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Abstract:

This study was performed to evaluate of active biodegradable packaging applied to low-sodium beef burgers stored at -18.C for 120 days. It was tested four treatments: Beef burgers with 25% (B25) and 50% (B50) of sodium reduction and biodegradable packaging and burgers packed in a biodegradable packaging with 1% oregano essential oil and 25 (BO25) and 50% (BO50) of reduction of sodium. The composition of packaging did not influence the moisture, crude protein, total fat, ash, cooking loss and water activity. The active packaging of oregano oil showed higher color protection in burgers during the storage period. The texture showed a small variation (5-10%) between packagings at day 1, stabilizing in the other periods until the storage end. The burger samples with 25% reduction in sodium and packaging with 1% oregano essential oil (B25) had better taste and aroma, getting a acceptability index above 80% for both periods (30 and 120 days). The use of 1% of oregano essential oil incorporated into packaging reduced lipid oxidation of burgers analyzed in 14% when compared packaging without adding oregano. Microbiological analyzes were maintained stable during the entire storage. Thus, active packaging with oregano essential oil can maintain the quality of beef burger 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 essential ingredients in foods. In meat products, salt has an important role, and affect shelf life and palatability (Mattes, 1997). Furthermore, salt and fat jointly contribute to the sensory traits in meat

product (Ruusunen & Puolanne, 2005; Desmond, 2006). However, over the past several decades, some studies repot that a high level of sodium consumption may linked to a rise of blood pressure that affects the risk of mortality from cardiovascular disease (He & MacGregor, 2007; Armenteros, Aristoy, Barat, & Toldrá, 2009), and is also linked to stomach cancer and kidney disease (He & MacGregor, 2007; 2010). Thus, the sodium reduction in meat products it is necessary for the food industry around the world (Asaria, Chisholm, Mathers, Ezzati, & Beaglehole, 2007; Dötsch et al., 2009). With the objective of reduce chronic degenerative diseases caused by an excessive salt intake, in most of the developed countries, the intake of sodium chloride varies from 8 to 13 g by adults/day, much higher than the 5 g as recommended by the World Health Organization (WHO, 2003). However, to achieve a significant reduction of sodium in meat products 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 & MacGregor, 2010); however, its complete replacement is not possible, being limited to 50%, because above this level the bitter taste is intensified and the 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 & Vesna, 2011; Carvalho et al., 2013).

Beef burgers with low sodium content are more prone to lipid oxidation, which is together with microbial growth a major cause of deterioration of meat products (Bidlas & Lambert, 2008). A new alternative for an efficient preservation of these products are active antioxidant packages, whose main advantage is the release of antioxidants during storage blocking the lipid oxidation process (Gómez-Estaca, Lopez-de-Dicastillo, Hernandez-Munoz, Catala, & Gavara, 2014; Vital et al., 2016; Vital et al., 2018).

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

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

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

This study was realized to evaluate the efficacy of active packaging (oregano essential oil) to reduce lipid oxidation and preserved quality on beef burger burgers with 25 and 50% sodium chloride reduction and storaged in frozen for 120 days.

Materials and methods

Beef burgers processing and storage

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

Beef Burgers were prepared so that the replacement effects of sodium chloride (NaCl) by potassium chloride (KCl) plus aromatic herbs and species could be evaluated. Two seasonings were prepared: 25% decrease in sodium chloride rate and 50% decrease in sodium chloride rate, following the methodology described by Carvalho et al. (2013) and being the composition of both seasonings showed in Table 1. During

processing, beef burgers were weighed into 80 ± 0.5 g portions, with 1 cm thickness, 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.

Beef burgers were packed in active bags biodegradable measuring 10 x 10 cm content ecoflex 40%, glycerol 13%, cassava starch 47% and biodegradable active with or without 1% oregano oil. Films were produced in the Laboratory of Food Science and Technology Department at the State University of Londrina as described by Cestari et al. (2015).

The treatments consisted of 1) Beef burgers with 25% (B25) of sodium reduction and biodegradable packaging; 2) Beef burgers with 50% (B50) of sodium reduction and biodegradable packaging; 3) Beef burgers with 25% (BO25) of sodium reduction and biodegradable packaging with 1% oregano essential oil added films; 4) Beef burgers with 50% (BO50) of sodium reduction and biodegradable packaging with 1% oregano essential oils added films. All treatments were frozen (-18°C) and storaged for 120 days. Synthetic preservatives did not use during the production process and storage period.

