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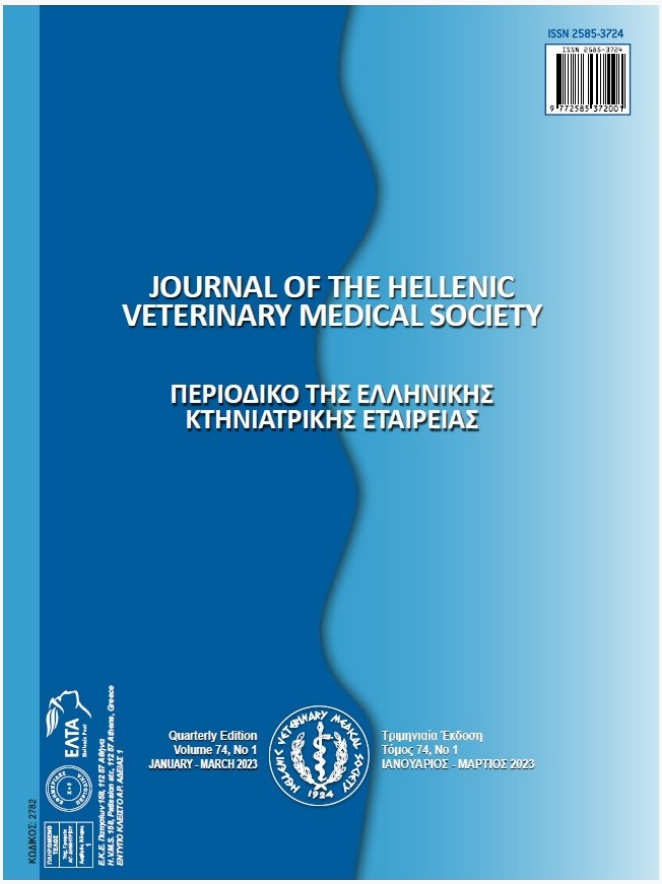

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Research article
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Effect of chitosanon microbial, chemical and sensory traitsof minced meat

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ABSTRACT: Chitosan is a naturally obtained biopolymer and has many applications in the food industry. The effect of chitosan (1% and 3%) added individually to meat on microbiological profile represented by aerobic plate count (APC), Psychrotrophic, mold and yeast counts; chemical (pH, TVB-N & TBA) and sensory traits (color, odor, appearance and consistency) was studied.Thus, the minced meat samples were divided into 3 groups. The first group was prepared as control (Untreated group) and the other two groups were treated with chitosan powder dissolved in glacial acetic acid at ratio of 1% and 3%. The results showed that both chitosan 1% and 3% had a significant destructive effect in all tested microbial groups, but chitosan 3% was more effective than the control group. From a chemical point of view, such concentrations of chitosan improved the chemical quality indices of minced meat. Organoleptically, the control samples were spoiled after the 4th day, while the usage of chitosan particularly at 3% extended the shelf life of the experimented minced meat till the 9th day. Finally, it can be concluded that chitosan had great effect on the quality and prolong the shelf life of minced meat as it improves the microbiological and chemical as well as sensory characteristics of minced meat.

Keywords: Chitosan; Minced Meat; Bacteriological profile; Chemical profile; Egypt.

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INTRODUCTION

Meat is very nutritious and provides a number of necessary nutrients that are difficult to get in the proper levels from other food sources (Geiker et al., 2021). Minced meat has a high economic value because it can be used in all processed products as burger, kofta and sausage. In contrast minced meat has high water activity (0.99%) which, in addition to the process of mincing of meat increase the surface area suitable for adherence and growth of both spoilage and pathogenic organisms (Rhoades et al. , 2009). Faulty processing procedures frequently result in microbial contamination of meat. Meat oxidation and microbial deterioration are the two main factors affecting the quality and shelf life of meat products(Lonergan et al. 2019).

Chitosan has been approved asGenerally Recognized as Safe (GRAS) and it has a broad-spectrum antimicrobial activity against Gram negative and Gram-positive bacteria as well as fungi (Garg et al., 2019).Chitosan is a biodegradable, renewable, non-toxic natural polymer that is often made from crustacean shell waste. It is a derivative of the widely available chitin (Kou et al., 2021).Chitosan was prepared through the alkaline deacetylation of chitin which is the second most abundant polysaccharide in nature after cellulose and can be found in the exoskeletons of crustaceans and mollusks, insect cuticles, fungi and a variety of animals and plants (Kaur and Dhillon, 2014).

The mechanism of action of chitosan was supposed to be different between Gram-positive and Gram-negative bacteria because of different cell surface characteristics (Matica et al., 2019).The main mechanism for chitosan's antibacterial activity appears to the electrostatic interactions between the polycationic structure of chitosan and the anionic groups on the surface of bacteria this results in change the cell wall (in Gram-positive bacteria) or outer membrane (in Gram-negative bacteria), which disrupts cytoplasmic membrane permeability and causes the loss of vital components like enzymes, nucleotides, and ions, as well as the death of the bacteria.(Chung et al., 2008) (Younes et al., 2015) (Zou et al., 2015).

Low molecular weight chitosan, both water soluble and chitosan-based small nanoparticles, have the potential to pass through the cell wall and inhibit mRNA synthesis and DNA transcription (Kong et al., 2010) (Matica et al., 2019). On the other hand, high molecular weight chitosan is able to bind to the negative charged elements present on the cell wall of bacteria resulting in the formation of an impermeable layer everywhere around the bacterial cell, changing cell permeability and blocking transport into the inside of bacteria (Zheng and Zhu, 2003) (Eaton et al., 2008)(Younes et al., 2015)

It is of great concern to mention that chitosan can prevent the growth of aerobic bacteria as it exhibits a coating film around the bacterial cell acting as an oxygen barrier and depriving bacteria from the needed oxygen (Helandar et al., 2001) (Rafaat et al., 2008).

Therefore, the present study was planned to spot light on:a) The effect of chitosan on microbial profile of minced meat sold in butcher shops and supermarkets in Kafr-Elshiekh governorate by assessing APC, psychrotrophs, yeast and mold counts; additionally, physicochemical tests as pH, Total Volatile Basic Nitrogen (TVBN),Thiobarbituric acid (TBA) and on sensory traits of minced meat. b)the enhancement of the quality of minced meat and prolongation of its shelf life was studied using 1% and 3% chitosan.

