Effects of chitosan on quality attributes fresh meat slices stored at 4^0C

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A B S T R A C T

The present study was conducted to evaluate the effect of natural substances as chitosan, thyme oil, lactic acid and acetic acid on microbial, chemical and sensorial quality of fresh meat slices during 18 day of storage at 4^0C and periodically analysed for total mould counts, pH, surface colour (Hunter L*, a* and b* values), 2-thiobarbituric acid reactive substance and Total Volatile Nitrogen were measured on days 0, 3, 6, 9, 12, 15 and 18 day of storage. The obtained results showed that addition of tested antifungal and antioxidants, individually, affected colour, lipid stability and sensory attributes of the meat slices compared to control during storage. Chitosan (1.5, 1.0 and 0.5 % w/v) showed the most intense red colour than other tested antifungal and antioxidants (thyme essential oil, lactic acid and acetic acid) when compared to the control. Also, the results showed that the initial total count (day 0) value for the fresh meat slices was $1x10^6$ CFU /g, then decrease gradually until 9 day not detected any colony by using chitosan 1.5%. While in treated samples with thyme oil 1.5% and lactic acid 1.5% ,the total mould values was $5.6x10^5$ $\pm5.5x10^3$ CFU /g at 15 days and $9.4x10^5$ $\pm3.9x10^3$ CFU /g at 12 days. Based on the results obtained in this study, the application of natural substances as chitosan, thyme oil, lactic acid and acetic acid were effective in preserving quality of fresh meat slices and is recommended in meat products.

Keywords: *Chitosan, Thyme essential oil, Colour, TBA, TVB, Antifungal.*

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1. INTRODUCTION

Microbial growth is generally responsible for the spoilage in meats and meat products together with biochemical and enzymatic deteriorations (Devlieghere *et al.*, 2004). In fact, yeasts and fungi contamination is one of the main factors determining the loss in fresh meat quality, since these products are very liable to be contaminated with microorganisms if they are not properly preserved and handled.

The most commonly used strategy used to extend fresh meat shelf life is the use of antimicrobial and antioxidant additives of synthetic origin, which are being questioned due to an increasing consumer demand for natural, healthy and safe preservatives.

Chitosan is a biocompatible polymer derived from shellfish, as a biological sanitizer arises from reports showing several beneficial effects such as antimicrobial and antioxidative

activities in foods (Friedman and Juneja, 2010). The use of chitosan in industry, agriculture and medicine is well described (Rabea *et al*., 2003; Senel and McClure, 2004 and Friedman and Juneja, 2010).

Thyme essential oil contains more than 60 ingredients, most of which have important antioxidant and antimicrobial properties (Baranauskiene *et al*., 2003). Thymol, rosmarinic acid and carvacrol are the most important active compounds of thyme essential oil.

Large amounts of food are lost every year due to spoilage by yeasts and fungi (Hassan *et al*., 2015). So that, preservative agents commonly used include weak organic acids such as acetic, lactic, benzoic and citric acids, which inhibit the microbial growth in various foods. The effect of organic acids on the fungal growth, which contaminate food and feed, has been investigated by several authors.

Therefore, the aim of our research was a trial to study effect of chitosan, thyme essential oil and organic acids in meat preservation, including fungal growth, chemical, sensory and colour qualities.

2. Materials and methods

2.1. Preparation of spore suspension of A. flavus:

The present study was carried out using a strain of *Aspergillus flavus* (GenBank accession number: KP137699) isolated previously at the Mycology Department - Animal Health Research Institute, Giza, Egypt. The *A. flavus was* subcultured and grown for 7 days on Czapek yeast extract. *A. flavus* culture was washed with 10 ml sterile distilled water in 2% Tween 80 with the aid of glass beads to help in the spore dispersion. The spore suspensions were standardized to $1x10^6$ spores/ml.

2.2. Preparation of antifungal (chitosan, thyme essential oil, lactic acid and acetic acid):

-Stock solution of chitosan (1.5, 1.0 and 0.5 % w/v) was prepared in 1.0 % (v/v) acetic acid.

