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The effects of natural antioxidants on colour, lipid stability and sensory evaluation of fresh beef patties stored at 4ºC

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Abstract

The effects of rosemary extract, chitosan and carnosine, added individually or in combination, on colour, lipid stability and sensory attributes of beef patties were investigated. All samples were stored for up to 15 days at 4 ± 1 ºC. Surface colour (Hunter L^*, a^* and b^* values), and lipid oxidation (2-thiobarbituric acid reactive substances) were measured on days 0, 1, 3, 6, 9, 12 and 15 of storage for raw beef patties. While, sensory attributes (odour, colour, texture, taste and overall acceptability) were carried out on cooked beef patties after 0, 3, 6, 9, 12 and 15 days of storage. The obtained results showed that addition of tested antioxidants, individually or in combination, significantly ($p < 0.05$) affected colour, lipid stability and sensory attributes compared to control during storage. Chitosan and its combinations with either rosemary extract or carnosine, and especially the former combination, showed the most intense red colour ($p < 0.05$) when compared to rosemary extract, carnosine or the control. Also, results showed that the combination of rosemary extract with chitosan provided the best antioxidative protection with regard to meat deterioration, indicating a possible synergistic effect.

Keywords: Natural antioxidants, Colour, Lipid Stability, Sensory evaluation, Beef patties

1. Introduction

Consumers demand high quality and convenient meat products, with natural flavour and taste, and they appreciate the fresh appearance of beef [22]. Colour is an important parameter that consumers use to judge the freshness and wholesomeness of beef. It has substantial influence on acceptability and purchasing decision at retail points [14]. Oxidative processes, which occur during raw material storage, processing, heat treatment and further storage of final products, are major non-microbiological factors involved in quality deterioration of meat during refrigerated storage. Oxidation induces modifications of muscle lipids and proteins and, therefore, affects the organoleptic and nutritional properties of meat and meat products. This is reflected in economic losses and health disorders [23,28]. Oxidative processes are also associated with discolouration of meat products, as lipid oxidation results in the formation of pro-oxidants capable of reacting with oxymyoglobin, which lead to metmyoglobin formation [17]. Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts [12]. The major strategies for preventing lipid oxidation are the use of antioxidants and restricting the access to oxygen during storage vacuum-packaging [56]. The antioxidant additives are added to fresh and processed meats to prevent oxidative rancidity, retard development of off-flavours, and improve colour stability [44]. Synthetic antioxidants like BHT and BHA have been successfully used to prevent the

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oxidation in meat products. However, the synthetic antioxidants used currently have been found to exhibit various health effects [52]. Reports of adverse health effects of these synthetic chemicals have lead to growing interest in the use of natural sources of antioxidants in meat products. In addition there has been growing interests in natural antioxidants because of their safety, consumer acceptability and greater application in extending the shelf life of foods.

Rosemary (*Rosemary officinalis* L.) extracts have been widely reported as having especially potent antioxidant effects. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which act as a hydrogen donor to interfere with the free radical chain reaction [2,4,20]. Other plant phenolic compounds can also act as metal chelators and singlet oxygen quenchers [29,52]. A large number of authors found rosemary to have a very favourable effect on slowing down the process of oxidation of fat in various meats, such as sausages [20,51], beef burgers [21] beef steaks [55], beef patties [35], pork patties [38,45], and chicken patties [48].

Chitosan is a deacetylated form of chitin and a straight-chain polymer of glucosamine and N-acetylglucosamine [43,58]. Chitin is obtained from the shell of crustaceans, the cuticles of insects and the cell wall of fungi, it is the second most abundant biopolymer in nature [30,58]. Antioxidant properties of chitosan in muscle foods have been documented, and recently there has been an increasing interest in the use of chitosan in commercial food applications [46]. The antioxidant property of chitosan is attributed to its ability to chelate free iron, released by myoglobin degradation during meat storage [26]. Effectiveness of chitosan on lipid oxidation of refrigerated ground beef was studied by Darmadji and Izumimoto, (1994) [8] who observed that the addition of chitosan at 1% was effective in minimizing lipid oxidation-induced quality deterioration. Furthermore, recent studies highlighted the effectiveness of chitosan in aerobically stored refrigerated fresh pork sausages [20,54] and frozen ground beef burgers [21] to minimize lipid oxidation and improve color stability.