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

Ingredients, %	B25	B50
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
Oreganum vulgare	0.02	0.02
Bixa orellana	0.10	0.10
Capsicum frutescens	0.01	0.01

1TSP (textured soy protein).

Chemical composition

The samples of beef burgers were thawed at 4 ± 1°C, minced, homogenized and analysed in triplicate on day one and 120. Beef burger moisture and ash content were determined according to ISO-R-1442 (International Organization for Standardization [ISO], 1997) and ISO-R-936 (International Organization for Standardization [ISO], 1998). Crude protein content was obtained through ISO-R-937 (International Organization for Standardization [ISO], 1978). The total fat content was quantified as described Bligh and Dyer (1959).

Cooking loss and water activity (aw)

Samples were thawed at a 4 ± 1°C and thermally processed by conventional dry cooking so that losses by cooking could be determined. Samples were weighed one by one on an electronic analytic scale. Beef burgers were grilled on an electric grill Multi-Britania 127V for approximately 5 minutes up to an internal temperature 70°C verified by a digital thermometer Incoterm (-50 and 300°C), 145 mm long with a 4 mm diameter. Samples were heated up to 25°C and weighed again. Cooking losses were calculated as Equation 1:

$$\% CL = \left(\frac{Thawed\ weight - cooked\ weight}{Thawed\ weight} \right) x\ 100 \quad (1)$$

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

Texture and color

The meat´s mechanical characteristics were determined by texture analyzer Stable Micro Sistemas Taxt Plus (Texture Technologies Corp., UK) and 25 kg charge cell. Analysis followed methodology by Research Center for Meat of USDA (Honikel, 1998). The analyses were performed at time 1, 60 and 120 days of storage. Each sample, was wrapped in aluminum paper and grilled in an electric grill (Multi Grill 2 Britânia 127V), up to 70ºC, measured by Incoterm thermometer (-50 and 300ºC), tube 145 mm long by 4 mm diameter. Six 1 cm^2 (transversal square section) samples, with fibers parallel to the longitudinal axis of each treatment, were taken per treatment six samples.

Color was determined by portable colorimeter Minolta® CR10, with integration sphere and 3º angle of vision, or rather, illumination D3 and illuminating D65, following CIE (1986) system. L*, a* and b* were determined in triplicate on the burger surface at 5°C after 1, 60 and 120 days of storage.

Thiobarbituric acid-reactive substances (TBARS) analysis and pH

Lipid oxidation was measured by TBARS formation according the method of Pfalzgraf, Frigg, and Steinhart (1995). Meat samples (10 g) were mixed with 20 mL 10% (w v^{-1}) trichloroacetic acid, centrifuged at 4000 rpm for 20 min. at 4°C, and the supernatants were filtered through filter paper. In total, an aliquot of 2 mL of 20mM 2 – TBA was added to 2 mL 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 calculated using a calibration curve. TBARS values were expressed as mg malonaldeyde MDA kg^{-1} of meat. The analyzes in triplicate were performed at time 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 pH-meter equipped with a penetrating electrode probe for five min. (Young, West, Hart, & Van Otterdijk, 2004). The pH mesuarements were performed on days 1, 30, 60, 90 and 120.

Microbiological analyses

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

Counts of coliforms at 35 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 three tubes series of Lauryl Sulfate Tryptose broth (Difco) that were incubated at 35°C for 48 hours. The tubes that presented gas production were transferred

to Green Bile Lactose broth (Difco) and incubated at 35°C for 48 hours, and to EC broth (Difco) and incubated at 45°C for 48 hours. The results are expressed as MPN g^{-1}.

Coagulase positive Staphylococci, counts were done using the spread-plating technique in Baird Parker Agar (Difco), and the plates were incubated at 35-37°C for 48 hours. Suspect colonies were submitted to coagulase test. The results are expressed as log CFU g^{-1}.

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 hours. Presumptive colonies were identified by biochemical tests. The results are expressed as log CFU g^{-1}.

