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NANO TECHNOLOGICAL ENHANCEMENT OF MEAT BALLS QUALITY

Mohebat A. Abd El - Aziz[1]
Hemmat M. Ibrahim
Nahla Abo EL-Roos
Badawi Anis
Rasha Elsabagh

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A B S T R A C T

Different concentrations of two nano-materials having very small size (~ 20 nm), Zinc oxide (Zn O) with concentrations (12 mM) and Titanium dioxide (Ti O2) with concentrations (12 mM) and Mixture between Zn O + Ti O2 (6 mM) were used for improving microbial, sensory and physic-chemical properties of meatballs during storage at refrigerated temperature for 20 days. Treated meat balls samples with nanoparticles show great improvement in their bacteriological status as the aerobic plate count, psychotropic bacterial count, coliform count and staphylococcus count have been decreased, also the score of Sensory characteristics of treated meatballs such as odor, color and texture were higher than those of control sample that spoiled at 9th day. Moreover, the physico- chemical properties of treated meat ball samples such as (PH, TBA and TVB-N) were significantly (P≤0.05) different from control one throughout all storage period.

1. INTRODUCTION

Meat products are highly susceptible to spoilage during all steps of processing which have undesirable effects on meat industry. The main case of deterioration of meat products during processing and storage are microorganism growth that lead to degradation of protein and release toxic compound, moreover lipid oxidation and accumulation of malonaldhyde (MDA), that is a product from lipid oxidation associated with toxic and mutagenic effects (Lorenzo et al., 2018). Increasing meat consumption around the world, provide concerns and challenges to meat hygiene and safety. Mostly these concerns are of a biological nature and include bacterial pathogens (Sofos & Geornaras, 2010). Meat industry is a bright example for production of perishable products as their shelf life may be reduced if no chemical preservatives are added (Banerjee, Verma, & Siddiqui, 2017). So, nanotechnology gives the food industry many new chances for improving the quality, safety, shelf life, and healthiness of foods (Huang et al., 2017).

Nanotechnology is relatively novel technology which may be the beginning of the second technical revolution. It is based on the characterization, fabrication and manipulation of structures or materials smaller than 100 nm (approximately1–100 nm in length), some large food companies such as Kraft, Nestlé and Unilever experiment with nanotechnologies in order to make better, tastier, safer and more acceptable products for consumers

[1] Corresponding author: Mohebat A. Abd El - Aziz
Email: pepovet79@yahoo.com

(Ozimek, Pospiech, & Narine, 2010), also it offers possibility of reducing preservatives and other undesirable or potentially harmful substances in food products, affects development of new or improved tastes, textures and bioavailability of nutrients and supplements, and also may extend shelf–life and keep products safe from microbial pathogens (Chaudhry & Castle, 2011). So, The advantage of nanotechnology has recently been applied to overcome these challenges of food and environmental issues(Jaiswal, Shankar, & Rhim, 2019).

Nanomaterial's currently used for meat applications generally; include the use of nanomaterial as food ingredients or additives that are used directly into food, or as a part of packaging materials (Rhim, Park, & Ha, 2013). Moreover, this new technology can be utilized to improve the stability of such micronutrients not only during processing but storage and distribution, as well (Chen, Weiss, & Shahidi, 2006).Nanomaterial's that commonly used as antibacterial agent in food industry are Titanium dioxide and Zinc oxide (Zn O) (Ti O_2) (Duncan, 2011).

Ti O_2 nanoparticles have been approved for use in human food by the American Food and Drug Administration (FDA) as it is nontoxic material having great broad spectrum antimicrobial property (Hashim, 2011). The antimicrobial effects of Ti O_2 NPs on bacterial growth of *E. coli* and *Staphylococcus aureus* have been studied (L. Wang, Hu, & Shao, 2017). Also, Zn O NPs are multifunctional inorganic nanoparticles has many features as physical and chemical stability, effective antibacterial activity and high catalysis activity (Matei, Cernica, Cadar, Roman, & Schiopu, 2008). The effectiveness of Zn O nanoparticles as antibacterial agent was increased when particle size has been decreased and its powder concentration increased (Emami-Karvani & Chehrazi, 2012).

Zn O nanoparticles have many advantage so, are useful for resolving the problems of food safety that resulted from the growth of pathogenic microorganisms, as they can decrease the development of bacteria, mold, and yeast that resulted in food spoilage during storage period. The function of Nano systems may act as antioxidants, which possible to reduce deterioration rates (Marcous, Rasouli, & Ardestani, 2017).

Generally deterioration of meat and meat products are caused by microbial growth along with the biochemical and enzymatic deterioration (Bonilla, Vargas, Atarés, & Chiralt, 2014). Total coliforms count was used as an indicator to assess the food safety and the product quality. Coliforms are microbiological indicators often employed to assess food safety and sanitation, and can be also used to evaluate foodstuff shelf life. Counting of psychotropic microorganisms is important to be evaluated in meat and meat products samples that kept under refrigeration as most strains of psychotropic bacteria are spoilage microorganisms and some of them are pathogenic (Jay, Loessner, & Golden, 2005).