Carnosine (b-alanyl-L-histidine), an endogenous dipeptide, is one of the most abundant nitrogenous compounds present in the non-protein fraction of vertebrate skeletal muscle and certain other tissues, is synthesized from b-alanine and L-histidine by carnosine synthetase [18,31,32]. Carnosine inhibits the catalysis of lipid oxidation by a wide range of pro-oxidants [5] and acts as a free radical-scavenger, metal chelator and hydrogen donor [7]. It also can interfere in the initiation step of oxidation, decrease the amount of preformed peroxides and react with some secondary products [27]. In recent years, there have been some interests in the antioxidant potential of carnosine in meats [6,34,47]. Research results indicated that carnosine (0.5–1.5%) inhibited lipid oxidation in beef [3,36], turkey [6], and pork [10,11].

The aim of this work was to assess the antioxidant properties of carnosine, chitosan and rosemary in beef patties during cold storage.

2. Materials and methods

2.1. Natural antioxidants and chemicals. L-carnosine, chitosan and 2-Thiobarbituric acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Rosemary extract (Guardian ™) was purchased from Danisco, Denmark. All other chemicals used were of analytical grade or the highest grade available and were obtained either from Sigma-Aldrich or Merck (Darmstadt, Germany).

2.2. Beef patties Preparation. Beef brisket and flank were purchased fresh from a local market (Ismailia, Egypt). Beef meat was trimmed of visible fat and connective tissue, their fat contents were determined and they were then frozen at -20 °C until processing. After thawing, they were cut in small cubes and ground separately using a conventional meat grinder (Bizerba, Wilhelm Kraut GmbH & Co KG, Germany) through a plate with 6 mm steel plate. The raw materials were then thoroughly mixed at an appropriate ratio to achieve a fat content of approximately 15% in the mixture and were ground again through a 4 mm steel plate. Portions of equal parts of the meat mixture were mixed with salt (2%) and the tested antioxidants (g/kg) according to the following formulations: (1) control (without antioxidant); (2) 0.2 g rosemary extract (ROSE); (3) 10 g chitosan (CHI); (4) 10 g carnosine (CAR); (5)

0.2 g (ROSE) plus 10 g (CHI); (6) 10 g (CHI) plus 10 g (CAR); and (7) 0.2 g (ROSE) plus 10 g (CAR).

Beef patties (50±1 g) were formed using a beef patties former (Italmans, Italy), and placed on styrofoam trays. The tray with the patties was introduced in a pouch made of a polyethylene. The patties were stored for 15 days at 4±1 ºC. All experiments were repeated three times in order to remove effects deriving from the initial quality of raw material.

pH, TBARS and colour were determined at zero, 1, 3, 6, 9, 12 and 15days of cold storage. Whereas, sensory attributes were performed on cooked samples after zero, 3, 6, 9, 12 and 15 days of cold storage.

*2.3. Proximate analysis.*Moisture, protein, fat and ash were determined only in the initial meat mixture used for the preparation of the experimental beef patties. The levels of moisture, protein, fat and ash were determined according to the methods described by AOAC, (2000) [1].

2.4. pH determination. The measurement of pH was carried out on 10 g of sample homogenized in distilled water (1:10 sample/water). The pH value of the sample was determined using a Jenway pH meter (Jenway 3010; Jenway LTD., Essex, UK) equipped with a J95, 924001 electrode (Jenway LTD., Essex, UK).

2.5. Lipid oxidation measurement. The extent of lipid oxidation was determined by 2-thiobarbituric acid reactive substance (TBARS) assay using the distillation method described by Tarladgis et al., (1960) [57], as modified by Shahidi et al., (1987) [53]. Distillates of 10 g samples were reacted with TBA reagent (0.02M aqueous solution of 2-thiobarbituric acid) and the absorbance of the resultant pink-coloured chromogen was measured at 532 nm using a spectrophotometer (6505 UV/Vis, Jenway Ltd., Felsted, Dunmow, UK). Values of absorbance at 532 nm were multiplied by 8.1 and expressed as TBARS numbers in mg malondialdehyde equivalents/kg sample [53].

