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ORIGINAL ARTICLE

Effects of zinc oxide nano particle on fortified tilapia mince during refrigerated storage (4 ± 1 °C)

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Abstract Microbial and zinc concentration changes in Tilapia (*Oreochromis niloticus*) mince, fortified with zinc oxide nano particle during storage for 18 days at 4 ± 1 °C were investigated. During the present study the samples treated with ZnO nano particles T_4 (60 ppm) and T_5 (80 ppm) were recorded to have the lowest ($p < 0.05$) Total Plate Count (TPC) figures among all the treatments suggesting a significant antimicrobial effect of ZnO nano partials. The control sample without fortification did not meet the daily requirement of Zn for an adult. The T_1 (inorganic ZnO @ 20 ppm) samples meet the dietary requirements till 3 days of storage where, sample T_3, T_4, T_5 and T_6 (ZnO nano particles @ 40 ppm, 60 ppm, 80 ppm and 100 ppm respectively) provides enough Zn till 6 days under storage at 4 ± 1 °C and in certain cases it is more than the dietary requirement of 10–15 mg/day for an adult individual. In the present study Zn concentration was significantly ($p < 0.05$) decreased in control. Hence, the intake of tilapia mince of these treatments may be adjusted accordingly to provide dietary Zn. Fortification of tilapia mince with Zn will not only improve the shelf life of the mince but also prove to be a good food fortification strategy to eradicate Zn deficiency.

Keywords Fortification · Zinc oxide nano particles · Tilapia mince · Microbial analysis

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Introduction

Fish and fish products are rich in protein, polyunsaturated fatty acids (PUFA), essential minerals, and vitamins but such perishable products have the limited shelf-life due to the rapid microbial growth and lipid oxidation (Mizielin´ska et al. 2018). With increasing the demand for fish products several innovative techniques have been developed for extending shelf-life, maintaining quality and safety of products. Arfat et al. (2015) reported the use of new types of nano-inorganic antimicrobial materials, due to their stability at high temperatures and pressure conditions. Mizielin´skav et al. (2018) suggested the use of Zinc Oxide (ZnO) nano particles in active food packaging systems as an antimicrobial agent and Zn as a micronutrient source. Zinc deficiency is one of the major nutritional health problems around the globe where the best sources like animal sources of zinc, are limited (Raya et al. 2016). The Food and Drug Administration (FDA 2011) listed ZnO as a generally recognized as safe (GRAS) material. According to Mostafa (2015), ZnO Nano-particles (NPs) act as a food additive and used as a source of zinc in the fortification of cereal based foods. The nano-sized ZnO can interact with bacterial surface and/or with the core of bacteria and enters inside the cell.Subsequently bactericidal mechanisms occurs (Seil and Webster 2012). ZnO-NPs are reported in several studies as non-toxic to human cells (Colon et al. 2006) and hold good biocompatibility to human cells (Padmavathy and Vijayaraghavan 2008). Rai and Bai (2011) reported antibacterial activities of ZnO NPs against broad spectrum pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella enteritidis, Salmonella typhimurium*, *Bacillus subtilis*, *Escherichia coli*, *Escherichia coli O157:H7*, *Listeria monocytogenes* and *Pseudomonas fluorescens*. Hence, fortification of food using ZnO may be a good solution to overcome Zn deficiency. Again, among different food alternatives, fish may prove to be a good choice for Zn fortification.

Tilapia is very popular and economically important edible fish species from nutritional point of view. As per the data released by the Global Aquaculture Alliance in 2019, the production of tilapia reached 6.5 million metric tons in 2019 growing at 4% compared to 2018 (GAA 2019). Due to the easy availability and low price, Tilapia has been in focus among freshwater fishes for development of mince and surimi. Majumder et al. (2017), demonstrated the development of dry surimi powder from the surimi prepared from tilapia. Minced meat, surimi and surimi powder may conveniently be used for Zn fortification, as preparation of homogenous blends are possible using them. Zn fortified mince, surimi or surimi powder then can conveniently be used to produce ready to eat or ready to cook products at reasonable price. Such fortified convenience products will in turn provide the necessary supplements of Zn for the consumers. ZnO nano particle used in fish products can improve the shelf-life, maintain the product quality and also overcome Zn deficiency.

The present study was focused to incorporate the zinc oxide nano particles in mince tilapia meat. Then the microbial and Zn concentration in mince was evaluated. The purpose of the present study was to access the microbiological changes and to determine the Zn concentration in tilapia mince during refrigerated storage (4 ± 1 °C). Generally, retail packs of mince are expected to be kept by the consumers under refrigeration within a temperature range of 1 to 4 °C. The upper limit of the refrigeration temperature was chosen to assess the minimum shelf life of the product under the upper temperature limit of refrigeration.

