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EFFECT OF TWO NATURAL ANTIOXIDANTS IN COMBINATION WITH EDIBLE PACKAGING ON STABILITY OF LOW FAT BEEF PRODUCT STORED UNDER FROZEN CONDITION

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Invited paper

Abstract: The chemical composition, expressible water (EW), water holding capacity (WHC), pH value, total acidity (as lactic acid), thiobarbituric acid (TBA), aerobic plate count, and sensory evaluation were determined in order to evaluate the effect of two different natural antioxidants combined with edible packaging on the stability of low fat beef product stored frozen for six months. The data showed that there were no changes in the chemical composition as a result of the addition of antioxidants. It also showed an increase in the (E.W) values as the storage time progressed correlated by decreasing in the (WHC) values. The increment in the (E.W) and the decrease in the (WHC) were the highest among the untreated samples. The data demonstrated decreasing in the pH values and increasing in the acidity values for the treated and the untreated samples. The reduction of pH and the increment of acidity were higher in the untreated samples compared by the treated ones. TBA values increased over time for all samples. The increment was rapid for the untreated sample. The reduction rate of color for the untreated sample was higher than the treated ones followed by the green tea treated sample, while flavor value for sample treated by the green tea was the lowest value compared with the other samples. The results indicated that the use of chitosan as edible film had delayed the proliferation of aerobic plate count.

Key words: antioxidants, edible packaging, chitosan, low fat beef product.

Introduction

Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors *(Fernandez-Lopez et al., 2005)*. During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbiological processes *(Wong et al., 1995)*. Oxidation is a major cause of that deterioration because of its negative effects on organoleptic qualities (flavor, color, etc.). Oxidation of lipids can also have a marked negative effect on nutritional value, and could be responsible for the production of toxic compounds *(McCarthy et al., 2001; and Nissen et al., 2004)*. Meat products, due to fat content are highly susceptible to lipid oxidation. Moisture, prooxidant pigments, storage, handling and display conditions contribute to lipid oxidation of meat products *(McCarthy et al., 2001; Lacroix et al., 2004; and Nissen et al., 2004)*. Due to detrimental effects of lipid oxidation on color, flavor, texture, and nutritional value of foods; addition of synthetic antioxidants such as BHT and BHA has been effective because of their low cost, high stability, and effectiveness. However, the use of such compounds has been related to health risks resulting in strict regulations over their use in food products and this has stimulated research for alternative antioxidant sources *(Lacroix et al., 2004)*.

With increased consumer concerns about the amount of chemicals in their foods, processors are looking for more natural ways to protect their products. In the last few years, there has been an increasing interest in the use of natural additives in preference to synthetic substances for the stabilization of fat-containing food stuff. Among the natural antioxidants, extracts of herbs such as rosemary and sage have played an important role *(Park et al., 2002; and Bekhit et al., 2003)*. The use of antioxidants like vitamin C and E had a significant effect in reducing oxidation of lipids and pigments of meat during storage *(Sahoo and Anjaneyulu, 1997)*. In view of the fact that natural spices are widely used in a variety of food products, it is important to know the effects they have on the keeping qualities of such products. A number of studies have been made on the bactericidal and bacteriostatic properties of spices to evaluate their effectiveness in preventing or retarding spoilage caused by microorganisms in addition to the antioxidant effect of spices on fats in certain foods *(Pruthi, 1980)*.

To obtain the optimum shelf-life of fresh red meat, it is necessary to limit microbial contamination. Microbial spoilage can be delayed by storage of meat at low temperature by effects on the growth rate of the organisms. Since frozen meat is highly susceptible to dehydration as a result of moisture losses and temperature fluctuations, the protection of frozen meat against fluctuations in temperature during storage is important from the standpoint of quality retention. An obvious approach is the use of suitable packaging materials to meet various criteria, such as protection against moisture migration and mechanical damage *(Kenawi, 1994)*.

Edible coating of biodegradable packaging is a new technology that has been introduced in food processing in order to obtain products with longer shelf life. Several applications for meat, poultry, and sea foods have been reviewed by *Lacroix et al. (2004)* with particular emphasis on the reduction of lipid oxidation, weight loss, moisture loss, microbial load, and volatile flavor loss. Edible films or coatings have been investigated for their abilities to retard moisture, oxygen, aromas, and solutes transports. It is one of the most effective methods to maintain food quality *(Pranoto et al., 2005)*.