- Stock solution of lactic acid and acetic acid (1.5, 1.0 and 0.5% v/v) were prepared in sterial distilled water.

- Stock solution of thyme essential oil was used as it is (1.5, 1.0 and 0.5% v/w).

2.3. Antifungal Activity test:

Lean beef meat were purchased from the butcher's shop at Benha City, Kalyobia governorate, Egypt, transferred directly to the laboratory in an ice box under complete aseptic condition without undue delay. The lean beef was boneless and trimmed of fat. Then, the beef was cutted into slices (weighing about 100 g with a size of about 5× 5 × 2 cm for each slice). Fresh lean beef meat slices were divided into three equal groups as following:

1. First group subjected to total mould count (*A. flavus*).
2. Second group was for organoleptic examination and
3. Third group was for chemical analysis.

First group was divided into 14 subgroup, while Second group and Third group were divided into 13 subgroup, each sample 100g. One ml of previously prepared spore suspension was added to each 100 g of beef meat slices and mixed well. Chitosan, thyme essential oil, lactic acid and acetic acid were added to the meat slice groups to achieve final concentrations of 0.5 %, 1% and 1.5% (% v/g). The all antifungal were mixed with the meat slice groups for a further 30 seconds to ensure even mixing. All samples with different treatments and controls were packed in polyethylene bags, labelled and stored at 4 ^{0}C. Mycological evaluation and analysis of stored meat slices under examination (total mould and yeast) was conducted every 3 days during storage period.

Determination of total mould count:

The technique was applied recommended by ISO 6887 all parts, (2017) and ISO 21527-1 :(2008)

Determination of pH: was done according to the technique recommended by Allen *et al*. (1997)

Determination of (TBA) (mg/kg): was adopted according to (E.O.S 63/8, 2006).

Determination of Total Volatile Nitrogen (TVN) (mg %): was carried out according to (E.O.S 63/10, 2006).

Color measurement: was done according to CIE (1976)

Beef meat slices samples were analyzed at Cairo University Research Park (CURP)/ Faculty of Agriculture, for the following traits: Beef meat slices color was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer. Color was expressed using the CIE L, a, and b color system (CIE, 1976). A total of three spectral readings were taken for each sample. Lightness (L*) (dark to light), the redness (a*) values (reddish to greenish). The yellowness (b*) values (yellowish to bluish) were estimated.

3. RESULTS

The results of the total mould values (*A. flavus*) of meat slices treated with chitosan, thyme, acetic acid and lactic acid (0.5%, 1% and 1.5%) during storage at refrigerator at 4 ± 1^0C for 15 days are given in Table (1). The initial total count (day 0) value for the fresh meat slices were $1x10^6$ CFU /g, then decrease gradually until 9 day not detected any colony by using chitosan 1.5%. While in treated samples with thyme oil 1.5% and lactic acid 1.5% the total mould values (*A. flavus*) was $5.6x10^5 \pm 5.5x10^3$ CFU /g at 15 days and $9.4x10^5 \pm 3.9x10^3$ CFU /g at 12 days (Table 2).

The colour parameters of different formulas of meat slices are summarized in Table 4. For L* value, fresh meat slices with lactic acid 0.5%, 1% and 1.5% had the highest value 42.19, 42.60 and 42.54 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 (0.5%, 1% and 1.5%) had the lowest value 37.62,36.77 and 35.96,respectively. On the other hand, L* values during storage of samples containing thymol and acetic acid (0.5%, 1% &1.5%) showed an increasing trend, which could be due to gradual protein decomposition, leading to increase of light scattering (McDougall ,1983).

Table 2 revealed that the pH value at zero day ranged from 5.51±0.11 in control samples to 4.30±0.03, 4.25±0.03, 4.23±0.23, 5.49±0.08, 5.36±0.07, 5.29±0.08, 4.99±0.01, 4.69±0.22, 4.40±0.06, 5.04±0.07, 4.72±0.05 and 4.45±0.04 for Chitosan 0.5%, Chitosan 1%, Chitosan 1.5%, Thymol 0.5%, Thymol 1%, Thymol 1.5 %, Acetic acid 0.5%, Acetic acid 1%, Acetic acid 1.5%, Lactic acid 0.5% , Lactic acid 1% and Lactic acid 1.5%, treated samples , respectively. These pH values indicated that all previous natural substances significantly ($P < 0.05$) lower than control samples at zero day.