Salmonella spp. was determined according to Downes and Ito (2001). Briefly, 25 g of each sample was homogenized with 225 mL of Lactose broth and incubated at 35°C for 18 to 24 hours, followed by selective enrichment in Selenite Cystine (Difco) and Rappaport Vassilids (Difco broth. Both cultures were plated on Hektoen Enteric Agar (Difco) and incubated at 35°C for 18 to 24 hours. 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 on their participating in the consumer analysis.

Consumer test was developed under standardized conditions on the sensory laboratory of the Food Engineering Department. Sensorial analysis was performed in two different days. In the 1st consumer test was evaluated beef burger with 30 days of storage, there were involve 80 consumers (40 males and 40 females, from 18 to 60 y old). In the 2nd consumer test was evaluated beef burger with 120 days of storage, participating 80 consumers (45 males and 35 females, from 18 to 60 y old), 90% of participants are regular consumers of beef burger.

Beef burgers were cooked on a double plate grill at 200º C until reach an internal temperature of 70ºC monitored by a thermometer. Beef burgers were divided on 2 x 2 cm portions and wrapped individually in aluminum foil and labeled with a unique three digit code. Samples were beef burgers served immediately following a randomized design in order to avoid carry over (Macfie, Bratchell, Greehoff, & Vallis, 1989). Each consumer scored four samples, one for each treatment, evaluating the acceptability for burgers 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) (Dutcosky, 2011). Afterwards, samples acceptance index was calculated by the following Equation 2 describe by Dick, Jong, and Souza (2011):

$$IA\% = \frac{x * 100}{n} \quad (2)$$

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

Statistical analyses

Data are represented as the means ± standard error of mean. Analyses were performed using statistical package SPSS Inc. (2005) (v.15.0) for Windows. It was applicate an Analysis of variance using a GLM procedure in which treatment and days of storage were considered fixed effects. Differences between group means were assessed using Tukey test ($p < 0.05$).

Results and discussion

Chemical composition

As it is shown in Table 2, time of storage (0 or 120 days) did not change chemical composition inside groups, as well as, there were not differences ($p > 0.05$) between treatments. The use of biodegradable packaging or biodegradable active packaging with essential oils on beef burgers with reduction of sodium did not affect the chemical composition, presenting those products an average of 61 of moisture, 2.5 of ashes, 19 of crude protein and 9% of total fat levels to the initial and end time of storage. A review article on meat products with reduced sodium from Oliveira et al. (2013) reported that the partial replacement of NaCl by KCl (25%) did not interfere in the water retention capacity, salty taste and the 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 a effect in those variables as it is shown in Table 3; keeping up within adequante values 0.96 a 0.98 for water activity and 24 a 28% for cooking (Bernadino Filho, Oliveira, & Gomes, 2012) loss.

Texture and color

In relation to texture treatments showed significant differences only in the initial period (day one). The treatments with addition of essential oils showed higher values, being the BO50 treatment that had a higher shear force. However, with the storage time (120 days) the texture values became 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 described by Scheeder et al. (2001) of 19.4 N kg^{-1}.

There were statistical differences between treatments on 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 tone darker. Burgers with active packaging (B25 and B50 treatments) were clearer than treatments without essential oils; this difference must to greater protection conferred by active packaging to the product in terms of browning and/or oxidation. L* values were comprised between 40 - 46, redness of meat (a*) between 11-16 and yellowness (b*) 11-16. In a study performed by La Storia et al. (2012) active packaging showed also increased protection on the color change of the meat surface during storage. Being normal rates for beef in natura above 35 (Page, Wulf, & Schwotzer, 2001), luminosity given to the burger over 40 is due to 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 buy meat products and its protection is of fundamental importance for the acceptability of the product by the consumer. Active packaging (B25

and B50) 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

Considering lipid oxidation, all treatments showed good control of the oxidative process during the product storage time (120 days) under freezing conditions, being values obtained lower than 0.44 mg MDA kg^{-1} of meat on all analyzed products (Table 4 and Figure 1). Values below those were found in similar studies with frozen burger with 30% sodium reduction conducted by Baker, Alkass, and Saleh (2013), where TBA levels were equal to or above 1 mg MDA kg-1 of meat, for treatments with rosemary and ginger extract in its 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 burger protection against lipid oxidation. The TBA values found may have also been influenced by the seasoning composition containing oregano leaves (Oreganum vulgare) with evidenced antioxidant potential as a study performed by Boroski et al. (2011) and the matte packaging, preventing the incidence of light to the product.