Microbial growth on the surface of food is a major cause of food spoilage and food-borne illness. Therefore, the concept of using edible active coating to inhibit spoilage and pathogenic microorganism has received considerable interest *(Wiles et al., 2000; Coma et al., 2001; Rodriguez et al., 2003; Kanatt et al., 2008; and Wunwisa and Mabumrung, 2008).*

The objective of this investigation was to determine the potential effect of two natural antioxidants rosemary and green tea in combination with edible packaging on stability of low fat beef product stored for six months under frozen condition.

Materials and Methods

Antioxidant powders, soy flour, and modified stach. Dried rosemary and green tea were obtained from a local market, and then powdered using electric grinder in order to get 80 mesh powder.

Hesco low fat soy flour was obtained from Hesco, Watertown, South Dakota, USA. Whereas, modified starch (TEXTAID A) was obtained from National starch &chemical company, Bridgewater, New Jersey, USA.

Preparation of chitosan solution. Chitosan edible film solution was prepared by dissolving 1g of chitosan powder in 100 ml of 1% glacial acetic acid solution. The solution was then filtered through a silk screen to remove undissolved material.

Preparation of low fat beef product. The beef (bottom round, 10 Kg) used in this study was obtained from a local market in El-Minia, Egypt, one hour after slaughter. The sample was trimmed, packed in low density polyethylene bags and held at 4±1 °C for 24 hours, cut into cubes and minced with a meat grinder using 8 mm (coarse) and 3 mm (fine) plates simultaneously to obtain ground beef.

Low fat beef product was prepared according to the following recipe (Table 1).

Table 1. Formulation of low fat beef product

Ingredient	Low fat beef product
Ground beef	66%
Minced fat	10%
Black pepper	1%
Onion powder	1%
Crushed ice (water)	8%
Salt	2%
Low fat soy flower	8%
Modified starch	4%

All the ingredients were individually mixed well, then divided into three equal portions. The first portion was left untreated as control, while rosemary powder was added to the second portion in the ratio of (2 %), whereas, green tea powder was added to the third portion in the same ratio. Each portion (untreated or treated) was divided into small balls 50 g each, then formulated in patties like shape (10 cm diameter, and 0.5 cm thickness). The samples were frozen at - 40°C for 2 hours, and immersed in chitosan solution for one minute (to form a thin film as primary package), then packaged in 2 mil low density polyethylene bags , and stored frozen at - 18°C for 6 months (Figure1).