The TBA test has been used widely to estimate the extent of lipid oxidation. The TBA value in beef slices treated with chitosan and thyme, acetic acid and lactic acid (0.5%, 1% and 1.5) were determined and compared during the 18 days of refrigerated storage. As shown in Table (5), lipid oxidation in samples (control samples) was more intense compared to the other treated samples, reaching 1.040, 1.326, 1.637 and 1.872 mg / kg on the 9^{th}, 12^{th} ,15^{th} and 18^{th} day of refrigerated storage. The greatest decrease was found in samples treated with chitosan (0.5%, 1% and 1.5) were 0.615, 0.489, 0.339, 0.777, 0.527, 0.461, 0.833, 0.592, 0.512, 0.892, 0.757 and 0.578 mg / kg on the 9^{th}, 12^{th}, 15^{th} and 18^{th} day of refrigerated storage.

Table (6) showed that the mean values of total volatile basic nitrogen(TVB-N) of meat slices

treated with chitosan 0.5%,chitosan 1%,chitosan 1.5%,thyme oil 0.5%,thyme oil 1%,thyme oil 1.5 %,acetic acid 0.5%,acetic acid 1%,acetic acid 1.5%,lactic acid 0.5%,lactic acid 1% and lactic acid 1.5% at 18th day of preservation are within the permissible limits 14.27, 13.20, 12.43, 15.09, 14.18, 13.26, 19.35, 18.22,17.92,19.87,19.49 and18.65 mg/100g, respectively. The mean values of total volatile basic nitrogen (TVB-N) increased rapidly with storage time in the control samples 26.44 mg/100g.

Table 1: Design of chitosan, thyme essential oil, lactic acid and acetic acid added to beef meat slices.

Treatments	
1st treatment	Meat slice Free from anything (control negative).
2nd treatment	Meat slice + *A. flavus* 10^6spore/ ml. (control positive)
3th treatment	Meat slice + 0.5% chitosan + *A. flavus* 10^6spore/ ml
4th treatment	Meat slice + 1% chitosan + *A. flavus* 10^6spore/ ml.
5th treatment	Meat slice + 1.5 % chitosan + *A. flavus* 10^6spore/ ml
6th treatment	Meat slice + 0.5% thyme oil + *A. flavus* 10^6spore/ ml
7th treatment	Meat slice+ 1% thyme oil + *A. flavus* 10^6spore/ ml.
8th treatment	Meat slice + 1.5 % thyme oil + *A. flavus* 10^6spore/ ml
9th treatment	Meat slice + 0.5% lactic acid + *A. flavus* 10^6spore/ ml
10th treatment	Meat slice + 1% lactic acid + *A. flavus* 10^6spore/ ml.
11th treatment	Meat slice + 1.5 % lactic acid + *A. flavus* 10^6spore/ ml
12th treatment	Meat slice + 0.5% acetic acid + *A. flavus* 10^6spore/ ml
13th treatment	Meat slice + 1% acetic acid + *A. flavus* 10^6spore/ ml.
14th treatment	Meat slice + 1.5 % acetic acid + *A. flavus* 10^6spore/ ml

Table 2: Antifungal activity of various concentration of different treated meat slices during stored at 4 ± 1 °C.

Table 3: Effect of chitosan and thymol, acetic acid and lactic acid on sensory attributes of treated meat slices during stored at 4 ± 1 °C.