The sensory parameters of foods is considered as the most important factor that control the demand of the consumers (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005). During lipid oxidation Reactions off odor can be formed also, off-flavor, changes in texture and changes in color, as the myoglobin has been oxidized causes discoloration, which affect the acceptance and consumer's choice (Gong, Parker, & Richards, 2010). Among all quality attributes, color is one of the most critical for the selection of many food products (Pires, de Souza, & Fernando, 2018). The reactions that occurred during oxidation of lipid can lead to the changes in acceptance parameters as odor, taste, color and flavor these changes occurred due to the myoglobin oxidation causes. Not only organoleptic changes are important during the development of lipid oxidation, but also the formation of toxic compounds such as aldehydes (Banerjee et al., 2017).

Thiobarbituric acid reactive substances (TBARS) assay quantify the malonaldehyde (MDA) present in the sample, being a typical indicator of lipid rancidity in meat products, providing useful information on lipid oxidation (Tornuk, Hancer, Sagdic, & Yetim, 2015).

The potential of hydrogen (pH) control the enzymatic reactions which support the growth of microorganisms during the food production chain. Control the pH value lead to production of a safer and well-preserved food. Increasing pH value favors the multiplication of bacteria, while decreasing pH inhibits those (Mendes, 2013).

The pH is estimated at 7.2 in live muscle, and the muscle is transformed into meat when the animal is slaughtered, the glycogen is converted to lactic acid, causing the pH to decrease. Values of pH ranged from 5.2 to 7 but in higher quality products usually vary between of 5.7 to 6 (lower pH variation) (Barbut, 2009).

2. MATERIALS AND METHODES

2.1 Meatball manufacture

In each trial, homogeneous dough were obtained from mixing of minced meat and all ingredients of spices then divided into 4 groups. 1st group Zn O (12mM) was added, 2nd group Ti O2 (12mM), 3rd group Mix (6mM Zn O+6mM Ti O2 50%:50%) and 4th group control one without nanoparticles. Meatballs (50 ± 2 g) were formed by hand, placed into plastic trays, sealed with one layer of a wrapping film, and stored at 4 ± 1 °C for 20 days. The samples were taken for analysis at 0, 3, 6, 9, 12, 15, 17 and 20 days of storage. This study was repeated in triplicate for three different sources of meat (Morsy, Elsabagh, & Trinetta, 2018).

2.2 Nanomateriales

Zinc oxide and Titanium dioxide nanoparticles have been Synthesized and Prepared at Spectroscopy Department, Physics Division in National Research Centre.

Synthesis and Preparation of Zinc Oxide nanoparticles:

Zinc oxide nanoparticles were prepared by dissolving 11.0 gm. zinc acetate hydrate (Zn (Ac) 2•2H2O, with 99.9% purity) in 500mL ethanol. Then 2.9 gm. sodium hydroxide was added into the solution through ultra-sonication, a transparent solution was obtained. After that the conical flask containing the transparent solution was put into a water tank with a constant temperature of 60ºC. To this solution, 10 ml distilled water was added into the conical flask. The solution was stirred for 30 min at 60ºC. The prepared Zn O nanoparticles were collected by centrifuging and drying at 60°C (H. Wang, Xie, Zhang, Cai, Yang, & Gui, 2007).

Physical properties:

Prepared nanoparticles were examined under TEM to evaluate its physical properties.

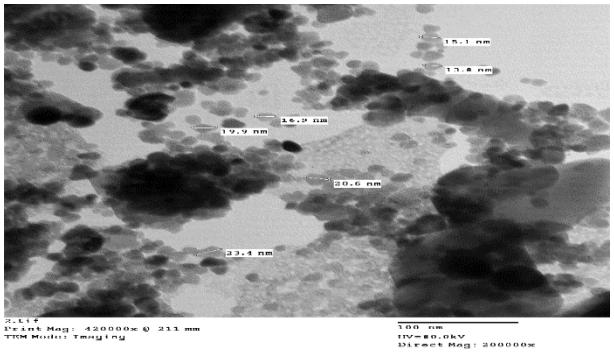

Figure 1. physical properties of zinc oxide nanoparticles under TEM

Appearance(color):white Size (TEM) : 20 ±5 nm
Purity: 99.9%. Appearance (form): powder.
Shape (TEM): Spherical shape
Solubility: stable colloid in mixture of ethanol and chloroform and water

Synthesis and Preparation of Titanium dioxide nanoparticles:

Titanium tetrachloride TiCl4 (Fluka 98%) was used as a starting material. Ti O2 nanoparticles were prepared by drop wise addition of 4 ml of TiCl4 into 400 ml of water/ethanol solution (3:1) at 0ºC with vigorous stirring. Subsequently, a dilute solution of NH4OH was used to adjust the pH value at 9. The solution was refluxed for 4 hrs. with continues stirring. Then the solution was cooled down to room temperature naturally. The Ti O_2 nanoparticles were obtained by centrifuging at 4000 rpm. The formed Ti O_2 was washed using acetone several times and then dried at 100ºC for 5 hrs. The powder was annealed at 400ºC in air for 2 hrs. By raising the temperature at a rate of 10ºC/min(Yin et al., 2001).

Physical properties

Prepared nanoparticles were examined under TEM to evaluate its physical properties.