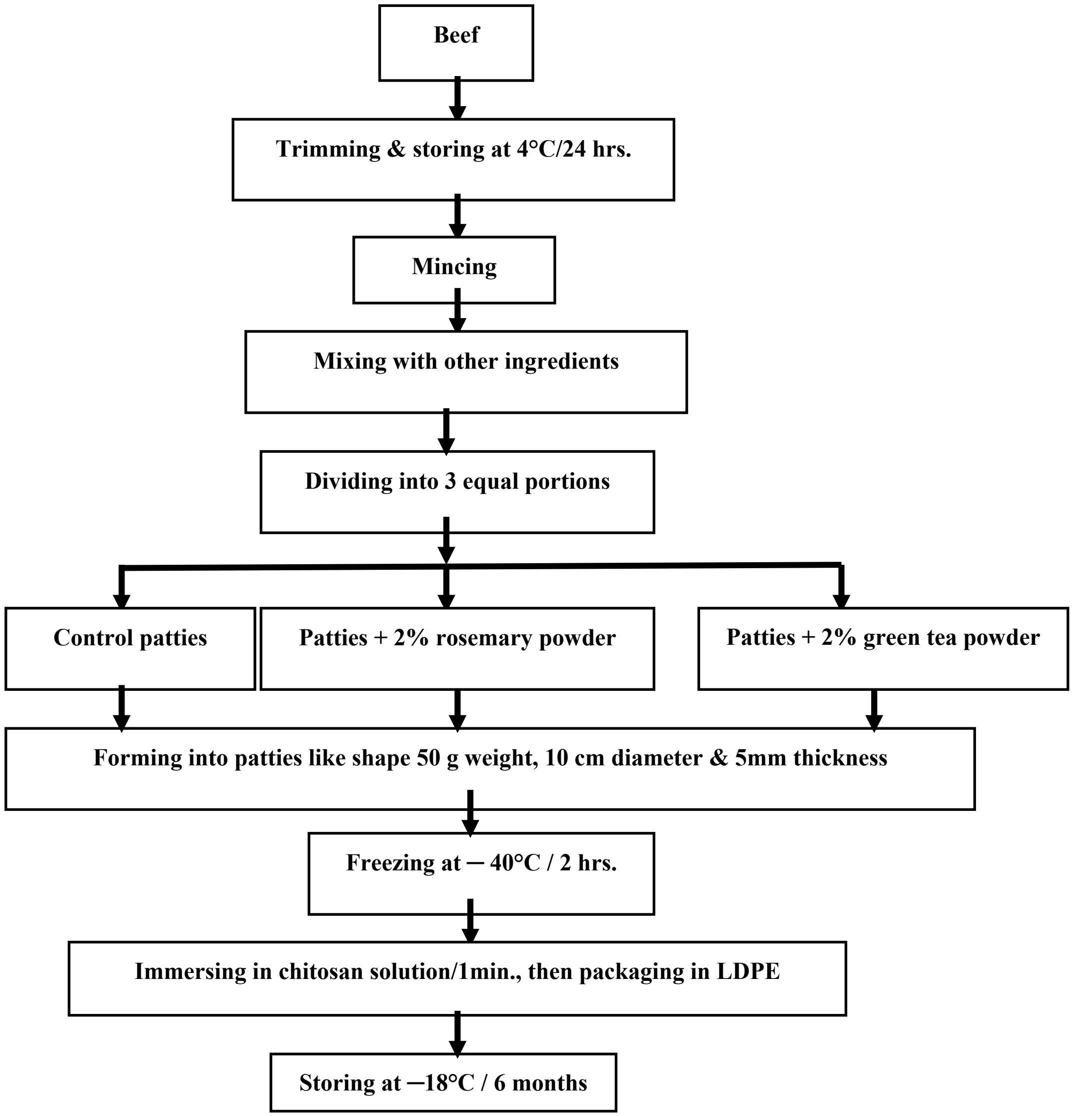

Figure 1. Flow diagram of production and storing of untreated and treated low fat beef product under frozen storing condition

Analytical methods. Moisture, crude protein, crude fat, ash, and carbohydrate contents were determined according to the methods of the AOAC (*AOAC, 1995*).

Determination of expressible water (EW) and water holding capacity (WHC). Expressible water (EW) was determined according to *Alvarez et al., (1992)*. Whereas, the water holding capacity (WHC) was calculated.

pH measurement. A slurry was prepared by blending the beef product (5g/50ml distilled water). The pH of this slurry was measured by using the glass-electrode method according to the AOAC *(AOAC, 1975)*.

Determination of total acidity. The acidity was determined by titration according to *Keeton and Melton, (1978)*.

Thiobarbituric acid (TBA) value. Frozen samples were tested separately. TBA-reactive substances were measured using the method of *Harold et al. (1981)*. Colorimetric absorbance at 530 nm was measured using a Spectronic 710 Spectrophotometer. Readings were converted to mg malonaldehyde /1000g beef product and reported as TBA values (mg TBA/1000g beef product).

Microbiological analysis. Twenty five grams of samples were aseptically homogenized in 225 ml of sterile buffered peptone water for 1 minute. Serial dilutions were made in the same sterile peptone water, and then used for microbiological analysis during the time of refrigerated storage.

Aerobic plate count. Aerobic plate counts (APC) were determined by incubating 0.1 ml of the sample homogenate at selected dilutions, for 48 hrs at 35ºC.

Sensory evaluation. Sensory evaluation for color, flavor, juiciness, and overall acceptability for the cooked untreated and treated beef products were carried out in order to determine the consumer acceptability for the product according to the methods described by *Larmond (1977)*. Ten judges were participated in this test. A numerical hedonic scale ranged between 1 and 10 (1 for very bad, and 10 for excellent) was used for sensory evaluation.

Results and Discussion

Muscle foods have low oxidative stability and are very susceptible to rancidity during production and storage. Numerous studies have indicated that lipid oxidation in meat and meat products may be controlled or minimized through the use of antioxidants.

Tables 2 and 3 - illustrates the chemical composition and some physical parameters of the untreated and treated beef product. The data showed that there are no noticeable changes in the chemical composition between the control and the samples treated by rosemary or green tea powders. The data also revealed that there are no big differences were observed regarding the physical parameters (weight, thickness, and diameter) for the treated and the untreated samples after cooking.