2.6. Colour measurement. Objective measurement of colour (CIE L^*, a^* and b^*) was performed at the surface of beef patties using a spectrophotocolorimeter (Konica Minolta Sensing, Inc. Osaka, Japan). In this coordinate system, the L^* value is a measure of lightness, ranging from 0 (black) to 100 (white), the a^* value ranges from -100 (greenness) to +100 (redness) and the b^* value ranges from -100 (blueness) to +100 (yellowness).

2.7. Sensory evaluation. Samples of beef patties were evaluated according to the method described by García et al., (2009) [19], ten experienced panelists (from both sexes in the age range of 25-55 years) were chosen from the staff members of the Department of Food Technology at Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Panelists were selected according to their habits, their familiarity with the patties to be analyzed, their sensitivity and the ability to reproduce the evaluation made. Moreover, they received a preparatory session prior to testing so that each panelist could thoroughly discuss and clarify each attribute to be evaluated. Seven beef patties were cooked on a preheated electric grill (WA-BBQ 01, White Whale, China) at approximately 180 ºC for 2 min, then turned over and cooked for another 2 min. The temperature of the centre of patties reached approximately 80 ºC. A beef patty of each sample was served to the panelists. Unsalted bread and water were also provided to clean the palate between samples.

A hedonic test was carried out using non-structured 9 point scales (0 = dislike extremely and 9 = like extremely) in which the panelists evaluated different attributes: odour, colour, texture, taste and overall acceptability.

2.8. Statistical analysis. All measurements were done in triplicates and data are presented as means ± SD. Analysis of variance (ANOVA) accompanied with Duncan test using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago) were conducted to identify the significance ($p < 0.05$) between means of treatments.

3. Result and discussion

3.1. Proximate analysis. Results of Proximate analysis of the meat mixture used for the preparation of beef patties are presented in Table 1. Our results are close to these observed by Georgantelis et al., (2007b) [21] who reported that Mean percent contents of moisture, protein, fat and ash for the meat mixture used in for the preparation of experimental beef burgers were 64.4%, 18.1%, 15.2% and 0.9%, respectively.

3.2. The pH values. The pH values of different formulas of beef patties are shown in Fig. 1. The pH values of all samples of beef patties prepared with chitosan and carnosine (CHI, CAR, ROSE + CHI, CHI + CAR, ROSE + CAR) were significantly ($p < 0.05$) higher compared to ROSE and control. These data are in agreement with Georgantelis et al., (2007a) [20] who reported that samples containing chitosan showed higher ($p < 0.05$) pH values compared to rosemary, α-tocopherol and controls. Also, Das et al., (2006) and Sánchez-Escalante et al., (2003) [9,50] mentioned that the pH values of the carnosine-treated samples were significantly ($p < 0.05$) higher than those of the control sample.

The pH value of all beef patty samples slightly decreased during the first 3 days of storage, whereas after day 3 there was a gradual increase. This decrease indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of endoprotease activity or the proteolytic microbial flora present in the raw meat [42].

3.3. Lipid oxidation. Determination of thiobarbituric acid reactive substances (TBARS) values has been found to be a good indicator for lipid oxidation in meat and meat products [16]. The TBARS values of different formulas of beef patties are shown in Fig. 2. TBARS formation increased rapidly with storage time in the control samples. Addition of the examined antioxidants showed significant ($p < 0.05$) effects compared to control during storage of beef patties. Samples containing rosemary extract and its combinations (ROSE, ROSE + CHI and ROSE + CAR) together with those containing (CHI + CAR) exhibited the lowest ($p < 0.05$) TBARS values compared to those containing the individual antioxidants (CHI, CAR), a fact that indicates the occurrence of a synergistic effect.

These results are in good agreement with Georgantelis et al., (2007a) [20] who observed lower TBARS values for fresh pork sausage treated with rosemary extract, chitosan and these combinations. The effectiveness of rosemary as an inhibitor of lipid oxidation in meat products has been documented [15,39,41,51]. Addition of rosemary and carnosine to beef patties resulted in significant ($p < 0.05$) reduction of TBARS during storage [49]. Darmadji and Izumimoto, (1994) [8] found that the TBA value of beef meat containing 10 g/kg chitosan was at the same level after 10 days of storage at 4 ºC as it was on day 0, whereas the respective value of control samples increased sharply. Carnosine exerted a significant ($p < 0.05$) inhibition of TBARS formation [13,49,50,60]. The inhibitory effects of carnosine on lipid oxidation might be due to scavenging free radicals and chelating transition metals [32,33].