Treatment	Control		Chitosan			Thymol			Acetic acid			Lactic acid		
	Control (-ve)	Control (+ve)	Chitosan 0.5%	Chitosan 1%	Chitosan 1.5%	Thymol 0.5%	Thymol 1%	Thymol 1.5 %	Acetic acid 0.5%	Acetic acid 1%	Acetic acid 1.5%	Lactic acid 0.5%	Lactic acid 1%	Lactic acid 1.5%
At zero day	$9.7x10^2 \pm 5.5x10^1$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$
3th	$1.4x10^3 \pm 8.5x10^2$	$1.1x10^6 \pm 1.9x10^2$	$9.6x10^5 \pm 3.3x10^2$	$8.7x10^5 \pm 5.5x10^2$	$3x10^5 \pm 3.6x10^2$	$9.9x10^5 \pm 4.2x10^3$	$9.6x10^5 \pm 5.2x10^3$	$6.1x10^5 \pm 9.5x10^3$	$9.9x10^5 \pm 5x10^2$	$9.8x10^5 \pm 6.4x10^2$	$9.7x10^5 \pm 3.5x10^3$	$9.9x10^5 \pm 1.7x10^3$	$9.8x10^5 \pm 6.6x10^3$	$9.6x10^5 \pm 2.8x10^3$
6th	$7.3x10^3 \pm 2.7x10^3$	$6.7x10^6 \pm 7.9x10^2$	$8.9x10^5 \pm 3.8x10^2$	$5.2x10^5 \pm 5.9x10^2$	$9.8x10^4 \pm 5.2x10^2$	$9.8x10^5 \pm 1.9x10^3$	$7.6x10^5 \pm 1.2x10^3$	$4x10^5 \pm 7.3x10^3$	$9.9x10^5 \pm 4.4x10^2$	$9.7x10^5 \pm 7.1x10^3$	$9.6x10^5 \pm 2.5x10^5$	$9.9x10^5 \pm 5x10^3$	$9.6x10^5 \pm 4.6x10^3$	$9.5x10^5 \pm 1.4x10^3$
9th	Spoiled	Spoiled	Spoiled	$4.4x10^5 \pm 2.2x10^2$	ND	Spoiled	$6.4x10^5 \pm 1.5x10^3$	$3.9x10^5 \pm 7.1x10^3$	Spoiled	Spoiled	Spoiled	Spoiled	$9.7x10^5 \pm 1.2x10^3$	$9.5x10^5 \pm 2.5x10^3$
12th				ND			$7x10^5 \pm 2.5x10^3$	$4.9x10^5 \pm 2.6x10^3$					Spoiled	$9.4x10^5 \pm 3.9x10^3$
15th							Spoiled	$5.6x10^5 \pm 5.5x10^3$						Spoiled

Item	control			chitosan			Thyme oil			Lactic acid			Acetic acid		
	0.5 %	1%	1.5 %	0.5 %	1%	1.5%	0.5 %	1 %	1.5 %	0.5%	1%	1.5%	0.5 %	1 %	1.5 %
Color	10	10	10	3	2	2	9	10	10	10	10	10	10	10	10
Odor	2	3	4	10	10	10	10	10	10	10	8	7	10	8	7

Table 4: Effect of chitosan and thymol, acetic acid and lactic acid on pH value and Hunter colour values (L*, a* and b*) of red meat slice during storage at 4 ± 1 °C at 3 days.

Treatment	pH	L*	A*	B*
Control	5.72±0.06	42.07±0.28	19.63±0.95	10.48±0.50
Chitosan 0.5%	4.39±0.06	37.62±0.35	8.59±0.13	6.91±0.14
Chitosan 1%	4.29±0.03	36.77±0.32	8.19±0.21	6.54±0.17
Chitosan 1.5%	4.25±0.03	35.96±0.19	8.09±0.05	6.28±0.09
Thymol 0.5%	5.51±0.07	41.11±0.23	19.28±0.56	9.83±0.33
Thymol 1%	5.41±0.06	37.86±1.45	18.49±0.73	8.74±0.35
Thymol 1.5 %	5.33±0.07	37.73±0.42	17.02±0.19	8.18±0.17
Acetic acid 0.5%	5.10±0.06	41.57±0.37	19.53±0.24	10.08±0.03
Acetic acid 1%	4.97±0.09	41.49±0.46	19.55±0.31	10.17±0.06
Acetic acid 1.5%	4.86±0.07	40.17±0.24	19.04±0.27	10.06±0.66
Lactic acid 0.5%	5.20±0.03	42.19±0.10	19.57±1.11	10.43±0.17
Lactic acid 1%	5.09±0.10	42.60±0.43	19.42±0.14	10.66±0.36
Lactic acid 1.5%	4.97±0.11	42.54±0.62	19.27±0.46	10.91±0.26

Table 5: TBA values of meat slices treated with different treatments during 18 days of refrigerated storage at 4 ± 1 °C (mg/kg).