Table 4 - represents the effect of storage time on moisture content (M.C), expressible water (E.W), and water holding capacity (WHC) for the untreated and treated beef product. There were no differences among (M.C) from different treatments observed at any time of examination during storage period. The data showed increasing in the (E.W) values as the storage time progressed correlated by decreasing in the (WHC) values. The increment in the (E.W) and the decrease in the (WHC) were the highest among the untreated samples compared by the samples treated by two natural antioxidants (rosemary or green tea). This gave the impression that the addition of rosemary or green tea powders increased the ability of water retention and the tenderness of the product. The data also revealed that there are no big differences in the (E.W) and the (WHC) values between rosemary and green tea treated samples.

Figure 2 - clearly illustrates the effect of storage time on the pH values for untreated and treated beef product. The data demonstrated decreasing in the pH values for the treated and the untreated samples. The reduction was higher in the untreated samples compared by the treated ones. This could be due to the effect of natural antioxidants which retarded the formation of free fatty acids *(Kenawi et al., 2004).*

Figure 3 - illustrates the effect of storage time and natural antioxidant (rosemary and green tea) treatment on the percentage acidity (as lactic acid) of the beef product. The data demonstrated that there is an increase in the acidity values for all samples along with storage time as a result of the increase of free fatty acids due to rancidity. The increment was the highest for the untreated sample and the lowest for the treated ones. Whereas, there were no observed differences between the rosemary or the green tea treated samples.

Table 2. Chemical composition of untreated and treated beef product*(wet weight basis)*

Component%	Untreated sample	Rosemary treated sample	Green tea treated sample
Moisture	66.92	66.83	66.53
Protein**	17.03	16.95	17.01
Crude fat	10.42	10.32	10.27
Ash	2.21	2.46	2.61
Fibers	0.39	0.42	0.50
Carbohydrates***	3.03	3.02	3.08
Energy (Kcal/100g)	174	173	173

* Means of three determinations. **Total nitrogen ×6.25. *** Calculated by difference.

Table 3. Weight, thickness and diameter of untreated and treated beef product *

Parameters	Untreate d sample		Rosemar y treated sample		Green tea treated sample	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
(Weight (g	50.00	36.82	50.00	35.03	50.00	35.28
Thickness ((mm	5.00	4.00	5.00	4.00	5.00	4.00
Diameter ((cm	10.00	7.20	10.00	7.20	10.00	7.20

* Means of three determinations.

Table 4. Effect of storage time on moisture content (M.C), expressible water (E.W), and water holding capacity (WHC) of untreated and treated beef product *

Treatments	Storage time	M.C %	E.W %	WHC %
Untreated sample	Zero time	66.92	10.92	56.00
	One month	66.87	17.85	49.02
	3 months	66.84	27.63	39.21
	6 months	66.61	31.89	34.72
Rosemary treated sample	Zero time	66.83	11.04	55.79
	One month	66.81	15.78	51.03
	3 months	66.78	21.98	44.80
	6 months	66.71	27.51	39.20
Green tea treated sample	Zero time	66.53	11.87	54.66
	One month	66.51	16.05	50.46
	3 months	66.51	22.01	44.50
	6 months	66.48	27.48	39.00

*Means of three determinations.

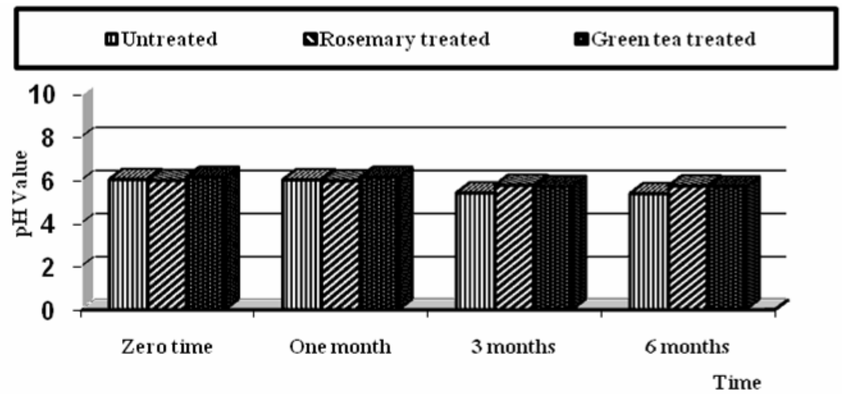

Figure 2. Effect of storage time on the pH values for untreated and treated beef product