MATERIAL AND METHODS

Experimentally, the effect of addition of chitosan at various concentrations on the microbial, chemical and sensory traits of minced meat was studied as a trial to prolong its shelf life.

This experiment was done in the Laboratoryof Food Analysis Center, Faculty of Veterinary Medicine,Benha University, Egypt, in 2019 and 2020.

Preparation of chitosan:

Chitosan was obtained from Sphinx for International Trade Company, Cairo, Egypt. Chitosan of low molecular weight from crab shells in powder form was used. Respectively, 1 and 3 grams of chitosan was dissolved in 100 ml (w/v) glacial acetic acid and stirred with a magnetic stirrer for 3 hours at 55ºC to induce1% and 3% concentration (Fernandes *et al.*, 2012).

Preparation of samples:

Fresh beef meat samples were purchased from a local butcher shops in Kafr El-Sheikh city, Egypt within 3 days of slaughter. Before mincing, meat samples were sterilized by soaking in 10 mg/l sodium hypochlorite for 60 min, followed by three times sterilized distilled water washes.

Treatment of samples with the chitosan (Barbosa *et al.*, 2009):

The samples were divided into 3 groups. The first group was prepared as control (Untreated group) and the other 2 groups were treated with chitosan at ratio of 1% and 3%**.**

Each treated sample was dipped for 15 min in the dipping emulsion solution (1% and 3% of pure chitosan extract to a final volume of 100 ml of sterile distilled water) then drained well for 5 min on a sterile stainless wire mesh screen. Control group were dipped in sterile distilled water.

The previous treated minced meat samples were labeled and each single sample was separately packaged in polyethylene bags. The experiment was conducted for 9 days of chilling storage at 2°C. Each group was subjected to bacteriological and sensory assessment at zero time (within 2 hours after treatment) then periodically regularly every day until spoilage was evident in each group. The scheme was replicated for 5 times.

Microbiological examination:

Preparation of samples (ISO 6887-2: 2017):

Ten grams of samples were taken aseptically and placed on a sterile homogenizer flask (ACE Homogenizer: Nissei model, Japan**)** containing 90 ml of sterile peptone water (0.1%) and homogenised at 2500 rpm for 2.5 minutes to produce a homogenate of 1/10 dilution.

One ml of the homogenate was transferred with a sterile pipette to another test tube containing 9 ml of sterile peptone water (0.1%) to give $10^{-2}$from which tenth fold serial dilutions were prepared up to 10^{-6}.

Aerobic plate count and psycrotrophic count (ISO 4833-1, 2013):

One ml of the previously prepared serial dilutions was aseptically inoculated into 2 sterile Petri dish, then about 15 ml of previously melted and cooledat 45°C standard plate count agar (obtained from Oxoid, Basingstoke, Hampshire, UK) were added and thoroughly mixed in a horizontal position till solidification.

For the APC, the inoculated as well as control plates were incubated in an inverted position at 37°C for 48 hrs.

For the psychotrophic count,the inoculated as well as control plates were incubated in an inverted position at 7°C for 10 days.

The plates with 30/300 colonies were selected and counted and the aerobic plate count was calculated/g.

Mold and Yeast count (ICMSF, 1996):

Accurately, 0.1 ml of the previously prepared serial dilutions was aseptically transferred into double sterile petri dishes, and then 10 ml of ofsabouraud dextrose agar (obtained from Oxoid, Basingstoke, Hampshire, UK) media previously melted and cooled at 45°C, were added and thoroughly mixed as well asincubated at 25°C for 7 days. During the incubation period, the inoculated plates were examined daily for the “star shape” colonies. Mold and yeast counts/g werethen calculated and recorded.

Chemical examination:

Determination of pH (Pearson, 2006)

Ten g of the sample were blended in 10ml of neutralized distilled water. The homogenate was left with continuous shaking for 10 minutes at room temperature. The pH value was measured by usage of an electrical pH meter (Bye model 6020, USA). Calibration of pH meter by using two buffer solutions of exactly known pH (alkaline pH 7.01, acidic pH 4.01). Therefore, pH electrode was washed with neutralized water and then introduced into the homogenate after the temperature correction system was adjusted.

Determination of Total Volatile Nitrogen (TVN)

The technique recommended by **AOAC (1995)** was carried out. In a clean dry beaker, 10 grams of the sample were macerated with 100 ml of distilled water and thoroughly mixed by a blender for 2 minutes. Then, 2 grams magnesium oxide were added and then mixed thoroughly. A macro Kjeldahl distillation apparatus (Labconco, U.K) was connected to the distillation flask containing 25 ml of 2% boric acid solution with few drops of methyl red indicator (obtained from Oxoid, Basingstoke, Hampshire, UK) with the receiver tube was dipped below the liquid. The mixture was boiled for 10 minutes and the distillation was continued for 25 minutes using the same heating rate. The condenser was then washed with distilled water and the distillate was titrated against 0.1N sulfuric acid. The Total Volatile Basic Nitrogen (mg/100g sample) was calculated as the titration multiply by 14.

Determination of Thiobarbituric Acid (TBA) value

The samples were analyzed for TBA according to the method described by Krishnan *et al.* (2014). Five grams of each sample was homogenized with 15 ml of deionized distilled water. Then, 1 ml of the homogenate was transferred to a test tube and 50 µL of Butylated hydroxyl toluene (7.2%) and 2 ml each of thiobarbituric acid (15m MTBA) and trichloroacetic acid (TCA 15%) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 minutes to develop color. The samples were subjected to cooling for 10 minutes, vortexed again and centrifuged for 15 min at 2500 ×g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of deionized water and 2 mL of TBA-TCA solution by using a spectrophotometer (UNICAM969AA Spectronic, USA). The amount of TBA was expressed as milligrams of malonaldehyde per kilogram of sample.

Sensory evaluation (Fik and Fik, 2007)

Sensory properties of raw minced meat samples were evaluated by a 6- member panel appropriately trained and tested in sensory sensitivity. Training sessions were concluded when individual scores did not vary by more than 1 unit from the mean score and the panelists were familiar with the evaluation system. Representative samples from the tested meat were randomly selected and served on porcelain plates in the laboratory (open area). Panel members were asked to evaluate the freshness grade using a 5-point scale-each attribute being scored from 1 to 5 points depending on specifications of sensory quality. The following properties were evaluated: color, odor, appearance and consistency.The overall sensory quality scores 5, 4, 3, 2, and 1 corresponded to the beef mince qualities evaluated as very good, good, acceptable, unacceptable and bad, respectively.