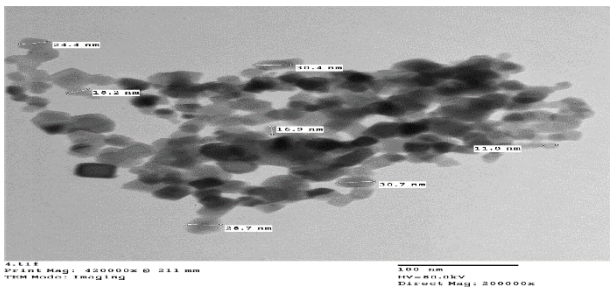

Figure 2. physical properties of titanium dioxide nanoparticles under TEM

Appearance(color):white. Appearance (form): powder.
Purity : 99.9% Size (TEM): 20 ± 5nm Shape (TEM): Spherical shape
Solubility : stable colloid in mixture of methanol, ethanol, isopropanol and water

Microbiological assay

Sample preparation (Preparation of serial dilution) according to (ISO, 1999)
Determination of aerobic plate count at 35^0c: surface plate method (Petran, Grieme, & Foong-Cunningham, 2015)
Determination of Staphylococci *spp.* count (FDA, 2001).
Determination of coliform counts (Feng, Weagant, Grant, & Burkhardt, 2002)
Determination of psychrotrophic bacterial counts (FDA, 2001).

Physical assay

PH measurements

The pH and acidity were determined according to the method described in (Zenebon, Pascuet, Tiglea, Zenebon, Pascuet, & Tiglea, 2008).

Chemical assay

Total volatile base nitrogen (TVB-N)

The content of TVB-N was performed according to the method described by (Egan, Kirk, & Sawyer, 1981).The TVB-N was expressed as mg N/100 g of sample.

Thiobarbituric acid (TBA)

The oxidative state of the samples was evaluated by the determination of the thiobarbituric acid reactive

substances (TBARS) values, according to (Rosmini et al., 1996) Results are expressed as mg of MDA/kg of sample. Sensory evaluation.

Selection of panelists as a) announcement and preliminary screening of 15 naive assessors ;b) pre-selection of 12 naive assessors who are to become initiated assessors; c) choosing of 6 the selected assessors in order to determine their ability to perform specific tests, who then become selected assessors; d)Training of selected assessors four sessions three hrs. of each, to become expert sensory assessors (ISO 8586, 2012). The panelists were able to perform descriptive sensory analysis for treated samples and control one and give reliable comparative judgments. The panel received a list of descriptors (color, odor and texture) to score on numerical and continuous scales from 0 (the lowest score for each attribute) to 10 (the highest score for each attribute) (Cullere, M., et al. 2018).

Sensory evaluation of meat balls samples (control and treated ones) was assessed under controlled condition of temperature (21^{o}c), light and humidity (65%) by six well trained panelists (30-35years, females having body weight (70±5 kg) who were working in Food Hygiene Department. The criteria used as the basis of the descriptive organoleptic assessment (color, odor and texture) with triangle test and the hedonic rating system. The scale points were as follows: (Excellent : 9 -Very very good : 8 - Very good : 7 - Good : 6 -Medium : 5 - Fair : 4 - Poor : 3 Very poor :2- Very very poor : 1) was used in the evaluation (ISO 13299, 2003) .
2.3 Statistical analysis

Experiments were run in triplicate for each treatment (three replicate in each independent experiment).They were performed under controlled condition of temperature, humidity and light by 6 well trained panelists. The challenge design was a 3×8 factorial in completely randomized design with fixed factors as (meatballs size, environmental condition during storage and analysis, analyst) and random factors as meat collected from different sources, meatballs coded with 3 numbers randomly. Three treatments of Zn O (12mM), Ti O_2 (12mM) and Mixture (Zn O 6mM+Ti O_2 6mM)) during 8 sampling days (Zero day, 3rd, 6th,9th,12th,15th, 17th and 20th day) at a refrigerated storage (3-5 °C).

Microbiological data were transformed into logarithms of the number of colony forming units (CFU/gm).

Sensory evaluation for (color, odor and texture) was assessed under controlled condition of temperature (21^{o}c), humidity 65% and light by 6 well trained panelists who were selected according to Totally 432 samples were examined on 8 sessions, 54 sample per session on 6 rounds per session at zero day, 3rd day, 6th day, 9th day, 12th day, 15th day and 17th and day. Every one of panelists take disposable dish contains three samples (two identical and another different) in triangle form randomly coded with three numbers and work sheet to give the score for each point.

Physic-chemical examination also occurs in randomized design in mixed model. All data were subjected to analysis of variance (ANOVA) using SPSS program for windows (Version 22) (SPSS Inc. Chicago, IL and USA). Means and standard deviations were calculated to remove diversity of results.
, and, F-values were significant at the $P\leq 0.05$ level, Multiple mean comparisons were done by applying Duncan's Multiple Range test (DMRT) that used for measuring the specific differences between pairs of means (Yin, Wu & Jiang, 2007).

3. RESULTS AND DISCUSSION

3.1 Bacteriological analysis

In the present study the bacteriological changes as (Aerobic plate count (APC), psychotrophic bacterial count (PTC), Staphylococcus spp. count and coliform count) of meat balls were evaluated throughout the storage period for 20 days at refrigerating conditions 4^{o}c (as shown in tables 1–4,respectively).