Treatment	At zero day	3th	6 th	9 th	12 th	15 th	18 th
Control	0.041±0.033	0.72±0.01	0.95±0.03	1.040±0.04	1.326±0.13	1.637±0.09	1.872±0.04
Chitosan 0.5%	0.037±0.004	0.44±0.02	0.56±0.02	0.615±0.43	0.777±0.54	0.833±0.58	0.892±0.62
Chitosan 1%	0.037±0.003	0.350±0.02	0.410±0.03	0.489±0.34	0.527±0.37	0.592±0.41	0.757±0.019
Chitosan 1.5%	0.034±0.024	0.27±0.02	0.30±0.02	0.339±0.24	0.461±0.32	0.512±0.36	0.578±0.401
Thymol 0.5%	0.037±0.026	0.609±0.42	0.699±0.48	0.789±0.55	0.869±0.60	1.015±0.70	1.605±1.11
Thymol 1%	0.038±0.027	0.548±0.38	0.780±0.54	0.793±0.55	0.858±0.60	0.991±0.69	1.507±1.04
Thymol 1.5 %	0.037±0.026	0.480±0.33	0.64±0.44	0.723±0.50	0.802±0.56	0.904±0.63	1.322±0.92
Acetic acid 0.5%	0.039±0.27	0.640±0.44	0.840±0.53	0.891±0.62	0.963±0.67	1.224±0.85	1.646±1.14
Acetic acid 1%	0.037±0.026	0.587±0.41	0.813±0.56	0.925±0.64	1.178±0.82	1.350±0.94	1.530±1.06
Acetic acid 1.5%	0.036±0.025	0.530±0.37	0.792±0.55	0.931±0.65	1.041±0.72	1.408±0.98	1.592±1.10
Lactic acid 0.5%	0.044±0.631	0.646±0.45	0.904±0.63	1.204±0.84	1.434±0.99	1.655±1.15	1.839 ±0.14
Lactic acid 1%	0.040±0.028	0.590±0.41	0.805±0.56	0.983±0.68	1.378±0.96	1.512±1.05	1.795±1.25
Lactic acid 1.5%	0.038±0.026	0.573±0.40	0.799±0.55	0.951±0.66	1.308±0.91	1.411±0.98	1.634±1.133

Table 6: TBA values of meat slices treated with different treatments during 18 days of refrigerated storage at 4 ± 1 °C (mg/kg).

Treatment	At zero day	3th	6 th	9 th	12 th	15 th	18 th
Control	6.18±0.09	17.53±0.55	19.26±0.22	20.04±0.58	21.15±0.21	24.41±0.37	26.44±3.39
Chitosan 0.5%	6.26±0.13	8.61±0.18	9.08±0.08	11.13±0.13	12.33±0.06	13.28±0.35	14.27±0.12
Chitosan 1%	6.11±0.58	7.96±0.30	8.65±0.23	10.68±0.17	12.02±0.24	12.71±0.43	13.20±0.12
Chitosan 1.5%	6.18±0.07	7.41±0.05	8.02±0.07	10.08±0.08	11.43±0.40	11.65±0.33	12.43±0.24
Thyme oil 0.5%	6.11±0.58	8.87±0.09	9.43±0.08	11.70±0.16	13.78±0.22	14.03±0.06	15.09±0.12
Thyme oil 1%	6.26±0.13	8.06±0.07	9.01±0.05	11.09±0.27	12.58±0.32	13.59±0.10	14.18±0.12
Thyme oil 1.5 %	6.18±0.09	7.59±0.19	8.35±0.03	10.71±0.20	11.83±0.31	12.38±0.14	13.26±0.02
Acetic acid 0.5%	6.30±0.10	10.28±0.34	12.40±0.07	14.3±0.42	15.87±0.20	17.23±0.15	19.35±0.05
Acetic acid 1%	6.22±0.07	9.79±0.11	11.78±0.19	14.49±0.31	15.20±0.10	16.94±0.10	18.22±0.17
Acetic acid 1.5%	6.18±0.06	9.65±0.23	11.70±0.09	13.81±0.26	15.11±0.10	16.91±0.02	17.92±0.41
Lactic acid 0.5%	6.58±0.19	11.37±0.28	13.00±0.02	14.34±0.23	16.72±0.12	18.0±0.06	19.87±0.33