Statistical Analysis

The obtained results were statistically evaluated by application of student t-test and Analysis of Variance (ANOVA) test according to Feldman *et al.* (2003).

Measurement of reduction percentage was calculated according to Abidi et al. (2014) through the equation: × 100.

Datawere presented as mean ± standard error of mean (SEM) and the significant values were set at P< 0.05.

RESULTS

Aerobic Plate Count

As indicated in Table (1), Mean count of APCat zero time was $8.6 \times 10^3 \pm 1.5 \times 10^3$cfu/g for all examined samples , while mean count of APC at 1st day at chilling was $4.5 \times 10^4 \pm 0.6 \times 10^4$, $1.3 \times 10^4 \pm 0.2 \times 10^4$, $9.6 \times 10^3 \pm 1.8 \times 10^3$ cfu/g for control, 1% chitosan , 3% chitosan treated samples respectively, with reduction 71.1% ,78.7 % relative to control samplesrespectively.

By the 3rd day, the mean APC values were $7.4 \times 10^6 \pm 1.1 \times 10^6$, $4.9 \times 10^4 \pm 0.7 \times 10^4$, $2.1 \times 10^4 \pm 0.4 \times 10^4$cfu/g for control, 1% chitosan , 3% chitosan treated samples respectively , with reduction 99.3% , 99.7% relative to control samples respectively.At 4th day control sample give signs of spoilage, but both samples treated with either chitosan 1% or chitosan 3% show extending shelf life of treated samples till 7th day of chilling for chitosan 1% and 9th day for chitosan 3%.

Table (1): Effect of chitosan addition on APC and extending shelf life of minced meat samples (n=5).

Treatment / Storage time+	Control	1% Chitosan		3% Chitosan+	
		Count	R%	Count	R%
Zero time	$8.6\times10^3\pm 1.5\times10^3$	$8.6\times10^3\pm 1.5\times10^3$	------	$8.6\times10^3\pm 1.5\times10^3$	------
1st day	$4.5\times10^4\pm 0.6\times10^4$	$1.3\times10^4\pm 0.2\times10^4$	71.1	$9.6\times10^3\pm 1.8\times10^3$	78.7
2nd day	$8.7\times10^5\pm 1.9\times10^5$	$3.0\times10^4\pm 0.5\times10^4$	96.5	$1.4\times10^4\pm 0.3\times10^4$	98.4
3rd day	$7.4\times10^6\pm 1.1\times10^6$	$4.9\times10^4\pm 0.7\times10^4$	99.3	$2.1\times10^4\pm 0.4\times10^4$	99.7
4th day	S	$7.6\times10^4\pm 1.0\times10^4$	------	$4.3\times10^4\pm 0.6\times10^4$	------
5th day	S	$1.4\times10^5\pm 0.2\times10^5$	------	$5.9\times10^4\pm 0.7\times10^4$	------
6th day	S	$3.6\times10^5\pm 0.5\times10^5$	------	$8.8\times10^4\pm 1.9\times10^4$	------
7th day	S	$9.2\times10^5\pm 2.1\times10^5$	------	$2.7\times10^5\pm 0.4\times10^5$	------
8th day	S	$4.9\times10^6\pm 0.8\times10^6$	S	$6.5\times10^5\pm 1.1\times10^5$	------
9th day	S	S	S	$9.7\times10^5\pm 2.0\times10^5$	------

R%*= Reduction % S= spoiled

APC should not exceed 10^6 (EOS, 2005) No. 1694/2005 for minced meat.

+ ANOVA test indicated significant differences (P<0.05)

By the 5th day, the mean APC were $1.4\times10^5 \pm 0.2\times10^5$, $5.9\times10^4 \pm 0.7\times10^4$cfu/g in 1% chitosan and 3% chitosan treated samples respectively.

By the 8th day , the mean APC were $4.9\times10^6 \pm 0.8\times10^6$, $6.5\times10^5 \pm 1.1\times10^5$cfu/g in 1% chitosan and 3% chitosan treated samples respectively and then samples treated with chitosan 1% give signs of spoilage, but samples treated with chitosan 3% extending shelf life with mean APC $9.7\times10^5 \pm 2.0\times10^5$cfu/g then the samples give signs of spoilage.

Psychrotrophic count

In Table (2), Mean psychrotrophic count of minced meat samples were $7.3\times10^3 \pm 1.1\times10^3$cfu/g for all examined samples (control and treated one). While at 1st day were $2.1\times10^4 \pm 0.4\times10^4$, $9.8\times10^3 \pm 2.0\times10^3$, $8.0\times10^3 \pm 1.4\times10^3$cfu/g for control, chitosan 1%, chitosan3%treatedsamples respectively with reduction 53.3% , 61.9% relative to control samples respectively.

For the control sample , the mean psychrotrophic count by 2nd day $5.9\times10^5 \pm 0.7\times10^5$ and then $3.8\times10^6 \pm 0.6\times10^6$ cfu/g then start signs of spoilage by 4th day of chilling meanwhile by 2nd day mean Psychrotrophic count became $1.7\times10^4 \pm 0.3\times10^4$, $1.1\times10^4 \pm 0.2\times10^4$cfu/g for 1% chitosan and 3% chitosan treated samples, with reduction 97.1% . 98.1%relative to control samples.

By the 3th day, the mean Psychrotrophic count were $3.2\times10^4 \pm 0.5\times10^4$, $1.5\times10^4 \pm 0.3\times10^4$ cfu/g in 1% chitosan and 3% chitosan treated samples respectively with reduction 99.2% , 99.6% relative to control samples respectively.

By the 5th day, the mean Psychrotrophic count were $8.2\times10^4 \pm 1.5\times10^4$, $3.1\times10^4 \pm 0.6\times10^4$cfu/g in 1% chitosan and 3% chitosan treated samples respectively.