Aerobic bacterial count

Adding nanoparticles (Zn O and Ti O2) to meat balls during processing lead to decrease the initial total bacterial load throughout chiller storage period .During chiller storage, APC has been decreased ~3log CFU in samples treated with (Zn O (12mM)and Ti O2(12mM)), decreased ~2 log CFU in samples treated with mixture (Zn O and Ti O_2(6mM)),While control one increase ~3log CFU as seen in table (1) with significance difference $P\leq 0.05$. Based on the results obtained Zn O (12mM) was the most effective material for destruction of total bacterial count. As the antibacterial properties of zinc oxide (Zn O) nanoparticles were based on prevention of DNA replication and interfered with vital cellular processes through interfering with enzymes (Duncan, 2011) followed by Ti O_2(12mM) as its antibacterial activity was related to production of reactive oxygen especially hydroxyl free radicals and peroxide formed that resulted in destruction of bacterial cell (Xing et al., 2012). The synergistic effect of the mixture between (Zn O and Ti O2 (6mM)) was not present as reported by (Marcous et al., 2017) who concluded that there is no synergistic action between Zn O and Ti O2 mixture.

Table 1. Effect of nanoparticles on of the Aerobic plate count of the examined untreated (control) and treated samples of meat balls during chiller storage at 4ºC.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	4.46±0.2^{a}	4.83±0.8^{b}	5.75±0.4^{ab}	6.29±0.5^{cb}	6.83±0.6^{d}	7.1±0.2^{e}	7.58±0.42^{f}	7.89±0.41^{f}
Zn O (12mM)	4.46±0.2^{a}	4.37±0.15^{b}	3.61±0.11^{c}	3.32±0.22^{c}	2.42±0.31^{dc}	2.1±0.11^{d}	1.98±0.35^{fg}	1.55±0.11^{fc}
Ti O_2 (12Mm)	4.46±0.2^{a}	4.43±0.18^{b}	3.72±0.12^{c}	3.55±0.23^{c}	2.61±0.41^{dc}	2.3±0.12^{d}	2.11±0.21^{fg}	1.96±0.15^{fc}
Mixture	4.46±0.2^{a}	4.49±0.20^{b}	3.82±0.15^{c}	3.63±0.13^{c}	2.72±0.44^{dc}	2.35±0.15^{d}	2.23±0.32^{fg}	1.99±0.26^{fg}

Psychrotrophic bacterial count (PTC)

The ability of Psychrotrophic bacteria to survive and multiply under chiller storage (4°C) made it very relevant for safety and stability of meat balls. The counts are shown in table (2) proved that all results of treated samples are significantly different $P \leq 0.05$ to control one. PTC has been decreased ~3log CFU in samples treated with Zn O 12mM , decreased ~2 log CFU in samples treated with Ti O2(12mM), mixture (Zn O and Ti O2(6mM)),while control one increase ~2log CFU(6.79 log CFU)which accompanied by slime formation and putrefactive odor as result obtained by (Abdou, Galhoum, & Mohamed, 2018). The results obtained proved that Zn O (12mM) was the most effective compound to protect the meat balls from spoilage during chiller storage followed by Ti O2(12mM), mixture between (Zn O and Ti O2(6mM)).

Table 2. Effect of nanoparticles on of the Phychrotrophic bacterial count of the examined untreated (control) and treated samples of meat balls during chiller storage at 4ºC.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	4.35±0.25^{a}	4.71±0.35^{b}	4.93±0.4^{ab}	5.25±0.25^{cb}	5.61±0.12^{d}	5.89±0.45^{e}	6.35±0.15^{f}	6.79±0.11^{f}
Zn O (12mM)	4.35±0.25^{a}	4.16±0.10^{b}	3.68±0.15^{c}	3.33±0.11^{c}	3.15±0.18^{dc}	2.58±0.22^{d}	2.1±0.16^{fg}	1.98±0.22^{fg}
Ti O_2 (12mM)	4.35±0.25^{a}	4.21±0.15^{b}	3.73±0.15^{c}	3.52±0.15^{c}	3.33±0.22^{dc}	2.69±0.28^{d}	2.22±0.14^{fg}	2.1±0.30^{fc}
Mixture	4.35±0.25^{a}	4.25±0.25^{b}	3.88±0.20^{c}	3.63±0.17^{c}	3.48±0.23^{dc}	2.73±0.15^{d}	2.4±0.11^{fc}	2.25±0.42^{fc}

Staphylococci bacterial count

As shown in table (3) the antibacterial effect of nanoparticles against staphylococci has been proved as the counts of treated sample were significantly ($P \leq 0.05$) different to control one. Staphylococci count becomes 1.41, 1.69 and 1.83 (log CFU/gm.) for Zn O 12mM , Ti O2(12mM) and mixture (Zn O and Ti O2(6mM)) respectively, while the count for control one become 5.40 log CFU/gm. Zn O has great antimicrobial effect against staphylococci because of the high affinity between it and surface membrane of the bacteria (Sawai, 2003) and higher sensitivity proved by (Premanathan, Karthikeyan, Jeyasubramanian, & Manivannan, 2011).