Materials and methods

Live tilapia (*Oreochromis niloticus*) were bought from the market by simple random sampling (Garia fish market, Kolkata) and used for the present study. The fishes were brought to the laboratory in iced condition, within an hour and processed immediately. The fishes (9 kg) were beheaded, descaled, filleted and skinned manually. Fillets were cut from the fish under good hygienic and sanitary conditions to prevent any cross contamination. The waste was first put in a bone separator with a 2 mm drum to obtain the meat. The separated meat was then minced in a mincer and after that the separated mince was subjected to water washing as per Rawdkuen et al. (2009).

Synthesis of zinc oxide nano particles and incorporation in tilapia mince

The nano zinc particles have been obtained from Department of Animal Nutrition (Faculty of Veterinary Animal Sciences), West Bengal University of Animal and Fishery Sciences, Kolkata 700037. The different varieties of particles were synthesized by benign wet chemistry approach and FESEM study reveals their size within the range of 40–50 nm. Zinc oxide nano particle in powder form was weighed in digital electronic weight machine and added in different concentrations like 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm (w/w) in the minced meat designated henceforth as T_2,T_3,T_4,T_5,T_6 and inorganic zinc sulphate, weighing 20 ppm added to the minced meat marked as T_1. The control mincemeat was without addition of inorganic ZnO or ZnO nano particle.

Biochemical analysis

The pH value was recorded using a pH meter, according to the method of Benjakul et al. (1997). Fish sample (0.5 g) was centrifuged with 4.5 ml of distilled water at 1800 rpm for 1 min and the homogenate used for pH determination. TVBN was measured by the method described by AOAC (1995).

Microbiological analysis

TPC (Total Plate Count) was determined according to standard American Public Health Association method (APHA 2001). It was determined as mean log 10 cfu g^{-1} of fish samples. Physiological saline was prepared and 10 g sample was mixed into it. Nutrient agar was used to make the media. Serial dilution of the sample was done and technique of pour plating was followed. The incubation of plates were done for 24 h at 37 °C. All materials used were sterilized except sample and all the procedure was done under aseptic condition.

Sensory analysis

Sensory evaluation of fillets was performed during storage by a sensory panel composed of 15 experienced members. They were required to evaluate the raw fillets based on the color, flavour, texture and overall acceptability using a 7 points hedonic scale: 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike 5 = like slightly, 6 = like moderately, and 7 = like very much as suggested by Siah and Tahir (2011).

Table 1 Changes in TVBN (mg N/100 g) of tilapia mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ±1 °C)

Days	TVBN (mg N/100 g)						
	C	T_1	T_2	T_3	T_4	T_5	T_6
0	11.18 ± 0.63	10.51 ± 1.51	10.24 ± 0.59	10.12 ± 0.34	10.795 ± 0.33	10.57 ± 0.14	10.76 ± 0.11
3	12.41 ± 0.14	11.92 ± 0.94	11.35 ± 0.30	10.575 ± 0.28	10.95 ± 0.28	10.855 ± 0.97	12.30 ± 1.80
6	15.49 ± 0.95	13.68 ± 0.13	12.39 ± 0.01	11.68 ± 0.57	11.815 ± 0.43	11.78 ± 0.34	12.38 ± 0.35
9	16.82 ± 3.73	13.99 ± 0.14	15.02 ± 0.32	13.36 ± 0.61	12.99 ± 0.03	12.975 ± 0.21	13.17 ± 0.57
12	16.95 ± 4.62	17.03 ± 0.94	15.61 ± 0.38	12.3 ± 3.75	14.745 ± 3.10	13.37 ± 1.65	14.74 ± 1.84
15	25.52 ± 1.22	20.59 ± 1.42	19.37 ± 0.36	17.95 ± 0.51	15.68 ± 2.14	16.05 ± 0.13	17.12 ± 0.20
18	32.65 ± 0.97	27.64 ± 0.52	26.34 ± 0.59	17.965 ± 2.81	17.565 ± 1.28	18.225 ± 1.32	18.97 ± 0.41

* *Results are mean of five determinations* (n = 5) *with s.d*

\# *Values of mean vary significantly* ($p < 0.05$) *between the treatments and storage days*