The mean Psychrotrophic count continue to increase till it reached $1.3\times10^6 \pm 0.2\times10^6$cfu/g and sensory characters of spoilage appear for 1% chitosan by 8th day of chilling samples, and for $8.0\times10^5 \pm 1.5\times10^5$cfu/g for 3% chitosan (pre-spoilage signs of samples appeared). chitosan showed a clear antimicrobial effect ($P < 0.05$) on the 7th day of storage for chitosan 1% in addition to extending the shelf life till 9th day on chitosan 3% treated samples.

Total Mold count

As shown in table (3), total mold count at zero time was $2.0\times10^2 \pm 0.3\times10^2$in all examined samples. Meanwhile, the counts of mold increased directly with progress in chilling period in control sample till reach $8\times10^2 \pm 1.4\times10^2$, $1.6\times10^3 \pm 0.3\times10^3$, $5.1 \times 10^3 \pm 0.6\times10^3$cfu/g for 1st day, 2nd day and 3rd day, respectively.

In samples treated with1% chitosan, the mold count became $4.0\times10^2 \pm 0.6\times10^2$cfu/g in the 1st day with reduction 50 %relative to control samples .While in the second day , total mold count became $7.0\times10^2 \pm 1.2\times10^2$cfu/g with reduction 56.3 % and still to increase till reach $2.0\times10^3 \pm 0.4\times10^3$cfu/g with reduction 60.8 % at 3rd day of storage.

By the 4th day, the mold count in chitosan 1% treated samples were $7.4\times10^3 \pm 1.0\times10^3$cfu/g then became $8.9\times10^3 \pm 1.8\times10^3$, $1.3\times10^4 \pm 0.2\times10^4$, $3.9\times10^4 \pm 0.5\times10^4$cfu/g in the 5th , 6th and 7th day respectively , In the 8th day , the mold count became $6.0\times10^4 \pm 1.2\times10^4$cfu/g and signs of spoilage appear .

In samples treated with 3% chitosan, the mold

Table (2): Effect of chitosan addition on psychrotrophic count of minced meat samples (n=5).

Storage time+ \ Treatment	Control	1% Chitozsan		3% Chitosan+	
		Count	R %	Count	R %
Zero time	$7.3\times10^3 \pm 1.1\times10^3$	$7.3\times10^3 \pm 1.1\times10^3$	------	$7.3\times10^3 \pm 1.1\times10^3$	------
1st day	$2.1\times10^4 \pm 0.4\times10^4$	$9.8\times10^3 \pm 2.0\times10^3$	53.3	$8.0\times10^3 \pm 1.4\times10^3$	61.9
2ndday	$5.9\times10^5 \pm 0.7\times10^5$	$1.7\times10^4 \pm 0.3\times10^4$	97.1	$1.1\times10^4 \pm 0.2\times10^4$	98.1
3rdday	$3.8\times10^6 \pm 0.6\times10^6$	$3.2\times10^4 \pm 0.5\times10^4$	99.2	$1.5\times10^4 \pm 0.3\times10^4$	99.6
4th day	S	$5.0\times10^4 \pm 0.8\times10^4$	------	$1.9\times10^4 \pm 0.4\times10^4$	------
5th day	S	$8.2\times10^4 \pm 1.5\times10^4$	------	$3.1\times10^4 \pm 0.6\times10^4$	------
6th day	S	$1.7\times10^5 \pm 0.3\times10^5$	------	$6.5\times10^4 \pm 0.9\times10^4$	------
7th day	S	$5.8\times10^5 \pm 0.7\times10^5$	------	$8.9\times10^4 \pm 1.6\times10^4$	------
8th day	S	$1.3\times10^6 \pm 0.2\times10^6$	S	$4.1\times10^5 \pm 0.6\times10^5$	------
9th day	S	S	S	$8.0\times10^5 \pm 1.5\times10^5$	------

R%*= Reduction % S= spoiled

+ ANOVA test indicated significant differences ($P<0.05$).

Table (3): Effect of chitosan addition on total mold count of minced meat samples (n=5).

Treatment / Storage time+	Control	1% Chitosan		3% Chitosan +	
		Count	R %	Count	R %*
Zero time	$2.0\times10^2\pm 0.3\times10^2$	$2.0\times10^2\pm 0.3\times10^2$	------	$2.0\times10^2\pm 0.3\times10^2$	------
1st day	$8.0\times10^2\pm 1.4\times10^2$	$4.0\times10^2\pm 0.6\times10^2$	50.0	$3.0\times10^2\pm 0.4\times10^2$	62.5
2nd day	$1.6\times10^3\pm 0.3\times10^3$	$7.0\times10^2\pm 1.2\times10^2$	56.3	$4.0\times10^2\pm 0.5\times10^2$	75.0
3rd day	$5.1\times10^3\pm 0.6\times10^3$	$2.0\times10^3\pm 0.4\times10^3$	60.8	$6.0\times10^2\pm 0.8\times10^2$	88.2
4th day	S	$7.4\times10^3\pm 1.0\times10^3$	------	$1.1\times10^3\pm 0.2\times10^3$	------
5th day	S	$8.9\times10^3\pm 1.8\times10^3$	------	$2.6\times10^3\pm 0.4\times10^3$	------
6th day	S	$1.3\times10^4\pm 0.2\times10^4$	------	$4.1\times10^3\pm 0.7\times10^3$	------
7th day	S	$3.9\times10^4\pm 0.5\times10^4$	------	$9.4\times10^3\pm 2.1\times10^3$	------
8th day	S	$6.0\times10^4\pm 1.2\times10^4$	S	$1.7\times10^4\pm 0.3\times10^4$	------
9th day	S	S	S	$3.4\times10^4\pm 0.5\times10^4$	------

R%*= Reduction % S= spoiled
+ ANOVA test indicated significant differences (P<0.05).

Table (4): Effect of chitosan addition on total yeast count of minced meat samples (n=5).