Table 3.Effect of nanoparticles on Staphylococci count of the examined untreated (control) and treated samples of meat balls during chiller storage at 4ºC.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	3.25±1.1^{a}	3.47±1.2^{b}	3.91±0.6^{ab}	4.12±0.2^{cb}	4.61±0.4^{d}	4.94±0.3^{e}	5.1±0.15^{f}	5.4±0.4^{f}
Zn O (12mM)	3.25±1.1^{a}	3.11±0.2^{b}	2.81±0.3^{c}	2.53±0.11^{c}	2.33±0.2^{dc}	2.15±0.11^{d}	1.53±0.1^{fg}	1.41±0.01^{fg}
Ti O_2 (12Mm)	3.25±1.1^{a}	3.15±0.3^{b}	2.92±0.3^{c}	2.78±0.12^{c}	2.54±0.2^{dc}	2.29±0.15^{d}	1.81±0.1^{fg}	1.69±0.02^{fg}
Mixture	3.25±1.1^{a}	3.18±0.2^{b}	2.95±0.4^{c}	2.81±0.15^{c}	2.63±0.25^{dc}	2.42±0.2^{d}	2.17±0.11^{fc}	1.83±0.01^{fg}

Coliform bacterial count

As shown in table (4) the antibacterial effect of nanoparticles against coliforms has been proved as the counts of treated sample were significantly ($P \leq 0.05$) different to control one. During chiller storage nanoparticles prevent multiplication and growth of coliform bacteria so the counts decrease to 1.43, 1.92 and 2.14 (log CFU/gm.) for Zn O 12mM, Ti O2 (12mM) and mixture between (Zn O and Ti O2 (6mM)) respectively, while the count for control one become 6.86 log CFU/gm.

Table 4. Effect of nanoparticles on coliform of the examined untreated (control) and treated samples of meat balls during chiller storage at 4ºC.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	3.53±0.12^{a}	3.71±0.16^{b}	4.25±0.18^{ab}	4.83±0.22^{cb}	5.41±0.24 ^{d}	5.65±0.35 ^{e}	6.39±0.21^{f}	6.86±0.19^{f}
Zn O (12mM)	3.53±0.12^{a}	3.33±0.14^{b}	3.26±0.11^{c}	2.75±0.11^{c}	2.49±0.15^{dc}	2.25±0.16^{d}	1.71±0.11^{fg}	1.43±0.10^{fc}
Mixture	3.53±0.12^{a}	3.45±0.13^{b}	3.39±0.13^{c}	2.81±0.11^{c}	2.63±0.18^{dc}	2.44±0.15^{d}	2.1±0.14^{fg}	1.92±0.11^{fc}
Ti O_2 (12Mm)	3.53±0.12^{a}	3.46±0.11^{b}	3.4±0.15^{c}	2.86±0.14^{c}	2.71±0.19^{dc}	2.56±0.21^{d}	2.44±0.16^{fg}	2.14±0.12^{fg}

3.2 Physical quality criteria

PH

As shown in table (5) that there were significant differences ($P \leq 0.05$) among treated and non-treated samples During chilling storage at 4 °C, the pH values were 7.2, 7.4 and 7.6 for Zn O 12mM , Ti O2(12mM) and mixture (Zn O and Ti O_2(6mM)), respectively and 8.05 for control one at the end of challenge study .The pH values increase as the bases of alkaline volatile increase (e.g., tri-methylamine and ammonia) formed by either endogenous or microbial enzymes (Badee, Moawd, ElNoketi, & Gouda, 2013).This increase in PH support the growth of lipolytic and putrefactive microorganism lead to meat ball spoilage in 9th day of chiller storage.

Table 5. Changes in physical quality criteria (PH) of meat balls after treatment with nanoparticles during storage at 4 °C.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	5.71±0.01^{a}	6.19±0.03^{ab}	6.5±0.015^{c}	6.7±0.12^{d}	6.79±0.12^{e}	7.15±0.15^{f}	7.9±0.2^{h}	8.05±0.20^{fc}
Zn O (12mM)	5.71±0.01^{a}	5.9±0.02^{ab}	6.05±0.012^{cb}	6.33±0.01^{dc}	6.45±0.11^{ec}	6.6±0.05^{fg}	6.7±0.06^{hg}	7.2±0.11^{fd}
Ti O2 (12mM)	5.71±0.01^{a}	5.95±0.01^{ab}	6.15±0.014^{cb}	6.4±0.01^{dc}	6.47±0.13^{ec}	6.7±0.07^{fg}	6.8±0.08^{hg}	7.4±0.21^{fd}
Mixture	5.71±0.01^{a}	6.06±0.01^{ab}	6.27±0.011^{cb}	6.5±0.01^{dc}	6.55±0.10^{ec}	6.89±0.09^{fe}	7.1±0.07^{fd}	7.6±0.11^{fc}

3.3 Chemical quality criteria

TVB – N

One of the most chemical criteria that have importance from quality view is total volatile nitrogen (TVN).During the 20-day of challenge study, TVB-N increased due to protein degradation which occurred from bacterial or enzymatic degradation. As seen in table (6) TVB – N gradually increased during chiller storage 4ºc with significant ($p \leq 0.05$) difference between treated and control sample. TVB-N values were 22.2, 30.4 and 32.9, (mg/100gm) for Zn O (12mM), Ti O2 (12mM) and mixture (Zn O and Ti O_2(6mM)) respectively and 45.4 mg/100mg for control one at the end of challenge study. The maximum permissible limit for TVN 20 mg/100mg (EOS, 2005) ,this mean that control sample must rejected at 9th day of chiller storage4ºc while sample treated with Zn O 12mM was accepted till 17 day of chiller storage 4ºc followed by Ti O2(12mM), mixture (Zn O and Ti O2(6mM)) which still accepted till15day.