Measurement of Zn concentration of fish tissue

A modified wet-digestion method was described by Churnoff (1975) and followed to prepare the fish tissue samples for the determination of Zn concentration. The mince meat 5 g of each treatment was weighed in electronic balance and kept in 100 ml beaker with 10 ml concentrated HNO_3 for overnight at room temperature. The acidified tissue samples were placed on a hot plate at 85 ± 5 °C. Then, a mixture of concentrated H_2SO_4 and $HClO_4$ (3:2) of about 5 ml was added gradually. The digestion was carried out until the samples turned into a pale transparent solution. The digested samples were cooled and filtered through an acid soaked Whatman filter paper (22–45 µ) and was adjusted to the required volume of about 20-25 ml with distilled water. The Zn content of the sample was detected in atomic absorption spectrophotometer (Varian AA.240) using three standard solutions (0.5 mg/l, 1.0 mg/l, 1.5 mg/l) of the metal. A series of standard metal solutions in the optimum concentration range was prepared by appropriate dilution of the stock metal solutions with water containing 1.5 mg/l concentration HNO_3/l. Stock standard solutions were procured from commercial suppliers. Based on total amount of the tissue sample taken, the actual concentration of the metal was calculated and results were expressed in µg/g.

Statistical analysis

All the data were checked for normal distribution with normality plots prior to analysis of variance (ANOVA) to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of Microsoft Office Excel (2016) and R software (Version 2.14.1). Tukey HSD was used to determine significant differences between treatments.

Results and discussion

Biochemical analysis during storage

Spoilage of fish is accompanied by the release of several volatile compounds like dimethyl amine, trimethyl amine, ammonia, trimethyl amineoxide etc. These compounds are produced by both bacterial and endogenous enzymes. The concentration of these compounds in the tissue may indicate the degree of spoilage, particularly in the later stage of spoilage. The TVBN values on the initial day in the tilapia mince were 11.18 ± 0.63, 10.51 ± 1.51, 10.24 ± 0.59, 10.12 ± 0.34, 10.795 ± 0.33, 10.57 ± 0.14 and 10.76 ± 0.11 mg N/100 g for C, T1, T2, T3, T4, T5 and T6 respectively (Table 1). The values thereafter exhibited significant ($p < 0.05$) (Fig. 1) increase in all samples during the storage period at 4 ± 1 °C reaching a value of 32.65 ± 0.97 mg N/100 g in control samples on day 18. Samples with a TVB-N value of 25 mg N/100 g are considered perfect quality, samples with 30 mg N/100 g are good quality, samples with 35 mg N/100 g are rated marketable quality and samples with TVB-N value above 35 mg N/100 g are considered spoiled (Ludorf and Meyer 1973). The control samples (C) thus crossed the limit of being good quality and showed signs of spoilage. In the mince samples treated with inorganic ZnO or ZnO nano particles the TVBN values never crossed 30 mg N/100 g suggesting good quality of mince on day 18 of storage. This significant lowering ($p < 0.05$) (Fig. 2) of TVBN values in the treatments over the control may be due to the effect of ZnO nano particle fortification. Zhang et al. (2017) also reported similar findings while studying the characterization of micro emulsion nanofilms based on tilapia fish skin gelatine (FSG) and ZnO.

nanoparticles incorporated with ginger essential oil (GEO) for packaging application. He concluded a