Treatment / Storage time+	Control	1% Chitosan		3% Chitosan +	
		Count	R %	Count	R %*
Zero time	$1.1\times10^3\pm 0.2\times10^3$	$1.1\times10^3\pm 0.2\times10^3$	------	$1.1\times10^3\pm 0.2\times10^3$	------
1st day	$1.9\times10^3\pm 0.4\times10^3$	$1.5\times10^3\pm 0.3\times10^3$	21.1	$1.2\times10^3\pm 0.3\times10^3$	36.8
2nd day	$7.0\times10^3\pm 1.2\times10^3$	$3.3\times10^3\pm 0.5\times10^3$	52.9	$1.8\times10^3\pm 0.3\times10^3$	74.3
3rd day	$2.2\times10^4\pm 0.5\times10^4$	$6.9\times10^3\pm 1.1\times10^3$	68.6	$2.7\times10^3\pm 0.4\times10^3$	87.7
4th day	S	$8.1\times10^3\pm 1.7\times10^3$	------	$4.0\times10^3\pm 0.6\times10^3$	------
5th day	S	$2.3\times10^4\pm 0.4\times10^4$	------	$7.5\times10^3\pm 1.2\times10^3$	------
6th day	S	$4.9\times10^4\pm 0.8\times10^4$	------	$1.1\times10^4\pm 0.3\times10^4$	------
7th day	S	$8.1\times10^4\pm 1.6\times10^4$	------	$3.0\times10^4\pm 0.5\times10^4$	------
8th day	S	$2.2\times10^5\pm 0.4\times10^5$	S	$7.3\times10^4\pm 1.0\times10^4$	------
9th day	S	S	S	$9.2\times10^4\pm 2.1\times10^4$	------

R%*= Reduction % S= spoiled
+ ANOVA test indicated significant differences (P<0.05).

count became $3.0\times10^2 \pm 0.4\times10^2$cfu/g in the 1st day with reduction 62.5 % relative to control samples .While in the second day , total mold count became $4.0\times10^2 \pm 0.5\times10^2$cfu/g with reduction 75.0 % and still to increase till reach $6.0\times10^2 \pm 0.8\times10^2$cfu/g with reduction 88.2 % at 3rd day of storage.

By the 4th day, the mold count in chitosan 3% treated samples were $1.1\times10^3 \pm 0.2\times10^3$cfu/g then became $2.6\times10^3 \pm 0.4\times10^3$, $4.1\times10^3 \pm 0.7\times10^3$, $9.4\times10^3 \pm 2.1\times10^3$cfu/g in the 5th , 6th and 7th day respectively , In the 8th day , the mold count became $1.7\times10^4 \pm 0.3\times10^4$ and continue to increase till reach finally to $3.4\times10^4 \pm 0.5\times10^4$cfu/g and signs of spoilage appear.

Total Yeast count

Results in table (4) indicated that the total yeast count at zero time in all examined samples were $1.1\times10^3 \pm 0.2\times10^3$cfu/g while total yeast count at 1st day at chilling was $1.9\times 10^3 \pm 0.4\times10^3$, $1.5\times10^3\pm 0.3\times10^3$,$1.2\times10^3 \pm 0.3\times10^3$ cfu/g for control, 1% chitosan , 3% chitosan treated samples respectively with reduction 21.1 % , 36.8 % relative to control samples respectively.

By the 3rd day, the total yeast count was $2.2\times10^4 \pm 0.5\times10^4$, $6.9\times10^3 \pm 1.1\times10^3$, $2.7\times10^3\pm0.4\times10^3$cfu/g for control, 1% chitosan , 3% chitosan treated samples respectively with reduction 68.6% , 87.7 % relative to control samples respectively.At 4th day control sample give signs of spoilage, but both samples treated with either chitosan 1% or chitosan 3% extending shelf life of treated samples till 7th day of chilling for chitosan 1% and 9th day for chitosan 3% treated samples.

By the 5th day, the count became$2.3\times10^4 \pm 0.4\times10^4$, $7.5\times10^3 \pm 1.2\times10^3$cfu/g in 1% chitosan and 3% chitosan treated samples respectively. In the 8th day , the chitosan 1% treated samples show signs of spoilage and the count become $2.2\times10^5 \pm 0.4\times10^5$cfu/g while in chitosan 3% treated samples were $7.3\times10^4 \pm 1.0\times10^4$, $9.2\times10^4 \pm 2.1\times10^4$cfu/g in the 8th and 9th day respectively then the samples spoiled.

The pH value

As shown in table (5), the mean values of pH at zero time were 5.7 ± 0.01 for all examined samples. The pH value was 5.89 ± 0.02 , 6,19 ± 0.02 , 6,41 ± 0.01 in 1st , 2nd and 3rd day in the control samples then the samples show signs of spoilage.The pH value in the chitosan 1% samples was 5.83 ± 0.02 , 5.89 ± 0.01 , 5.94 ± 0.01 , 6.01 ± 0.01 , 6.07 ± 0.01 , 6.10 ± 0.02 , 6.19 ± 0.01 , 6.33 ± 0.03 in the 1st , 2nd , 3rd , 4th , 5th , 6th , 7th and 8th day respectively then the samples show signs of spoilage. The pH value in the chitosan 3% samples was 5.81 ± 0.01 , 5.85 ± 0.02 , 5.88 ± 0.01 , 5.90 ± 0.01 , 5.93 ± 0.01 , 5.98 ± 0.02 , 6.04 ± 0.01 , 6.12 ± 0.02 , 6.18 ± 0.02 in the 1st , 2nd , 3rd , 4th , 5th , 6th , 7th , 8th and 9th day respectively then the samples show signs of spoilage.

TVB-N values

As demonstrated in table (6), the quantity of TVB-N at zero time was 1.8±0.09 mg/100g in all examined samples then gradually increased in all groups.In control samples, the TVB-N was 9.64 ± 0.37, 16.33 ± 0.81, 25.06 ± 0.94 mg/100g in 1st, 2nd and 3rd day respectively then the samples give signs of spoilage.In the 1% chitosan treated samples , the TVB-N was 3.95 ± 0.14 , 5.41 ± 0.16 , 8.17 ± 0.32 , 10.59 ± 0.28 , 13.02 ± 0.51 , 16.44 ± 0.57 , 19.10 ± 0.72 , 24.56 ± 0.96mg/100g in 1st , 2nd, 3rd , 4th , 5th , 6th , 7th and 8thday respectively then the samples give signs of spoilage at 9th day.

Chitosan 3% treated groups were in the range of permissible level in all days and the content of TVB-N was not over 20mg/100g . The TVB-N was 3.05 ± 0.10 , 4.99 ± 0.11 , 6.76 ± 0.23 , 9.12 ± 0.30 , 11.14 ± 0.36 , 12.83 ± 0.45 , 14.52 ± 0.61 , 17.46 ± 0.67 , 19.27 ± 0.80mg/100g in 1st , 2nd , 3rd , 4th , 5th , 6th , 7th , 8th and 9th day respectively then the samples give signs of spoilage in the next day.