Table 6. Changes in physicochemical quality criteria TVB-N of meat balls after treatment with nanoparticles during storage at 4 °C.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	17th day	20th day
Control	3.46±0.12^{a}	8.36±0.25^{b}	15.46±0.12^{c}	21.82±0.52^{d}	25.2±0.38^{e}	31.7±0.18^{f}	38.1±0.02^{a}	45.4±0.30^{a}
Zn O (12mM)	3.46±0.12^{a}	5.52±0.12^{ab}	7.53±0.13^{b}	9.88±0.35^{cd}	11.12±0. 11^{ed}	15.8±0.13f^{g}	20.1±0.15^{b}	22.2±0.10^{d}
Ti O_2 (12mM)	3.46±0.12^{a}	6.8±0.11a^{b}	9.62±0.16^{cb}	12.29±0. 11^{ed}	15.28±0.12^{cd}	19.1±0.21^{d}	25.8±0.11^{bc}	30.4±0.11^{de}
Mixture	3.46±0.12^{a}	6.98±0.19^{ab}	10.68±0.18^{cb}	13.37±0.11^{ed}	15.44±0.14^{cd}	20.05±0.11^{d}	25.1±0.14^{bc}	32.9±0.12^{de}

TBA

Also thiobarbituric acid (TBA).considered an important factor from quality view as its increase means that malonaldehyde has been formed due to fatty acid oxidation process (Abdel-Hamied, Nassar, & El-Badry, 2009).These compounds (aldehydes) have rancid flavor occurred also due to enzymatic or microbial action. As seen in table (7) TBA gradually increased during chiller storage 4ºc with significant ($p \leq 0.05$) difference between treated and control sample. TVB-N values were 0.92, 1.1 and 1.1, (mg malonaldehyde /kg) for Zn O (12mM) , Ti O2(12mM) and mixture (Zn O and Ti O2(6mM)) respectively and 1.90 mg malonaldehyde /kg for control one at the end of challenge study. The maximum permissible limit for TBA is 0.9 mg malonaldhyde/kg in some meat products(EOS, 2005). This mean that control sample must rejected at 9th day of chiller storage4ºc while sample treated with Zn O 12mM was accepted till 20 day of chiller storage 4ºc.

Table 7. Changes in physicochemical quality criteria TBA of meat balls after treatment with nanoparticles during storage at 4 °C.

Group	Zero day	3rd day	6thday	9th day	12th day	15th day	17th day	20th day
Control	0.20± 0.01^{a}	0.44± 0.02^{b}	0.64±0.01^{b}	0.95±0.02^{cb}	1.20±0.005^{f}	1.60±0.03^{e}	1.80±0.012^{a}	1.90±0.15^{f}
Zn O (12mM)	0.20± 0.01^{a}	0.37±0.03^{ab}	0.39±0.02^{ab}	0.41±0.002^{c}	0.58±0.01^{fc}	0.61±0.001^{ef}	0.80±0.01^{ab}	0.92±0.02^{g}
Ti O_2 (12mM)	0.20± 0.01^{a}	0.38±0.03^{ab}	0.40±0.1^{ab}	0.44±0.003^{c}	0.61±0.006^{fd}	0.85±0.004^{fd}	0.91±0.02^{dc}	1.1±0.01^{fg}
Mixture	0.20± 0.01^{a}	0.40±0.01^{ab}	0.41±0.002^{ab}	0.46±0.006^{c}	0.65±0.002^{fd}	0.89±0.002^{fd}	0.97±0.004^{dc}	1.1±0.06^{fg}

3.3. Sensory evaluation

The acceptability (odor, texture and color) of meat balls during storage at 4^{o}c is shown in table 8. The results showed that there were significant differences ($p \leq 0.05$) between treated samples and control one as control sample spoiled at 9th day of storage and continue decrease during all storage period. Changes in color, odor and texture during storage occurred due to lipid oxidation and protein degradation (Sirocchi et al., 2017).The data also reveals that the shelf life of meat balls extended till17 day and spoiled at 20 day of chiller storage when treated with Zn O (12mM) then samples treated with Ti O2 (12mM) and mixture (Zn O and Ti O2(6mM)) spoiled at17day of chiller storage. Generally, all organoleptic data were in agreement with microbiological, physical and chemical quality indices present in tables (1, 2, 3, 4, 5, 6 and 7).

Table 8. Effect of treatment with nanoparticles on Acceptability of meat balls during storage at 4^{o}C up to 20 days.

Groups	Zero day	3rd day	6thday	9th day	12th day	15th day	17th day	20thday
Control	8.5±0.2 ^{a}	7±0.1 ^{b}	5±0.2 ^{c}	4±0.01^{e}	3±0.15^{d}	2.5±0.1^{d}	2.1±0.1^{d}	1.5±0.5^{d}
Zn O(12mM)	8.4±0.3 ^{a}	8.2±0.2 ^{ab}	8.1±0.2 ^{d}	7.9±0.1 ^{b}	6.9±0.3 ^{b}	6.5±0.2 ^{b}	5.2±0.3^{f}	4.1±0.2 ^{fc}
Ti O2(12mM)	8.45±0.1 ^{a}	8.3±0.1 ^{ab}	7.7±0.1 ^{d}	7.5±0.1 ^{d}	6.4±0.1^{b}	5.5±0.1 ^{b}	4.1±0.2^{fc}	3.1±0.1^{c}
Mixture	8.4±0.02 ^{a}	8.3±0.4 ^{ab}	7.6±0.3 ^{d}	7.4±0.2 ^{d}	5.6±0.1^{b}	5.1±0.3^{b}	3.6±0.1^{b}	2.2±0.1^{c}

4. CONCLUSION

As a conclusion using of nanoparticles as Zn O and Ti O_2 enhance the quality meat balls. Moreover, it was evident that Zn O nanoparticles were more effective than Ti O_2 as an antibacterial and antioxidant and can also enhance the shelf life time of meat products (meat balls). Also this study proved that no synergistic action appeared between Zn O and Ti O_2.