Lactic acid 1%	6.34±0.04	11.07±0.14	12.53±0.10	13.87±0.03	15.64±0.19	17.55±0.29	19.49±0.27
Lactic acid 1.5%	6.25±0.05	10.67±0.33	12.24±0.36	13.27±0.16	15.22±0.34	17.11±0.55	18.65±0.29

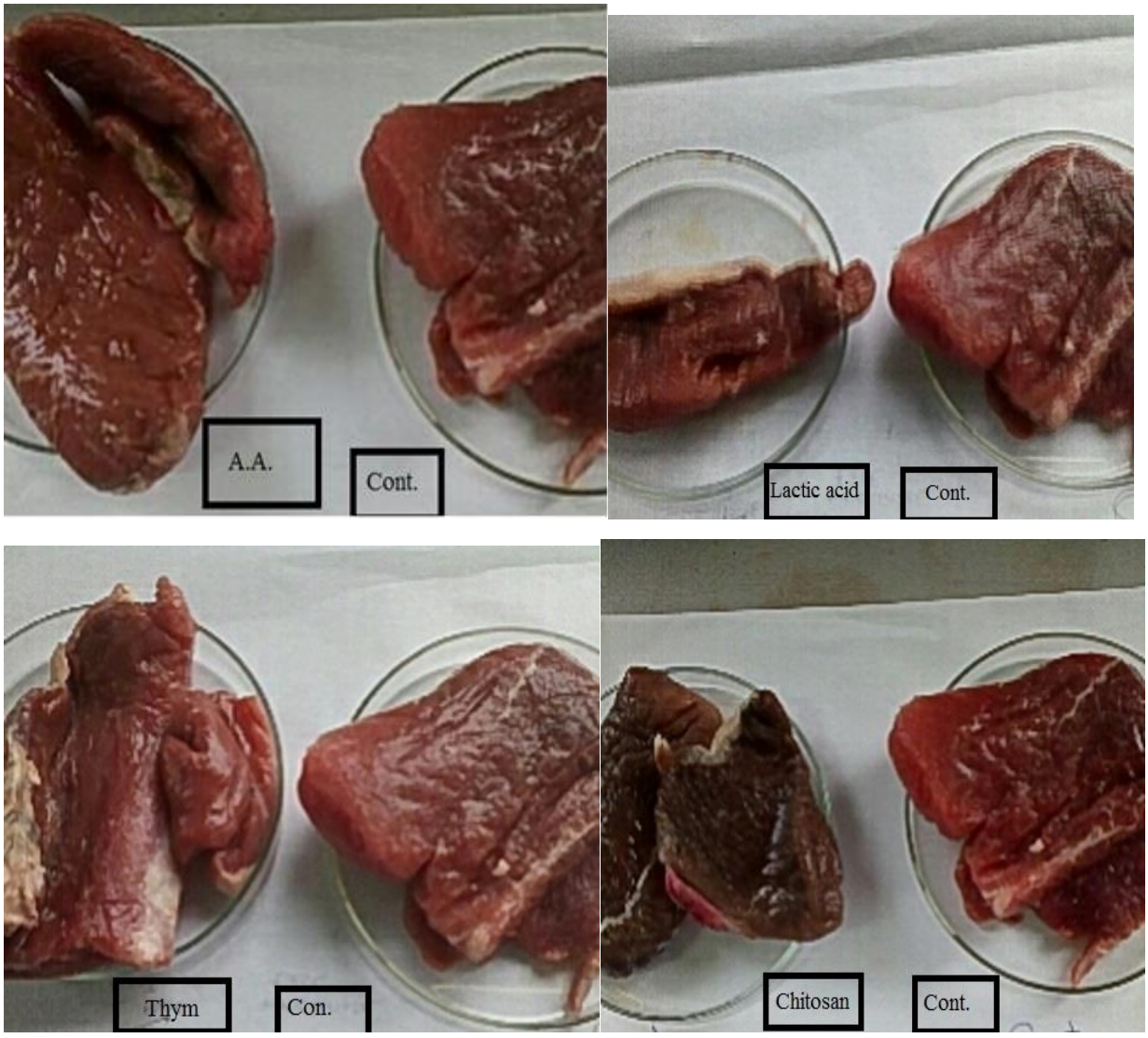

Fig.1. Show meat colour of (a): acetic acid, (b) lactic acid), (c) thyme essential oil and (d) chitosan treatment.

4. DISCUSSION

Meat and especially meat products are highly susceptible to both microbial growth and lipid oxidation because of their large surface to weight ratio, leading to rapid spoilage and development of rancid or warmed-over flavour, respectively (Jay *et al*., 2005). Because there is an increasing consumer demand for minimally processed foods without chemical preservatives, the food industry is facing a constant challenge to develop alternative 'natural' methods to extend product shelf life and improve safety (Karabagias *et al*., 2011).

Low molecular weight water-soluble chitosan (LMWS-chitosan) strongly inhibited the growth of *A. fumigatus, A. parasiticus, B. cinerea, F. solani, F. oxysporum*, and *P. verrucosum* Park *et al.* (2008). The antifungic mechanism of chitosan involves cell wall morphogenesis with chitosan molecules interfering directly with fungal growth. Microscopic observation reported that chitosan oligomers diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth. The intensity of degradation action of chitosan on fungal cell walls is also dependent upon the concentration, degree of acetylation and local pH. Studies conducted on *Rhizoctonia solani* revealed that the percentage of fungus germination decreased with increasing the chitosan concentration in the medium (Goy *et al*., 2009).

The possible mechanism of action of essential oil components on the growth of fungi was reported in several studies. It is generally accepted that the essential oil components act on the functionality and the structure of the cell membrane. Low concentrations