TABLE 2.
Chemical analysis of beef burger packaged with active films.

Item	Beef burger Storage Day	B25	B50	BO25	BO50
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; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl. The samples were analysed. Analyses statical were using package SPSS Inc. (2005) (v.15.0) for Windows.

TABLE 3.
Activity water, cooking loss, texture and colours analysis of beef burger packaged with active films.

Item	Beef burger Storage days	B25	B50	BO25	BO50
Activity water	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
Cooking loss, %	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, Newton	1	18.90 ± 0.39^{b}	19.01 ± 0.91^{b}	20.33 ± 1.23^{ab}	21.66 ± 1.16^{a}
	60	19.41 ± 1.39	19.49 ± 0.78	19.81 ± 0.21	19.35 ± 2.39
	120	21.40 ± 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; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

TABLE 4.
TBARS (mg malonaldeyde kg^{-1} of meat) values and pH analysis of beef burger packaged with active films.

Item	Beef burger Storage Day	B25	B50	BO25	BO50
TBARS	1	0.323 ± 0.02 abC	0.363 ± 0.04 a C	0.269 ± 0.01 c B	0.289 ± 0.01 bc C
	30	0.329± 0.01 ab C	0.367± 0.04 aC	0.270± 0.02 c B	0.293± 0.01 bc C
	60	0.379 ± 0.02 ab B	0.399± 0.02 a BC	0.344± 0.02 b A	0.340 ± 0.02 b B
	90	0.392± 0.02 ab AB	0.422± 0.02 a AB	0.358± 0.02 b A	0.372± 0.02 bAB
	120	0.429± 0.02 ab A	0.443± 0.02 a A	0.369± 0.02 c A	0.392± 0.02 bc A
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 a A	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 b A	5.82± 0.07 ab A	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; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

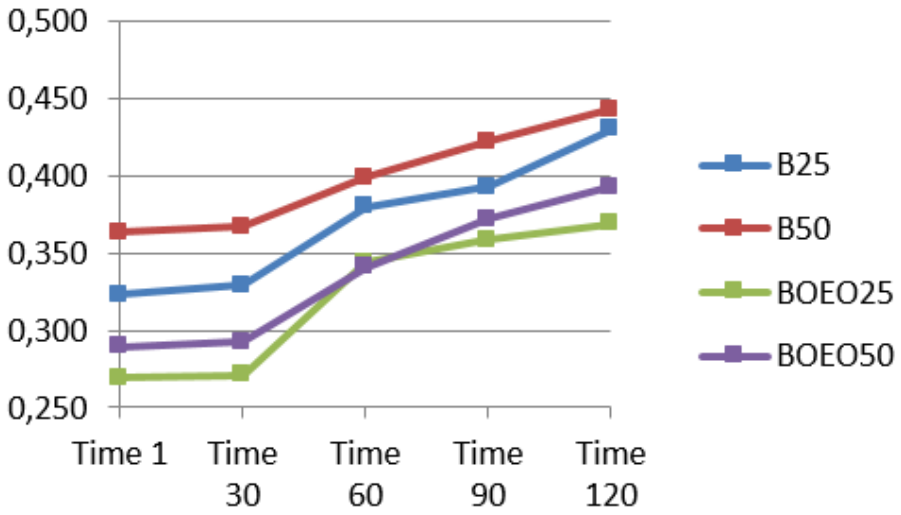

FIGURE 1.
TBARS (mg malonaldeyde kg-1) beef burger packaged with active films. B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

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

A study with restructured chicken steaks with packaged with active film 1% oregano shown 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.

PH values during the storage period showed small tendency to increase for all treatments starting with lower pH values (from 5.47 to 5.52) and ending the storage period with higher pH values (from 5.76 to 5.80), although this variation was within pH values considered normal (from 5.5 to 5.8) according Savell, Mueller, and Baird (2005). In a study performed by Emiroğlu et al. (2010) with ground beef the results were similar to our study varying from pH 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 as Table 5.

MPN of coliforms at 35°C ranged from 15 to 1.100 MPN g^{-1} among treatments and days of storage (Table 5). The counts of coliforms at 45°C were < 3 MPN g^{-1} on all treatments and all days of storage, while Coagulase positive Staphylococci and Sulfite reducing clostridia, the UFC counts were < 10^1 and < 10 UFC g^{-1}, respectively (Table 5). *Salmonella* spp. was absent in 25 g, were not detected in beef burger in all treatments during the period of storage (Table 5).