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References:

Abdel-Hamied, A., Nassar, A., & El-Badry, N. (2009). Investigations on antioxidant and antibacterial activities of some natural extracts. *World Journal of Dairy & Food Sciences, 4*(1), 1-7.

Abdou, E. S., Galhoum, G. F., & Mohamed, E. N. (2018). Curcumin loaded nanoemulsions/pectin coatings for refrigerated chicken fillets. *Food Hydrocolloids, 83*, 445-453.

Badee, A., Moawd, R., ElNoketi, M., & Gouda, M. (2013). Improving the quality and shelf-life of refrigerated chicken meat by marjoram essential oil. *J. Appl. Sci. Res, 9*, 5718-5729.

Banerjee, R., Verma, A. K., & Siddiqui, M. W. (2017). Control of lipid oxidation in muscle food by active packaging technology. In *Natural Antioxidants* (pp. 363-402): Apple Academic Press.

Barbut, S. (2009). Pale, soft, and exudative poultry meat—Reviewing ways to manage at the processing plant. *Poultry science*, *88*(7), 1506-1512.

Bonilla, J., Vargas, M., Atarés, L., & Chiralt, A. (2014). Effect of chitosan essential oil films on the storage-keeping quality of pork meat products. *Food and bioprocess technology*, *7*(8), 2443-2450.

Chaudhry, Q., & Castle, L. (2011). Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries. *Trends in Food Science & Technology*, *22*(11), 595-603.

Chen, H., Weiss, J., & Shahidi, F. (2006). Nanotechnology in nutraceuticals and functional foods. *Food technology*.

Duncan, T. V. (2011). Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *Journal of colloid and interface science*, *363*(1), 1-24.

Egan, H., Kirk, R., & Sawyer, R. (1981). Chemical analysis of foods. *Churchill Liningstone, 439*.

Emami-Karvani, Z., & Chehrazi, P. (2012). *Antibacterial activity of Zn O nanoparticle on Gram-positive and Gram-negative bacteria*.

EOS. (2005). Minced Meat Mixed With Soybean Protein. (Egyptian Organization for Standardization and Quality)E.S: 2097/2005

FDA. (2001). Bacteriological analytical manual online. *Available from:< Available from: http://www. fda. gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949. htm>. Acessed: fev*, *2*, 2015.

Feng, P., Weagant, S. D., Grant, M. A., & Burkhardt, W. (2002). BAM: Enumeration of Escherichia coli and the Coliform Bacteria. *Bacteriological analytical manual*, *13*.

Fernández-López, J., Zhi, N., Aleson-Carbonell, L., Pérez-Alvarez, J. A., & Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*, *69*(3), 371-380. https://doi.org/https://doi.org/10.1016/j.meatsci.2004.08.004.

Gong, Y., Parker, R., & Richards, M. (2010). Factors affecting lipid oxidation in breast and thigh muscle from chicken, turkey and duck. *Journal of food biochemistry*, *34*(4), 869-885.

Hashim, A. A. (2011). *Advances in nanocomposite technology*: BoD–Books on Demand.

Huang, X., Dai, Y., Cai, J., Zhong, N., Xiao, H., McClements, D. J., & Hu, K. (2017). Resveratrol encapsulation in core-shell biopolymer nanoparticles: Impact on antioxidant and anticancer activities. *Food Hydrocolloids*, *64*, 157-165.

ISO. (1999). Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1:General rules for the preparation of the initial suspension and decimal dilutions. . *1st edition,, 6887-1:1999(E)*.

ISO 13299 (2003) Sensory analysis — Methodology — General guidance for establishing a sensory profile.

ISO 8568. (2012). Sensory analysis – General guidelines.for the selection, training and monitoring of selected assessors and expert sensory assessors.Switzerland: Genève8586.

Jaiswal, L., Shankar, S., & Rhim, J.-W. (2019). Chapter 3 - Applications of nanotechnology in food microbiology. In V. Gurtler, A. S. Ball & S. Soni (Eds.), *Methods in Microbiology* (pp. 43-60): Academic Press.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Indicators of food microbial quality and safety. *Modern food microbiology*, 473-495.

Kikuchi, Y., Sunada, K., Iyoda, T., Hashimoto, K., & Fujishima, A. (1997). Photocatalytic bactericidal effect of Ti O2 thin films: dynamic view of the active oxygen species responsible for the effect. *Journal of photochemistry and photobiology A: Chemistry*, *106*(1-3), 51-56.

Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., . . . Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, *106*, 1095-1104. https://doi.org/https://doi.org/10.1016/j.foodres.2017.12.005.

Marcous, A., Rasouli, S., & Ardestani, F. (2017). Low-density polyethylene films loaded by titanium dioxide and zinc oxide nanoparticles as a new active packaging system against Escherichia coli O157: H7 in fresh calf minced meat. *Packaging Technology and Science*, *30*(11), 693-701.