Table (5): Effect of chitosan addition on pH values of minced meat samples at chilling temperature "2°C" (n=5).

Storage time+ \ Treatment	Control	1% Chitosan	3% Chitosan +
Zero time	5.76 ± 0.01	5.74 ± 0.01	5.73 ± 0.01
1st day	5.98 ± 0.02	5.83 ± 0.02	5.81 ± 0.01
2nd day	6.19 ± 0.02	5.89 ± 0.01	5.85 ± 0.02
3rd day	6.41 ± 0.01	5.94± 0.01	5.88± 0.01
4th day	S	6.01 ± 0.01	5.90 ± 0.01
5th day	S	6.07 ± 0.02	5.93 ± 0.01
6th day	S	6.10 ± 0.02	5.98 ± 0.02
7th day	S	6.19 ± 0.01	6.04 ± 0.01
8th day	S	6.33 ± 0.03	6.12 ± 0.02
9th day	S	S	6.18 ± 0.02

pH should not exceed 6.2 (EOS, 2005) .
S= spoiled
+ ANOVA test indicated significant differences (P<0.05).

Table (6): Effect of chitosan addition on TVB-N values (mg/100g) of minced meat samples at chilling temperature "2°C" (n=5).

Storage time+ \ Treatment	Control	1% Chitosan	3% Chitosan +
Zero time	1.87± 0.09	1.86± 0.09	1.84± 0.09
1st day	9.64 ± 0.37	3.95 ± 0.14	3.05 ± 0.10
2nd day	16.33 ± 0.81	5.41 ± 0.16	4.99 ± 0.11
3rd day	25.06 ± 0.94	8.17± 0.32	6.76± 0.23
4th day	S	10.59 ± 0.28	9.12 ± 0.30
5th day	S	13.02 ± 0.51	11.14 ± 0.36
6th day	S	16.44 ± 0.57	12.83 ± 0.45
7th day	S	19.10 ± 0.72	14.52 ± 0.61
8th day	S	24.56 ± 0.96	17.46 ± 0.67
9th day	S	S	19.27 ± 0.80

TVB-N should not exceed 20 mg/100g (EOS, 2005).
+ ANOVA test indicated significant differences (P<0.05).

Table (7): Effect of chitosan addition on TBA values (mg/Kg) of minced meat samples at chilling temperature "2ºC" (n=5).

Storage time+ \ Treatment	Control	1% Chitosan	3% Chitosan +
Zero time	0.08± 0.01	0.07± 0.01	0.07± 0.01
1st day	0.47± 0.37	0.15 ± 0.01	0.10 ± 0.01
2nd day	0.84± 0.81	0.22 ± 0.01	0.16 ± 0.01
3rd day	1.13 ± 0.94	0.29 ± 0.01	0.21 ± 0.01
4th day	S	0.35 ± 0.02	0.28 ± 0.01
5th day	S	0.46 ± 0.02	0.37 ± 0.02
6th day	S	0.60 ± 0.04	0.46 ± 0.03
7th day	S	0.78 ± 0.03	0.59 ± 0.02
8th day	S	0.92 ± 0.05	0.70 ± 0.02
9th day	S	S	0.86 ± 0.03

TBA should not exceed 0.9 mg/Kg (EOS, 2005) .
+ ANOVA test indicated significant differences (P<0.05).

TBA values

In table (7), The quantity of TBA at zero time was 0.08 ± 0.01 mg/Kg in all examined samples then gradually increased in all groups. In control samples, the TBA was 0.47 ± 0.37 , 0.84 ± 0.81 , 1.13 ± 0.94 mg/Kg in 1st , 2nd and 3rd day respectively then the samples give signs of spoilage.In the 1% chitosan treated samples , the TBA value was 0.15 ± 0.01 , 0.22 ± 0.01 , 0.29 ± 0.01 , 0.35 ± 0.02 , 0.46 ± 0.02 , 0.60 ± 0.04 , 0.78 ± 0.03 , 0.92 ± 0.05 mg/Kg in 1st , 2nd , 3rd , 4th , 5th , 6th , 7th and 8th day respectively then the samples give signs of spoilage at 9th day.

Chitosan 3% treated groups were in the range of permissible level in all days and the content of TBA was not over 0.9 mg/ Kg . The TBA was 0.10 ± 0.01 , 0.16 ± 0.01 , 0.21 ± 0.01 , 0.28 ± 0.01 , 0.37 ± 0.02 , 0.46 ± 0.03 , 0.59 ± 0.02 , 0.70 ± 0.02 , 0.86 ± 0.03 mg/Kg in 1st , 2nd , 3rd , 4th , 5th , 6th , 7th , 8th and 9th day respectively then the samples give signs of spoilage in the next day.

Sensory traits

The data illustrated in Table (8) declared that the final score for control group was (5) very good, (4-4.4) good, (2.6-3.6) acceptable, (1) bad at zero, 1st, 2nd, 3rd day of chilling storage at 2°C respectively. There was no significant difference (p< 0.05) between all examined groups at zero time.

Regarding chitosan 1% treated group, the final score was 5 (very good) at zero day, (4-4.8) good for 1st, 2nd ,3rd, 4th day , (2.6-3.8) acceptable for 5th, 6th, 7th, (1) bad for 8th day, meanwhile, signs of spoilage was cleared by 9th day of chilling storage at 2°C , the control samples were decomposed.

In concern to chitosan 3% samples , the final score was (4.6-5) very good at zero, 1st, 2nd days , good (3.6-4.4) for 3rd , 4th ,5th , 6th day , acceptable (2.6-3.8) for 7th, 8th (1.6-2.4) bad for 9th day where signs of spoilage was cleared at chilling storage at 2°C. There were significant difference (P<0.05) in the sensory traits of control group and the other treated groups by chitosan 1% or chitosan 3%.

The final score is obtained from the average of color, odor, appearance and consistency.