Table 1. Proximate analysis of the meat mixture used for the preparation of beef patties

Component (%)	Mean ± SD
Moisture	64.6 ± 0.25
Protein	18.8 ± 0.15
Fat	15.1 ± 0.33
Ash	0.90 ± 0.01

Data represent averages of three independent repeats ± standard deviation

Table 2. Effect of natural antioxidants on Hunter colour values ($L*$, $a*$ and $b*$) of beef patties during storage at 4 ± 1 °C

Storage time (days)	Treatment	$L*$	$a*$	$b*$
0	CON	49.36±0.158e	13.98±0.125d	13.73±0.108d
	ROSE	51.12±0.057ab	13.72±0.044e	13.85±0.070c
	CHI	50.79±0.071c	15.67±0.151a	14.12±0.062a
	CAR	49.61±0.049d	13.78±0.047e	13.79±0.070cd
	ROSE + CHI	51.15±0.053a	15.34±0.061c	14.04±0.059ab
	CHI + CAR	50.72±0.146c	15.51±0.032b	13.98±0.066b
	ROSE + CAR	50.99±0.162b	14.02±0.078d	13.72±0.082d
1	CON	49.49±0.065f	12.32±0.07f	11.78±0.104e
	ROSE	50.66±0.072c	13.55±0.076d	12.34±0.036d
	CHI	50.82±0.021b	15.33±0.086b	13.81±0.073a
	CAR	50.36±0.041e	13.09±0.055e	12.41±0.062d
	ROSE + CHI	51.11±0.078a	15.44±0.089a	12.66±0.159c
	CHI + CAR	50.56±0.095d	15.3±0.092b	12.88±0.050b
	ROSE + CAR	50.86±0.092b	13.9±0.072c	12.38±0.027d
3	CON	49.83±0.032f	10.52±0.088f	11.03±0.077e
	ROSE	51.75±0.051a	12.32±0.041d	12±0.075d
	CHI	50.5±0.026d	14.59±0.055a	12.24±0.065b
	CAR	50.92±0.079c	11.98±0.111e	12.18±0.073b
	ROSE + CHI	50.86±0.062c	14.65±0.094a	12.03±0.038cd
	CHI + CAR	50.12±0.095e	14.12±0.089b	12.55±0.033a
	ROSE + CAR	51.32±0.083b	12.56±0.046c	12.1±0.032c
6	CON	50.73±0.065d	9.68±0.076f	10.65±0.072f
	ROSE	52.04±0.046a	12.02±0.106d	11.43±0.089d
	CHI	50.01±0.103f	14.02±0.081b	11.86±0.076c
	CAR	51.33±0.065c	11.04±0.067e	11.26±0.038e
	ROSE + CHI	50.21±0.079e	14.2±0.073a	11.4±0.025d
	CHI + CAR	49.73±0.047g	13.78±0.047c	12.28±0.036a
	ROSE + CAR	51.83±0.057b	11.98±0.050d	12.04±0.051b
9	CON	51.2±0.109d	8.19±0.141f	10.56±0.095f
	ROSE	52.77±0.054a	11.36±0.071d	11.16±0.094c
	CHI	49.31±0.035f	13.52±0.047b	11.73±0.048a
	CAR	51.99±0.075c	10.21±0.057e	10.91±0.080e
	ROSE + CHI	49.92±0.026e	13.76±0.1a	10.98±0.036de
	CHI + CAR	48.86±0.079g	13.48±0.089b	11.31±0.063b
	ROSE + CAR	52.21±0.037b	11.5±0.041c	11.02±0.028d
12	CON	52.02±0.041d	7.4±0.067f	10.83±0.055c
	ROSE	52.96±0.031a	10.7±0.095d	11.37±0.055a
	CHI	48.85±0.086f	12.81±0.054a	11.09±0.041b
	CAR	52.17±0.051c	9.58±0.056e	10.16±0.093d
	ROSE + CHI	49.13±0.058e	12.87±0.051a	10.87±0.034c
	CHI + CAR	47.98±0.101g	12.63±0.019b	11.06±0.026b
	ROSE + CAR	52.51±0.110b	11.03±0.037c	10.83±0.056c
15	CON	52.1±0.085d	6.22±0.054g	10.36±0.041d
	ROSE	53.37±0.058a	10.25±0.042e	10.97±0.103ab
	CHI	48.2±0.045f	11.65±0.047c	10.93±0.089b
	CAR	52.61±0.076c	8.92±0.092f	10.05±0.052e
	ROSE + CHI	48.62±0.065e	12.31±0.089b	11.04±0.044a
	CHI + CAR	47.91±0.071g	12.43±0.09a	10.95±0.053b
	ROSE + CAR	52.78±0.065b	11.18±0.049d	10.59±0.035c