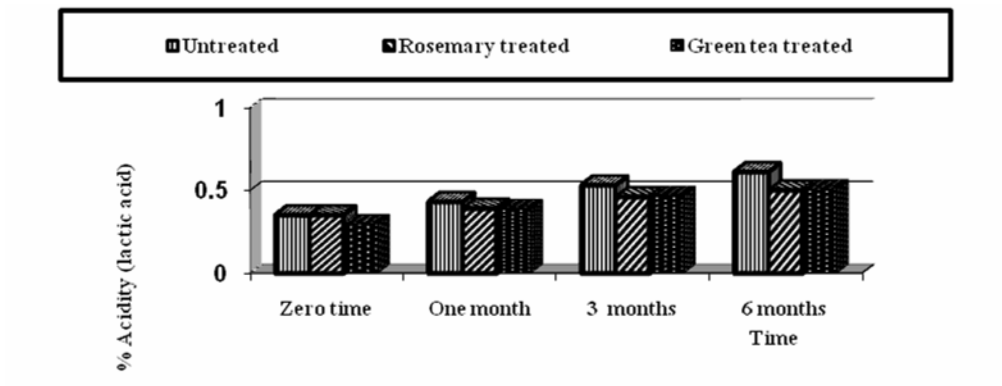

Figure 3. Effect of storage time on the total acidity values for untreated and treated beef product

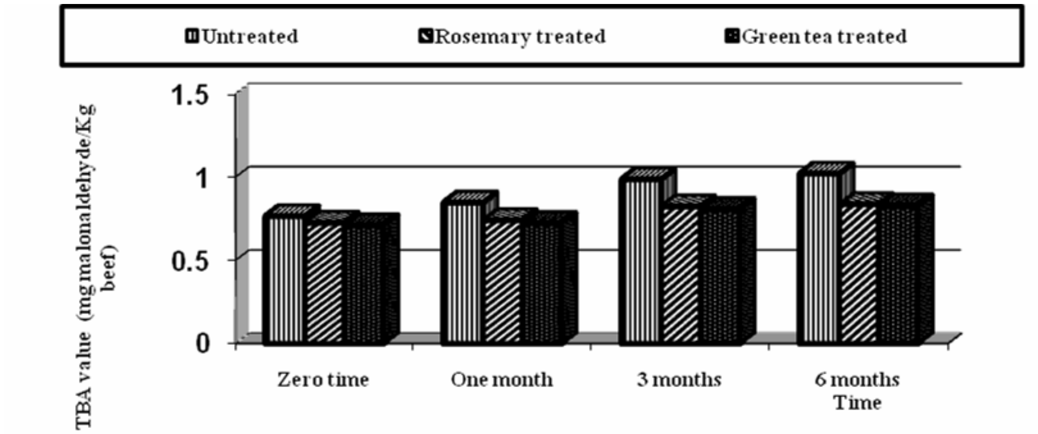

Figure 4. Effect of storage time on the TBA values for untreated and treated beef product

Lipid oxidation is a major cause of chemical spoilage in food systems. It is considered as one of the major causes of quality deterioration of processed meat. Figure 4, illustrates the effect of storage time and the natural antioxidants on the TBA values for the beef product. TBA values increased over time for all samples. The increment was rapid for the untreated sample and the greatest change occurring between the third and the sixth month of storage, whereas the beef treated samples presented overall lower levels of changes in the TBA values. This could be explained by the effect of both rosemary and green tea as natural antioxidants. Green tea treated sample showed less TBA value compared with rosemary treated one. In general the effect of a certain potential antioxidants might vary considerably depending on a complex interaction between various factors.

Table 5. Effect of storage time on the sensory evaluation of untreated and treated beef product

Treatments	Storage time	Color	Flavor	Juiciness	Over all acceptability
Untreated sample	Zero time	9.00	8.00	8.00	8.50
	One month	8.50	8.00	7.50	8.00
	3 months	7.50	7.50	7.00	7.50
	6 months	7.00	7.00	7.00	7.00
Rosemary treated sample	Zero time	9.00	8.00	8.00	8.50
	One month	9.00	8.00	8.00	8.50
	3 months	8.50	8.00	7.50	8.00
	6 months	8.00	7.50	7.00	7.50
Green tea treated sample	Zero time	8.50	7.50	8.00	8.00
	One month	8.50	7.50	8.00	8.00
	3 months	8.00	7.50	7.50	8.00
	6 months	8.00	7.00	7.00	7.50

* Means of ten determinations.