DISCUSSION

Chitosan has a strong antimicrobial activity against Gram-positive,Gram-negative bacteria in addition to fungi. Aerobic plate count (APC) is considered the most reliable index of meat quality, sanitary processing and storage life of meat products (ICMSF, 1980)

As showed in Table (1), control samples give signs of spoilage at 4th day, but both samples treated with either chitosan 1% or chitosan 3% extending shelf life of treated samples till 7th day of chilling for chitosan 1% and 9th day for chitosan 3% samples.Chitosanexhibited strong action on APC especially at concentration 3%.

Chitosan can prevent the growth of aerobic bacteria (APC) as it exhibits a coating film around the bacterial cell acting as an oxygen barrier and depriving bacteria from the needed oxygen.The microbial inhibitory effect of chitosan depends on its molecular weight, concentration, and type of microorganisms. High molecular weight chitosan is able to bind to the negative charged elements present on the cell wall of bacteria resulting in the formation of an impermeable layer everywhere around the bacterial cell, changing

Table (8): Sensory traits of control and chitosan treated minced meat stored at 2 ºC (n=5).

Trait / Storage time+	Color (5)	Odor (5)	Appearance (5)	Consistency (5)	Overall (5)	Grade
Control:						
Zero time	5	5	5	5	5	Very good
1st day	4.4	3.6	3.8	4.2	4.0	Good
2nd day	3.6	2.8	3.4	2.6	3.1	Acceptable
3rd day	1.2	1.2	1.0	1.4	1.2	Bad
4th day	S	S	S	S	S	Spoiled
5th day	S	S	S	S	S	Spoiled
6th day	S	S	S	S	S	Spoiled
7th day	S	S	S	S	S	Spoiled
8th day	S	S	S	S	S	Spoiled
9th day	S	S	S	S	S	Spoiled
1% Chitosan:						
Zero time	5	5	5	5	5	Very good
1st day	4.8	4.4	4.6	4.4	4.5	Good
2nd day	4.4	4.0	4.6	4.2	4.3	Good
3rd day	4.4	4.0	4.2	4.2	4.2	Good
4th day	4.0	3.6	4.4	4.0	4.0	Good
5th day	3.8	3.4	3.6	3.8	3.7	Acceptable
6th day	3.4	3.2	3.2	3.4	3.3	Acceptable
7th day	3.4	2.8	3.0	3.2	3.1	Acceptable
8th day	1.8	1.2	1.2	1.4	1.4	Bad
9th day	S	S	S	S	S	Spoiled
3% Chitosan±:						
Zero time	5	5	5	5	5	Very good
1st day	5.0	4.6	4.6	4.8	4.8	Very good
2nd day	4.8	4.8	4.6	4.6	4.7	Very good
3rd day	4.6	4.6	4.4	4.4	4.4	Good
4th day	4.2	4.4	4.2	4.4	4.3	Good
5th day	4.2	4.0	4.2	4.0	4.1	Good
6th day	4.2	3.6	4.0	4.2	4.0	Good
7th day	3.8	3.6	3.8	4.0	3.8	Acceptable
8th day	3.2	2.8	3.4	3.4	3.2	Acceptable
9th day	2.4	1.6	2.6	1.8	2.1	Bad

5: Very good **4:** Good **3:** Acceptable
2: Unacceptable **1:** Bad **S:** Spoiled
+ ANOVA test indicated significant differences (P<0.05).

cell permeability, and blocking transport into the inside of bacteria (Zheng and Zhu, 2003) (Yuan, Lv, et al., 2016). .

The current results were in agreement with those recorded by Sagoo et al,(2002) who reported a shelf life extension of 2 days in a minced pork mixture after the addition of chitosan (0.6%).Soultos *et al.*,(2008) reported a reduction of 0.5 and 1 log CFU/ g in aerobic plate count of fresh sausages after 1 day of storage at 4°C with the addition of chitosan at a concentration of 0.5 and 1%, respectively. Moreover, Chounou *et al.*, (2013) also reported that the addition of chitosan at a concentration of 1% reduced the aerobic plate count of minced beef by 0.4 to 2 $\log_{10}$ CFU /g during storage at 4°C.Costa et al.(2021) reporteda decrease in the initial population of Enterobacteriaceae in meat samples packed with chitosan in comparison to samples packed with commercial membranes.Psychrotrophic bacteria are mainly responsible for the spoilageof meat products at refrigerated temperatures (Bazargani-Gilaniet al., 2015).

As showed in Table (2), signs of spoilage start by 4th day of chilling in controlsamplesmeanwhilesigns of spoilage appear for 1% chitosan by 8th day of chilling and by 9th day for 3% chitosan treated samples. Chitosan showed a clear antimicrobial effect (P <

0.05).The current findings were supported by Sallam and Samejima (2004) who reported that the initial psychrotrophic count in ground beef samples were ranged from 4.0 to 4.14 log10 CFU/g at zero storage day. Also it was similar to the results of Mehdizadeh and Langroodi (2019) who reported a decrease in the number of psychrotrophs in chicken specimens with a combination of chitosan coating containing propolis extract and thyme essential oil. There were significant differences in psychrotrophic count among the examined groups where chitosan showed a clear antimicrobial effect ($P < 0.05$) on the 7th day of storage this is due to the fact that chitosan and its enzymatic hydrolyzates inhibite growth of Gram-negative bacteria as it acted mainly on the outer surface of the bacteria (No *et al. ,*2002).At a lower concentration (< 0.2 mg/mL), the polycationic chitosan does probably bind to the negatively charged bacterial surface to cause agglutination, while at higher concentrations, the larger number of positive charges may have imparted a net positive charge to the bacterial surfaces to keep them in suspension (Entsar *et al.*, 2003) . Chitosan also hydrolyzes the bacterial cell membrane by affecting the surrounding peptidoglycan layer and this increasing the antimicrobial effect of chitosan (Mehdizadeh and Langroodi, 2019) .

As demonstrated in table (3),the samples treated with1% chitosan and 3% chitosan , the mold count appeared with reduction 60.8 % and 88.2 % respectively at 3rd day of storage while in table (4) the total yeast count appear with reduction 68.6 % and 87.7% respectively at 3rd day.

In general, chitosan has an effect on yeasts and molds,and lactic acid bacteria by approximately 1-3 log cfu g^{-1} for 18 days at 7°C. Chitosan treatment increased the shelf-life of chilled skinless sausages from 7 to 15 days. Addition of 0·3 and 0·6% chitosan to an unseasoned minced pork mixture reduced total viable counts, yeasts and molds, andlactic acid bacteria by up to 3 log cfu g^{-1} for 18 days at 4°C compared with the untreated control. The results indicated that chitosan was an effective inhibitor of microbial growth in chilled comminuted pork products (Sago *et al.*,2013).