CON: control (without antioxidant); ROSE: 0.2 g rosemary extract/kg meat; CHI: 10 g chitosan/kg meat; CAR: 10 g carnosine/kg meat; ROSE + CHI: 0.2 g rosemary extract plus 10 g chitosan/kg meat; CHI + CAR: 10 g chitosan plus 10 g carnosine/kg meat; ROSE + CAR: 0.2 g rosemary extract plus 10 g carnosine/kg meat

$L*$ = lightness; $a*$ = redness; $b*$ = yellowness

Means in the same column for the same storage day with a different letter (a-g) differ significantly ($p < 0.05$)

Table 3. Effect of natural antioxidants on sensory attributes of beef patties during storage at 4 ± 1 °C

Storage time (days)	Treatment	Odour	Colour	Texture	Taste	Overall acceptability
0	CON	7.9±0.516a	8.53±0.381ab	7.85±0.626a	8.5±0.471b	8.03±0.583a
	ROSE	7.85±0.669a	8.5±0.333ab	8.25±0.354a	8.65±0.242ab	8.13±0.460a
	CHI	8.05±0.643a	8.6±0.316ab	8.33±0.409a	8.7±0.350ab	8.1±0.460a
	CAR	8.2±0.483a	8.4±0.516b	7.95±1.066a	8.55±0.284ab	8±0.408a
	ROSE + CHI	8.18±0.442a	8.8±0.258a	8.1±0.516a	8.8±0.258a	8.25±0.264a
	CHI + CAR	7.95±0.599a	8.75±0.264a	7.95±0.550a	8.75±0.264ab	8.15±0.412a
	ROSE + CAR	8.1±0.658a	8.65±0.242ab	8.1±0.658a	8.65±0.242ab	8±0.471a
3	CON	7.6±0.568ab	8±0.624b	7.9±0.460a	7.83±0.553b	7.1±0.516a
	ROSE	7.65±0.626ab	8.3±0.587ab	7.95±0.438a	8.1±0.316ab	7.4±0.738a
	CHI	7.5±0.577b	8.28±0.478ab	8.05±0.438a	8.15±0.580ab	7.45±0.497a
	CAR	8±0.527a	8.1±0.316ab	8.1±0.658a	7.9±0.460ab	7.25±0.425a
	ROSE + CHI	8.05±0.369a	8.5±0.408a	7.85±0.412a	8.25±0.425a	7.5±0.624a
	CHI + CAR	8±0.471a	8.23±0.299ab	7.75±0.486a	8.2±0.350ab	7.5±0.577a
	ROSE + CAR	8.05±0.550a	8.1±0.516ab	8.1±0.460a	8.15±0.412ab	7.3±0.587a
6	CON	6.3±0.753b	7.08±0.688c	7.25±0.486a	7.1±0.907b	7±0.527a
	ROSE	7.03±0.886a	7.8±0.919ab	7.5±0.527a	7.9±0.738a	7.45±0.438a