Matei, A., Cernica, I., Cadar, O., Roman, C., & Schiopu, V. (2008). Synthesis and characterization of Zn O–polymer nanocomposites. *International Journal of Material Forming*, *1*(1), 767-770.

Mendes, J. I. S. (2013). Qualidade nutricional e microbiológica de enchidos. Instituto Politécnico de Bragança, Escola Superior Agrária.

Morsy, M. K., Elsabagh, R., & Trinetta, V. (2018). Evaluation of novel synergistic antimicrobial activity of nisin, lysozyme, EDTA nanoparticles, and/or Zn O nanoparticles to control foodborne pathogens on minced beef. *Food control*, *92*, 249-254.

Ozimek, L., Pospiech, E., & Narine, S. (2010). Nanotechnologies in food and meat processing. *ACTA Scientiarum Polonorum Technologia Alimentaria*, *9*(4), 401-412.

Petran, R. L., Grieme, L. E., & Foong-Cunningham, S. (2015). Culture Methods for Enumeration of Microorganisms. In *Compendium of Methods for the Microbiological Examination of Foods*.

Pires, J. R. A., de Souza, V. G. L., & Fernando, A. L. (2018). Chitosan/montmorillonite bionanocomposites incorporated with rosemary and ginger essential oil as packaging for fresh poultry meat. *Food Packaging and Shelf Life*, *17*, 142-149. https://doi.org/https://doi.org/10.1016/j.fpsl.2018.06.011.

Premanathan, M., Karthikeyan, K., Jeyasubramanian, K., & Manivannan, G. (2011). Selective toxicity of Zn O nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(2), 184-192.

Rhim, J.-W., Park, H.-M., & Ha, C.-S. (2013). Bio-nanocomposites for food packaging applications. *Progress in polymer science*, *38*(10-11), 1629-1652.

Rosmini, M., Perlo, F., Pérez-Alvarez, J., Pagán-Moreno, M., Gago-Gago, A., López-Santoveña, F., & Aranda-Catalá, V. (1996). TBA test by an extractive method applied to 'paté'. *Meat Science*, *42*(1), 103-110.

Sawai, J. (2003). Quantitative evaluation of antibacterial activities of metallic oxide powders (Zn O, MgO and CaO) by conductimetric assay. *Journal of microbiological methods*, *54*(2), 177-182.

Sirocchi, V., Devlieghere, F., Peelman, N., Sagratini, G., Maggi, F., Vittori, S., & Ragaert, P. (2017). Effect of Rosmarinus officinalis L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. *Food chemistry*, *221*, 1069-1076.

Sofos, J. N., & Geornaras, I. (2010). Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of Escherichia coli O157:H7 in nonintact, and Listeria monocytogenes in ready-to-eat, meat products. *Meat Science*, *86*(1), 2-14. https://doi.org/https://doi.org/10.1016/j.meatsci.2010.04.015.

Steele, R., & Torrey, J. (1980). Principles and procedures of statistics: a biometrical approach. New York: McGraw & Hill. *Inc. p*, *593*.

Tornuk, F., Hancer, M., Sagdic, O., & Yetim, H. (2015). LLDPE based food packaging incorporated with nanoclays grafted with bioactive compounds to extend shelf life of some meat products. *LWT-Food Science and Technology*, *64*(2), 540-546.

Wang, H., Xie, C., Zhang, W., Cai, S., Yang, Z., & Gui, Y. (2007). Comparison of dye degradation efficiency using Zn O powders with various size scales. *Journal of Hazardous materials*, *141*(3), 645-652.

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, *12*, 1227-1249. https://doi.org/10.2147/IJN.S121956.

Xing, Y., Li, X., Zhang, L., Xu, Q., Che, Z., Li, W., . . . Li, K. (2012). Effect of Ti O2 nanoparticles on the antibacterial and physical properties of polyethylene-based film. *Progress in Organic Coatings*, *73*(2-3), 219-224.

Yin, H., Wada, Y., Kitamura, T., Kambe, S., Murasawa, S., Mori, H., . . . Yanagida, S. (2001). Hydrothermal synthesis of nanosized anatase and rutile Ti O2 using amorphous phase Ti O2. *Journal of Materials Chemistry*, *11*(6), 1694-1703.

Yin, L., Wu, C., & Jiang, S. (2007). Bio preservative effect of pediocin ACCEL on refrigerated seafood. Fisheries Science, 73, 907-912.

Zenebon, O., Pascuet, N. S., Tiglea, P., Zenebon, O., Pascuet, N., & Tiglea, P. (2008). Physicochemical methods for food analysis. *São Paulo: Instituto Adolfo Lutz*, 1020.

Mohebat A. Abd El - Aziz
Food Hygiene, Animal Health Research Institute
Egypt
pepovet79@yahoo.com

Nahla Abo EL-Roos
Food Hygiene, Animal Health Research Institute
Egypt
dr.nahlashawky@yahoo.com

Rasha El.sabagh
Food Hygiene and Control Department, Faculty of Veterinary Medicine
Benha University
Egypt
Rasha.alsbagh@fvtm.bu.edu.eg

Hemmat M. Ibrahim
Food Hygiene and Control Department
Faculty of Veterinary Medicine
Benha University
Egypt
drhemmat01@yahoo.com

Badawi Anis
Spectroscopy Department, Physics Division, National Research Centre
Egypt
badawi.ali@gmail.com