The pH value in Table (5) is an important physicochemical characteristic to decide the quality and shelf life of meat. The mean values of pH at zero time were 5.7 for all examined samples which came in accordance with that reported by Sallam and Samejima, (2004) who reported 5.65 -5.8 initial pH in ground beef samples at zero time of storage. There was a significant increase in pH of 1% chitosan treated group ($p< 0.05$) at the 4th day of storage at 2°C.On the contrary pH not exceed 6.2 till 6th day of storage at 2°C. Chitosan had a significant effect on pH, which attributed to the inhibition of bacterial growth on contrary, Chounou *et al.*(2013) noticed that the addition of chitosan at a concentration of 1% did not affect pH during storage at 4°C. Significant differences between groups were observed ($P< 0.05$) by the end of the storage period. The control group had the highest pH value which may be owed to the utilization of amino acids by bacteria, with the accumulation of ammonia as the end product of amino acid decomposition increased pH . Langroodi *et al.*,(2021) noticed that increasing the value of pH negatively affect quality of food especially sensory characters as taste, odor, color and texture.

The enzymatic action and microbial activity responsible for protein degradation and formation of total volatile nitrogen was studied in table (6). The quantity of TVB-N is a very good chemical indicator to assess the quality and freshness of the meat. The (TVB-N) at zero time was 1.8 in all examined samples then gradually increased in all groups. Chitosan 3% treated groups were in the range of permissible level reported by EOS 1694 (2005) which limited the content of TVB-N must be not over 20mg/100g. On contrary, the control group exceeded the permissible limit reached 25 by the 3th day of chilling at 2°Cwhich was higher than permissible limit. Meanwhile, Chitosan 1% and 3% treated groups reached to 24.5 and 19.27 mg% by the 8th and 9th day of chilling at 2°C . Consequently, thus will extending the shelf life for 5 days in chitosan 1% & 6 days for chitosan 3%. In other words, the control group significantly had a higher level of TVB-N ($p< 0.05$) which attributed to the higher and rapid psychrotrophic growth and multiplication in the control group led to degradation of protein and the formation of free amines. Protein, as the main constituent in meat, is uninterruptedly broken down by bacteria and finally produced a diversity of amines, including non-volatiles such as volatile amines and biogenic amines (Rukchon *et al.*,2011). Degraded consistency of meat during storage is due to lipid oxidation.This process is associated with the presence of free radicals contributing to the production of aldehydes responsible for major changes in meat color and rancid flavors (Guillén *et al.*,1998). A very recent study (Rezaeifar *et al.,*2020) indicates a decrease in TVB-N changes

in rainbow trout fillets coated with chitosan containing Lemon verbena essential oil and extract packaged in vaccum conditions . This mainly occurs due to the ability of phenolic compounds to inhibit the growth of bacteria by preventing oxidation of lipids and degradation of proteins. Langroodi *et al.,*(2021) said that endogenous enzymatic activity and microbial growth in meat leads to increase TVBN value during storage and this lead to protein decomposition and generation of volatile nitrogen compounds so using antioxidants antimicrobial agents can prevent lipid oxidation and microbial growth and this inhibit TVBN increasing during storage.

TBA considered one of important parameter to determine degree of lipid oxidation in meat and one of important factors associated with decreasing quality of meat as undesirable rancid flavor and poisoning. In the storage period, oxygen attack the double bond of fatty acids lead to lipid oxidation and free radical formation. TBA value is used to measure secondary oxidation product´s formation like malondialdehyde , alkenals and alkadienals (Shahidi et al, 2003). In table (7) showed that the minced meat samples were acceptable till 8th day using Chitosan 1% and 9th day by Chitosan 3% where TBA values were not exceed 0.9mg MDA/kg recommended by EOS 1694(2005). Moreover, the Chitosan group contains the lower value of TBA is due to the chelation of free iron that is released from meat hemoproteins during heat processing and storage (Georgantelis et al,2007) . The effect of chitosan on oxidative stability of minced beef was studied by Darmadji and Izumimoto**,**(1994) who observed that the addition of chitosan (1%) resulted in a 70% reduction in TBA values of meat after 3 days at 4°C. Moreover, Chounou *et al***,**(2013) noticed that the addition of chitosan at a concentration of 1% had a significant effect on the MDA which equal to 1.5 and 1 mg MDA/ kg in control and chitosan-treated samples during storage at 4°C.

The rate of lipid oxidation in fresh sausages was significantly decreased ($p<0.05$) by addition of increasing levels of chitosan, while samples containing both chitosan and nitrites showed the lowest malondialdehyde (MDA) values, indicating a synergistic antioxidative effect. Consequently, the samples containing the combination of nitrites and chitosan at any level deteriorated less rapidly and were judged as more acceptable than all the other samples (Soultos *et al.*,2008).Khorshidi *et al. ,*(2020) described the reasons for the rapid increase in the value of TBA in chicken samples as following; high content of unsaturated fatty acids in samples,high amount of phospholipids which can increase the pace of oxidation in the storage period and that chemical compounds that present in meat as pigments influence fat oxidation in the storage period.

There were significant difference ($P<0.05$) in the sensory traits of control group and the other treated groups by chitosan 1% or chitosan 3%.The sensory quality has a high influence on consumer satisfaction of the meat and purchase decision. Khorshidi *et al.,*(2020) declared that the reasons for occurrence of early degeneration in samples could be due to increase in number of microbes during storage period. Also, fat oxidation product´s and ammonia production as a result of decomposition of proteins by microorganisms and this is the reason for bad smell of meat and reduction of scores and total unacceptability of meat samples in the final storage days.

Dahaya *et al.,(*2021) proved that the addition of chitosan maintains good organoleptic properties of chicken meat color, texture and odor this is proved by Duncan´s Follow up test1% . It can be said that the improvement in the sensory characters was due to changes in the process of bacterial spoilage and as a result changes in activity of microorganisms responsible for food spoilage.

In conclusion, chitosan had an effect on the quality and prolonged the shelf life of minced meat as it improved the microbiological and chemical as well as sensory characteristics of such food article.

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