	CHI	7.13±0.775a	7.83±0.800ab	7.5±0.913a	7.65±0.747ab	7.3±0.675a
	CAR	7±0.707a	7.6±0.658bc	7.65±0.818a	7.4±0.516ab	7.4±0.460a
	ROSE + CHI	7.65±0.580a	8.2±0.350a	7.65±0.818a	8±0.577a	7.35±0.474a
	CHI + CAR	7.35±0.709a	7.95±0.369ab	7.1±0.775a	7.88±0.615a	7.4±0.615a
	ROSE + CAR	7.15±0.784a	7.8±0.633ab	7.4±0.658a	7.9±0.615a	7.2±0.538a
9	CON	5±0.817c	6.5±0.882b	7.15±0.669a	6±0.943b	6.1±0.699b
	ROSE	7.3±0.715a	7.13±0.810a	7.1±0.699a	7.5±0.624a	7±0.817a
	CHI	7.05±0.497a	7.03±0.712ab	7.25±1.137a	7.3±0.823a	6.9±0.810a
	CAR	6.3±0.587b	6.95±0.550ab	7.15±0.914a	6.95±0.685a	6.95±0.599a
	ROSE + CHI	7.3±0.715a	7.4±0.615a	7.3±0.823a	7.6±0.843a	7.1±0.316a
	CHI + CAR	7.05±0.832a	7.25±0.425a	7.15±0.818a	7.5±0.577a	7.05±0.798a
	ROSE + CAR	6.95±0.896ab	7.3±0.715a	7.45±0.643a	7.43±0.800a	7±0.850a
12	CON	4.35±0.669c	6.03±0.820c	6.45±0.643a	5.4±0.907c	5.4±0.615d
	ROSE	5.95±0.685a	7.2±0.888ab	6.8±0.633a	7.1±0.568ab	6.4±0.775abc
	CHI	5.75±0.791ab	6.9±0.394ab	6.9±0.994a	6.9±0.966ab	6.1±0.699bc
	CAR	5.1±0.394b	6.75±0.354b	6.75±1.339a	6.53±0.558b	5.9±0.615cd
	ROSE + CHI	6.05±0.725a	7.45±0.369a	7.05±0.599a	7.2±0.422a	6.7±0.422a
	CHI + CAR	5.95±0.798a	7.15±0.914ab	6.7±0.789a	7.2±0.483a	6.5±0.471ab
	ROSE + CAR	5.95±0.985a	7.13±0.907ab	6.9±0.658a	7.15±0.626a	6.6±0.775ab
15	CON	4.1±0.394c	5.9±1.049c	6.5±0.707a	4.7±0.633c	4.55±0.550c
	ROSE	5.65±0.747a	6.85±0.669ab	6.45±0.725a	6.85±0.852a	6.05±1.040ab
	CHI	5.4±0.615ab	6.5±0.333abc	6.75±0.890a	6.5±0.624ab	5.85±0.852ab
	CAR	4.8±0.422b	6.3±0.633bc	6.45±0.762a	6.05±0.896b	5.65±0.338b
	ROSE + CHI	5.95±0.762a	7.15±0.580a	6.8±0.856a	6.95±0.497a	6.5±1.202a
	CHI + CAR	5.45±0.685a	6.95±0.685ab	6.4±0.658a	6.9±0.615a	6.25±0.540ab
	ROSE + CAR	5.75±0.921a	6.8±0.949ab	6.7±0.789a	6.75±0.540a	6.3±0.919ab