result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane, whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death (Kocić-Tanackov and Dimić (2013). Different acetic and lactic acid concentrations (5 and 10 %) were studied by (Hassan *et al*., 2015) for antifungal activity against different strains of *A. flavus*. They determined that the increase of acid in the medium decreases the growth rate and extends the lag phase, when acetic acid and lactic acid (10%) were used the inhibition effect were 40.92% and 17.96%, respectively.

Meat colour is the first condition that consumer use to judge meat quality and acceptability. Anwer *et al*. (2013) reported that colour is an important factor in selection of meat products. Meat colour is one of the most important for consumers are indication of an originality and uprightness. Consumers will often refuse products in which the colour varies from the predictable appearance. Therefore, colour is frequently used to determine economic value of food.

In organoleptic examination the test revealed that, (as shown in Table 3,Fig 1) the colour of the control sample not changed, while the odour slightly changed in concentration 1.5% (score 4) after 3 days of preservation. In samples treated with chitosan and thymol, acetic acid and lactic acid (0.5%,1% and 1.5%),the colour changed into dark red after preservation only in case of chitosan (0.5%,1% and 1.5%) score 3,2 and 2, respectively. The odour slightly changed after preservation dilution 1% and 1.5% of lactic acid and acetic acid (score 8 and 7), respectively.

The pH value of all meat slices samples slightly decreased in chitosan than other treatments during the storage, this decrease indicates that some fermentation occurs during storage (Table 4,Fig 1). This result agree with Mokhtar *et al*. (2014) where reported that the pH value 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 endprotease activity or the proteolytic microbial flora present in the raw meat Mokhtar *et al*., (2012).