The study realized for Emiroğlu et al. (2010) with ground beef patties and active packaging show inhibition zone diameters yielded by soy protein based edible film disks against all test organisms (*Staphylococcus*

aureos (27.50 mm), *Escherichia* coli (32 mm), *Pseudomonas* (35.50 mm), *Lactobacillus* (22.5 mm) at even at minimum concentration 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 as shown in Table 5. Where salt is used to help preserve the product and antimicrobial action, partial or complete replacement by KCl is possible (Bidlas & Lambert, 2008).

Sensorial perception

As it is compiling in Table 6, two groups of consumers indicated their acceptability scores in the different times of storage of beef burgers. On 30 days storage, treatments with 25% sodium reduction had better acceptability compared to treatments with 50% sodium reduction, especially for taste attribute, in all attributes B25 treatment obtained a better result with 80.88% acceptability on I.A (Acceptability Index). In work performed by Mohamed and Mansour (2012) the addition of essential oils of rosemary and marjoram improved the sensory scores of beef patties during frozen storage period.

On 120 days storage all treatments improved their acceptability indices with the exception of B25 treatment, despite being evaluated by different consumers. Treatments with active biodegradable packaging with essential oil of oregano (BO25 and BO50) showed better results with acceptability index equal to or above 80%, and obtained the highest scores for taste and odor, given probably by the exposure time of the product in the packaging, which are pleasing to the consumer. The use of herbs as oregano on meat derivates composition let reduce the addition of salt meat products, such burgers and others, keeping consumer acceptability, if the quantity of added herbs will not exceed the limit of tolerance of consumers (Wang, Lee, & Lee, 2014). According to Table 6, with 120 days of storage , the index of acceptability of BOEO25 treatment compared to the B25 treatment was 11.88% higher, proving the positive influence of essencial oregano oil.

The use of biodegradable polymers from renewable sources such as cassava starch becomes a promising alternative to commercial application, due to its low cost and availability. The cassava starch combined with a biodegradable synthetic polymer retains its mechanical and barrier properties suitable for use in meat products as 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 and simultaneously improve the acceptability of the product.

TABLE 5
Microbiological analysis of beef burger during freezing storage.

Microorganisms	Samples	Days 1	30	60	90	120
Col. 35°C[a]	B25	1.100	240	43	93	93
	B50	1.100	210	23	93	15
	BO25	150	240	150	43	93
	BO50	460	240	460	150	93
Col. 45°C[a]	B25	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	B50	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	BOE025	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	BOE050	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Coagulase positive Staphylococci[b]	B25	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	B50	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	BO25	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	BO50	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
Clostridium reducers 46°C[b]	B25	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	B50	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	BO25	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
	BO50	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
Salmonella spp.	B25	AUS	AUS	AUS	AUS	AUS
	B50	AUS	AUS	AUS	AUS	AUS
	BO25	AUS	AUS	AUS	AUS	AUS
	BO50	AUS	AUS	AUS	AUS	AUS

aMPN g-1, bCFU g-1, B25 – B25 – Biodegradable packaging + 25% reduction NaCl; B50 – Biodegradable packaging + 50% reduction NaCl; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl.

TABLE 6.
Sensory analysis by consumers for beef burger with sodium reduction

Beef burger Attribute	B25	B50	BO25	BO50
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. 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; BO25 – Biodegradable packaging with oregano essential oil + 25% reduction NaCl; BO50 – Biodegradable packaging with oregano essential oil + 50% reduction NaCl. I.A. = Index of product´s acceptability.

Conclusion

Biodegradable packaging with 1% of oregano essential oil showed the best potential between treatments tested for reduction and stabilization of lipid oxidation in beef burgers for 120 days of storage under freezing, being effective for maintaining characteristics of quality of products as color or sensorial proprieties. The reduction of sodium in 25 and 50% did not affect the maintenance of the quality of the burgers during the storage period, as well as their physical and microbiological characteristics. Active packaging with 1% of oregano essential oil through this study proves its feasibility to control lipid oxidation in burgers during its shelf life, improving the 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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