CON: control (without antioxidant); ROSE: 0.2 g rosemary extract/kg meat; CHI: 10 g chitosan/kg meat; CAR: 10 g carnosine/kg meat; ROSE + CHI: 0.2 g rosemary extract plus 10 g chitosan/kg meat; CHI + CAR: 10 g chitosan plus 10 g carnosine/kg meat; ROSE + CAR: 0.2 g rosemary extract plus 10 g carnosine/kg meat

Means in the same column for the same storage day with a different letter (a-d) differ significantly ($p < 0.05$)

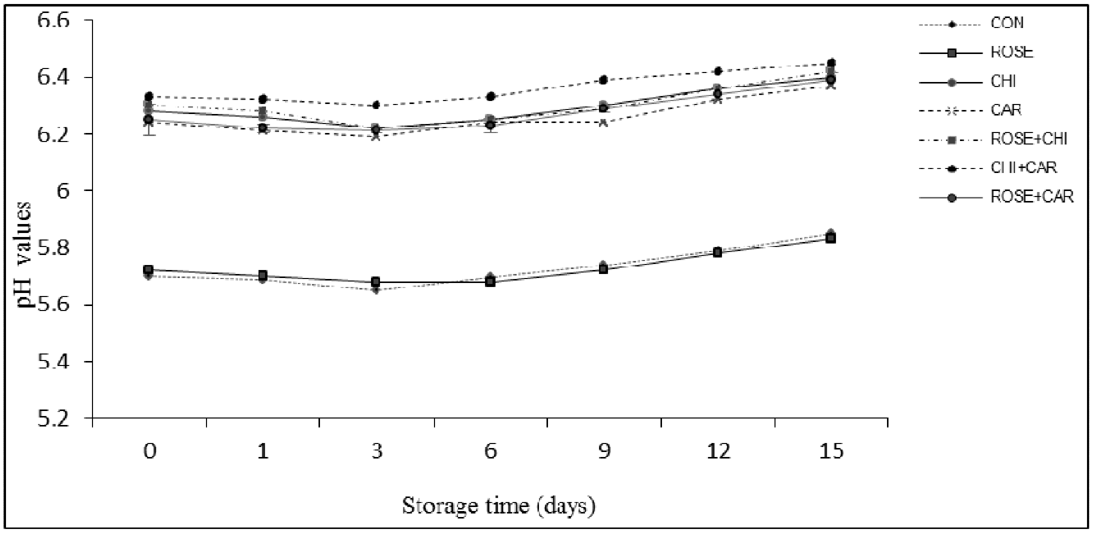

Figure 1. Effect of natural antioxidants on pH values of beef patties during storage at 4 ± 1 °C

CON: control (without antioxidant); ROSE: 0.2 g rosemary extract/kg meat; CHI: 10 g chitosan/kg meat; CAR: 10 g carnosine/kg meat; ROSE + CHI: 0.2 g rosemary extract plus 10 g chitosan/kg meat; CHI + CAR: 10 g chitosan plus 10 g carnosine/kg meat; ROSE + CAR: 0.2 g rosemary extract plus 10 g carnosine/kg meat

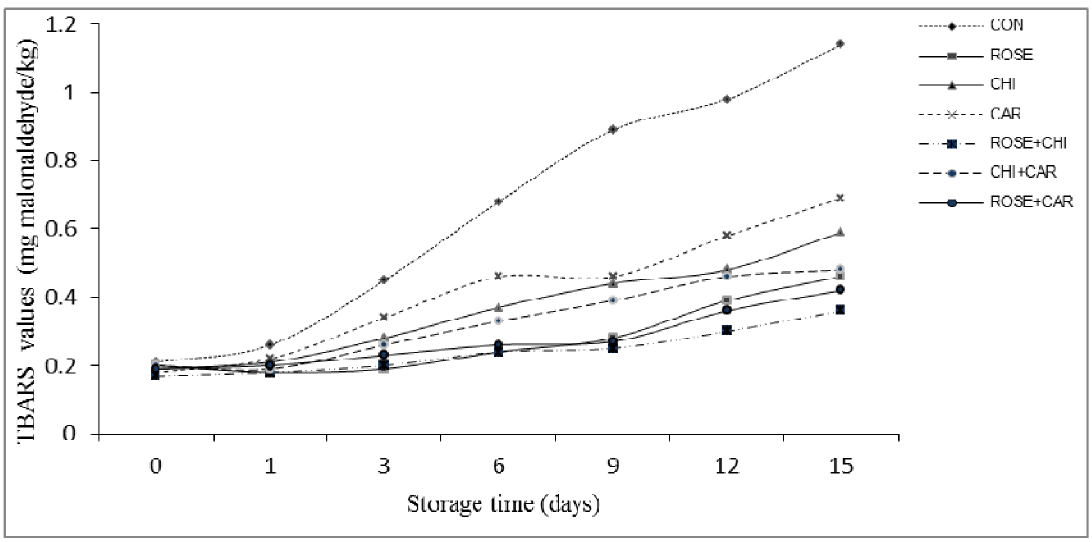

Figure 2. Effect of natural antioxidants on TBARS values of beef patties during storage at 4 ± 1 °C

CON: control (without antioxidant); ROSE: 0.2 g rosemary extract/kg meat; CHI: 10 g chitosan/kg meat; CAR: 10 g carnosine/kg meat; ROSE + CHI: 0.2 g rosemary extract plus 10 g chitosan/kg meat; CHI + CAR: 10 g chitosan plus 10 g carnosine/kg meat; ROSE + CAR: 0.2 g rosemary extract plus 10 g carnosine/kg meat

3.4. Colour evaluation. The colour parameters of different formulas of beef patties are summarized in Table 2. For L^* value, fresh beef patties with rosemary extract had the highest value ($p < 0.05$) compared to the other treatments and control.

