTRL+ST and CTRL+ST+HC samples were significantly increased ($p > 0.05$) by 0.70% and 0.91% after marination with salt, and with salt and carrageenan, respectively, in comparison with the raw meat. These increases are related to the salt concentration of the samples because of the marination.

Although the moisture of the meat was significantly increased ($p > 0.05$) in CTRL+ST and CTRL+ST+HC samples than the raw meat, the water activity was significantly reduced ($p > 0.05$) in CTRL+ST sample due to the salt addition. The absolute value of the water activity of CTRL+ST+HC sample was 0.013 lower than the raw meat, but they did not differ significantly ($p < 0.05$) due to the high standard deviations of the means.

3.4 LIPID OXIDATION ANALYSIS

The results of the lipid oxidation of the different samples (CTRL, CTRL+ST, CTRL+ST+HC, CTRL+ST+HC+ERI and CTRL+ST+HC+AST) measured by TBARS assay (mg of TBARS/kg of sample) are shown in Figure 4. The marination of meat with sodium chloride resulted in a significant oxidative increase ($p > 0.05$) when compared to control because sodium chloride (and other curing salts) accelerate lipid oxidation (De Lima Júnior et al., 2013). All other treatments were effective to reduce the lipid oxidation.

Figure 4. Results of the lipid oxidation of the different samples (CTRL: control, CTRL+ST: control with salt, CTRL+ST+HC: control with salt and hydrocolloid, CTRL+ST+HC+ERI: control with salt, hydrocolloid and sodium erythorbate, CTRL+ST+HC+AST: control with salt, hydrocolloid and astaxanthin) measured by TBARS methodology (mg of TBARS/kg of sample).



Carrageenan showed significant antioxidant action ($p>0.05$) when compared to control. Its positive interaction with saline-soluble proteins, trapping more water inside the meat, offers more lipid protection. Although carrageenan is not an antioxidant, it may delay or inhibit oxidation rates (Cabral, Shirahigue, de Arruda, Carpes, & Oetterer, 2011).

The CTRL+ST+HC+ERI sample showed the significant lowest oxidative value ($p>0.05$), although the CTRL+ST+HC+AST sample had a similar one. The similar antioxidant potential of samples marinated with astaxanthin than erythorbate can be justified by the high number of carotenoids present in its composition. Thus, the replacement of synthetic antioxidants with natural antioxidants, such as astaxanthin, is an alternative for consumers seeking a healthy diet.

3.5 SENSORY ANALYSIS

In the triangular test with 30 tasters, 15 of them identified the presence of carrageenan in one sample (CTRL+ST+HC), while 15 of them did not identify because of the other two samples (CTRL+ST) were indicated by 9 and 6 tasters. Thus, the number of assertive answers (15 of 30 tasters) was less than the tabulated minimum to accept the hypothesis ($p<0.05$) that the different sample was identified (Dutcosky, 2015). However, 12 of the 15 tasters identified a greater tenderness of the CTRL+ST+HC sample than the CTRL+ST samples (described in the space of the formulary delimited to the comments).

In the ordering preference test, 15, 10, and 5 tasters preferred the CTRL+ST+HC, CTRL+ST, and CTRL+ST+HC+AST samples, respectively. Thus, 50% of the tasters preferred the CTRL+ST+HC sample. However, the number of responses by the preferred sample was lower than the tabulated and, therefore, there was no significant difference between them ($p<0.05$) (Dutcosky, 2015).

Studies in the literature have indicated that tasters accepted the substitution of fat by cassava starch or carrageenan in sausage samples. In contrast, samples treated with wheat protein did not have favorable acceptability (below 50%) (Sampaio, Castellucci, Pinto Silva, & Torres, 2004). Tasters did not identify the difference between the cooked hams samples added with starch and carrageenan (1 to 2%), which were evaluated with mean grades between 6 and 7 (9-point hedonic sensory analysis) (Pedroso & Demiate, 2008). No significant differences ($p<0.05$) between meat products (made with pale, soft and exudative (PSE) turkey meat) added with collagen, soy protein isolate, or carrageenan were reported by the tasters (Daigle et al., 2005).

4 CONCLUSIONS

The marination of meat with hydrocolloids and sodium chloride was an effective alternative to improve the mass gain and to reduce the mass loss (i.e., to optimize the total mass variation) of meat cooked in a microwave. In general, carrageenan presented better results, especially lower mass loss and higher total mass variation than modified starch, collagen, and soy protein isolate. Samples marinated with salt, hydrocolloid (carrageenan), and antioxidant (sodium erythorbate or astaxanthin) presented lower lipid oxidation than the control sample (without salt, hydrocolloid, and antioxidant), minimizing oxidation because of the microwave processing. The antioxidant results of the astaxanthin (natural compost) showed similar values to the sodium erythorbate. In the triangular sensory analysis, the panelists did not identify the sample with hydrocolloid (carrageenan), and in the ordering preference test, there was no significant difference by a preferred sample (with salt; with salt and hydrocolloid; or with salt, hydrocolloid and antioxidant). Therefore, the meat marinated with salt, carrageenan, and astaxanthin, and microwave cooked showed promising results.

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