Regarding to The changes in L* values during the storage period, there was a significant increase in samples dipped in 1% chitosan (CS) compared with chitosan oligosaccharides (COS) (chitosan subjected to γ irradiation, 50 or 100 kGy) Zahran (2015). Also similar trend was reported by Jo *et al*. (2001) which could be the result of gradual protein decomposition, leading to the increase of light scattering.

Further L* values in meat and meat products are related to surface water, water vapour exchanges between the products and the environment and modifications of the different states of the heme pigments Fernández-López *et al*. (2000).

The values of meat slices were affected by the addition of the examined preservatives. Samples containing chitosan had a lower a* values than samples containing lactic acid, thymol and acetic acid throughout the whole period of storage. Similar results were observed by Georgantelis *et al*. (2007) and Mokhtar *et al*. (2014) who found that changes in L* values during storage, there was a decreasing trend in samples containing Chitosan and a decreasing trend was observed as regards to a* values, which is attributed to the gradual oxidation of myoglobin, conversion of myoglobin into metmyoglobin and accumulation of metmyoglobin with time. A significant decrease in chrome values (brownish colour development), which could be a drawback in terms of consumer acceptance.

The b* values of chitosan-treated samples (0.5%, 1% and 1.5%) were decrease than samples containing lactic acid, acetic acid and thymol, which could be attributed to the natural yellowish colour of chitosan affecting the meat slices colour. Similar observations have been also found by Georgantelis *et al*. (2007) in beef burger, Jo *et al*., (2001) and Youn *et al*., (1999) in pork sausage with added chitosan. During refrigerated storage, a decreasing trend in the control and all treated samples was observed. Zahran (2015) reported that initial measurements (day-1) of b*-values (yellowness) were higher ($p< 0.05$) in samples dipped in chitosan and chitosan oligomer compared with control and acetic acid 1%. Also, after 2 weeks of storage, b*- values increased in samples dipped in acetic acid 1% and chitosan 1% irradiated 50 kGy γ, but decreased in control, acetic acid 1% and chitosan 1% irradiated 100 kGy γ.

Beef muscle with greater lipid oxidation accelerated greater protein oxidation and metmyoglobin formation which lead variation in physicochemical quality of beef muscle. Chemical deterioration related to pH and lipid oxidations have greater effect in declining palatability and texture of beef and beef products. Fresh beef and beef products preserved under refrigeration state can still develop physicochemical deterioration slowly. The degree of lipid oxidations affected by various thawing methods during frozen state is still a potential problem and effective methods need to be explored (Rahman *et al.,* 2015).

The results of TBA are agreement with Georgantelis *et al*., (2007) who observed lower TBA values for fresh pork treated with chitosan. Also, Mokhtar *et al.* (2014) found that the TBA values of different treated samples (chitosan, rosemary extract and carnosine) TBA 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 ($p < 0.05$) TBA values compared to those containing the individual antioxidants (CHI, CAR), a fact that indicates the occurrence of a synergistic effect. Jin *et al*. (2016) reported that TBA measured as the lipid oxidation value increased with storage period in treated thyme essential oil samples during storage sausages. However, the TBA value did not show any consistent trends between treated samples during storage.

Egyptian organization for standardization and quality (E.O.S., 2006) had laid down the TVB-N level must not be more than 20 mg/100g in chilled meat.

Total volatile basic-nitrogen (TVB-N) is a substance produced in the process of meat spoilage, and its content in meat was proportional to the extent of meat spoilage. So it is an important index to evaluate the degree of meat freshness Shanmei and Gan (2016). Meat raw material is the natural source of the substrate from which biogenic amines are produced. It also is the largest component of the matrix in which the decarboxylation reactions take place and any conditions that alter its nature and characteristics will influence the formation of biogenic amines (Ruiz-Capillas and Jiménez-Colmenero (2004).

Accordingly, the presence of biogenic amines can cause several problems for susceptible consumers, such as nausea, respiratory disorder, hot flushes, sweating, heart palpation, headache, bright red rash, oral burning, hypo or hypertension, whose intensity is depend on quantitative and qualitative differences. The total volatile nitrogen bases (TVBN) in meat may be increased as the days of storage increased Sayed *et al*. (2009).

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