As regards to changes in L^* values during storage, there was a decreasing trend in samples containing Chitosan (CHI, ROSE+CHI and CHI+CAR). On the other hand, L^* values during storage of samples containing (ROSE, CAR, ROSE + CAR and CON) showed an increasing trend, which could be due to gradual protein decomposition, leading to increase of light scattering [40].

The *a** values of beef patties were significantly ($p < 0.05$) affected by the addition of the examined antioxidants. Samples containing chitosan, individually or in combination (CHI, ROSE + CHI and CHI + CAR) had a higher *a** values than samples containing ROSE, CAR, ROSE + CAR and CON throughout the whole period of storage. The best synergistic effect was obtained from the combination of ROSE + CHI. Similar results were observed by Georgantelis et al., (2007b) [21] who found that *a** values, colour stability and retention of red colour was significantly ($p < 0.05$) improved in samples from treatments containing chitosan. A decreasing trend was observed as regards to *a** values, which is attributed to the gradual oxidation of myoglobin and accumulation of metmyoglobin with time [13,24,37]. Sánchez-Escalante et al., (2001) [49] reported that samples with rosemary and carnosine had significantly ($p < 0.05$) higher *a** values than of the control during storage. Similar results were observed by Sebranek et al., (2005) [51] who found that 2500 ppm of rosemary extract was more effective in maintaining colour than the control, and appeared to be more effective than the BHA/BAT treatment. Sánchez-Escalante et al., (2003) [50] reported that addition of carnosine maintained the red colour of fresh meat for 8 days longer than control without added carnosine. The colour stabilizing effects of carnosine may be the result of its ability to chelate transition metals involved in free radical generation and/or free radical scavenging, thereby delaying the oxidation of oxymyoglobin to metmyoglobin [34].

The *b** values of chitosan-treated samples (CHI, ROSE + CHI and CHI + CAR) were significantly ($p < 0.05$) higher than samples containing ROSE, CAR, ROSE + CAR and CON, which could be attributed to the natural yellowish colour of chitosan affecting the patties colour. Similar observations have been also found by Georgantelis et al., (2007b) [21] in beef burger, Jo et al., (2001) [25] and Youn et al., (1999) [59] in pork sausage with added chitosan. During refrigerated storage, a decreasing trend in the control and all treated samples was observed.

*3.5. Sensory evaluation.*The effect of different antioxidants on the sensory properties of beef patties during storage are presented in Table 3. In general, all beef patty samples treated with antioxidants had significantly ($p < 0.05$) higher odour, colour, taste and overall acceptability scores than the control. As regards to texture, no significant ($p>0.05$) differences were observed between the treated samples and the control. These data are in agreement with Mohamed and Mansour, (2012) [41] who reported that the flavour scores of beef patties prepared without addition of antioxidants were significantly ($p < 0.05$) lower than those of other formulas, and panelists detected a rancid flavour in beef patties formulated without addition of antioxidants during storage. Among the treated samples, the containing ROSE + CHI showed highest sensory properties scores, followed by ROSE + CAR, CHI + CAR, ROSE, CHI and CAR. This intensity order of the protective ability of the antioxidants on meat quality, as measured by sensory evaluation, consistently agreed with their effectiveness in preventing both myoglobin and lipid oxidations. Similar conclusion was observed by Georgantelis et al., (2007b) [21] who mentioned that beef burger samples from treatments containing chitosan had a more intense red colour than samples containing only rosemary extract and α-tocopherol or the controls. Sánchez-Escalante et al., (2001) [49] reported that the presence of rosemary, both alone and with ascorbic acid, extended the fresh meat odour during storage. Also, Badr, (2007) [3] found that samples containing carnosine had a significant ($p < 0.05$) effect in maintaining an acceptable red colour of irradiated and non-irradiated ground beef and raw patties during refrigeration and frozen storage.

4. Conclusion

Inclusion of rosemary extract (ROSE), chitosan (CHI) and carnosine (CAR), added individually or in combination, in beef patties can enhance colour, reduce lipid oxidation and improve the sensory attributes. Moreover, the antioxidant combinations are superior to individual addition of antioxidants in inhibiting the colour change, lipid oxidation and improve the sensory attributes. The combination of ROSE + CAR provided the best antioxidant protection against meat deterioration during 15 days of cold storage.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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