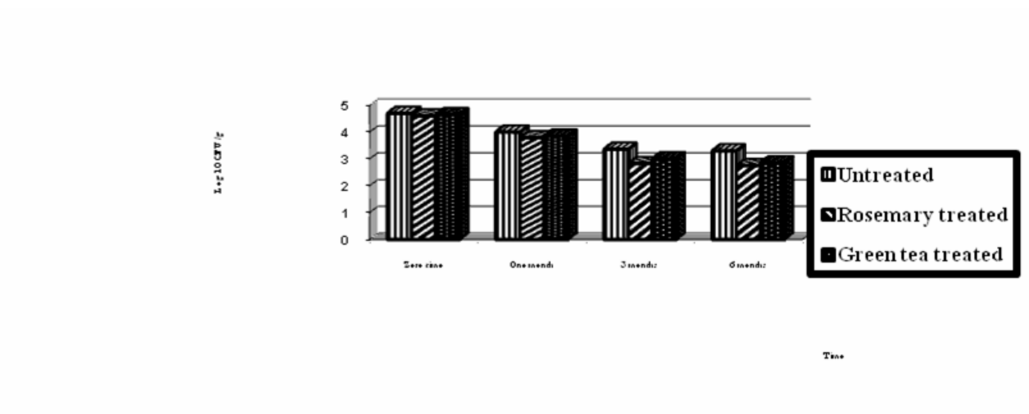

Figure 5. Effect of storage time in combination with edible packaging on the total plate count of untreated and treated beef product

Table 5 - represents the effect of storage time and natural antioxidants treatment in addition to edible packaging material on the sensory evaluation of low fat beef product. The data showed that values for color, flavor, juiciness and overall acceptability for all samples were decreased with the progressing of storage time. The reduction rate of color for the untreated sample was higher than the treated ones followed by the green tea treated sample. Regarding the flavor, sample treated by the green tea had the lowest value compared with the other samples and this could be due to effect of the distinct bitter flavor for the green tea.

Chitosan as a natural polysaccharide is generally considered as safe, biocompatible and biodegradable material. It has broad range of applications edible film in the food industry.

Figure 5 - illustrates the effect of storage time in combination with chitosan as an edible packaging on the total plate count of untreated and treated beef product.

The data showed reduction in the total bacterial count for all treated and the untreated samples all over the time of storage. The reduction was higher in the natural antioxidants treated samples than the untreated one. There were no differences among microbial counts from different antioxidants were observed at any time of determination during storage period. In general the application of chitosan as an edible primary packaging was very effective in reducing all bacterial counts in both untreated and treated beef product during the frozen storage. Both chitosan as antimicrobial agent and low storage temperature interacted and produced impact effect and resulted in reduction of the total bacterial count.

Uticaj dva prirodna oksidanta u kombinaciji sa jestivim pakovanjem na stabilnost duboko zamrznutih goveđih proizvoda niskog sadžaja masti

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Rezime

U cilju procene efekta dva različita prirodna antioksidansa kombinovana sa jestivim pakovanjem na stabilnost goveđih proizvoda sa niskim sadržajem masti, smrznutih tokom šest meseci, određen je hemijski sastav, voda (EW), spososobnost zadržavanja vode (WHC), pH vrednost, ukupna kiselost (mlečna kiselina), tiobarbituratna kiselina (TBA), ukupan broj aeroba i senzorna ocena. Podaci su pokazali da nije došlo do promene hemijskog sastava kao rezultat dodavanja antioksidanata. Takođe se EW vrednost tokom vremena skladištenja povećavala praćena opadanjem WHC vrednosti. Povećanje EW i smanjenje WHC vrednosti bilo je najveće kod netretiranih uzoraka. Podaci pokazuju opadanje pH vrednosti i povećanje kiselosti kod treterinih kao i kod netretiranih uzoraka. Redukcija pH

vrednosti i povećanje kiselosti bili su veći kod netretiranih uzoraka u poređenju sa tretiranim. TBA vrednost se tokom vremena povećavala kod svih uzoraka. Ovo povećanje je bilo rapidno kod netretiranih uzoraka. Stepen redukcije boje netretiranih uzoraka je bio veći od tretiranih, praćen zelenim čajem tretiranim uzorcima, dok je ocena ukusa za uzorke tretirane zelenim čajem bila najniža u poređenju sa ostalim uzorcima. Rezultati ukazuju da je upotreba kitozana kao jestivog omotača odložila proliferaciju aerobnih bakterija.

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