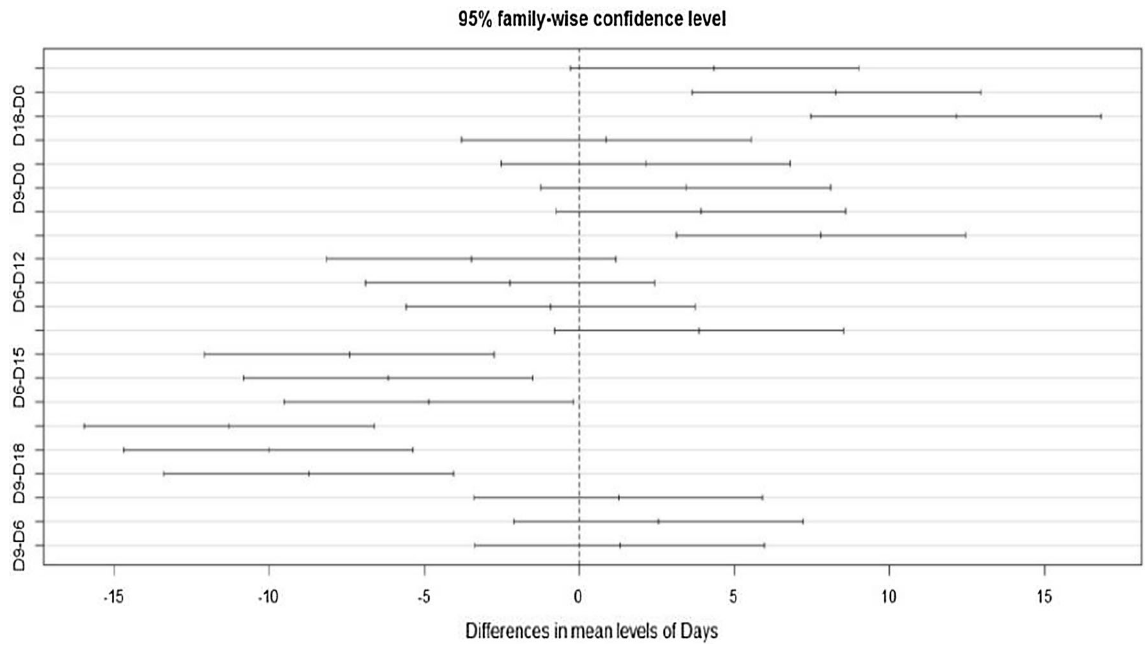


Fig. 1 Plot of Tukey HSD of changes in TVBN in Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

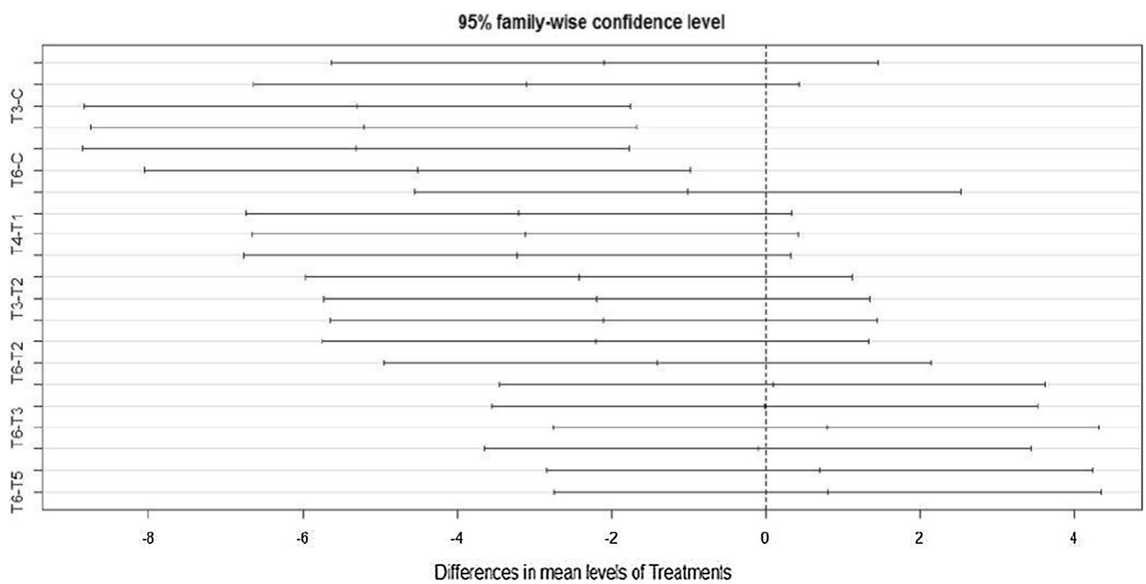


Fig. 2 Plot of Tukey HSD of changes in TVBN in Tilapia fish mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ± 1 °C)

significant lower ($p < 0.05$) TVB-N content in FSG-ZnO NP films with GEO as compared to unpackaged and the control ones.

Table 2, of changes in pH indicate that during refrigerated storage pH value increased with increasing storage time. On 0 day of storage, pH value of tilapia mince samples C, T1, T2, T3, T4, T5 and T6 were 6.49 ± 0.20, 6.44 ± 0.03, 6.46 ± 0.04,6.47 ± 0.02, 6.45 ± 0.03, 6.46 ± 0.01 and 6.47 ± 0.10 respectively. Tukey HSD of the mean pH values revealed (Fig. 3) a significantly ($p < 0.05$) lower pH in all treated samples except T1, T3 and T6 as compared to control till the end of storage period. After 18 days of storage at 4 ± 1 °C, pH increased in control (C) sample reaching a value of 7.19 ± 0.01, followed by T1 (7.01 ± 0.01), T3 (7.01 ± 0.01) and T6 (7.00 ± 0.02). Fresh fish muscle pH is most frequently in

Table 2 Changes in pH value of tilapia mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ± 1 °C)

Days	pH						
	C	T_1	T_2	T_3	T_4	T_5	T_6
0	6.49 ± 0.20	6.44 ± 0.03	6.46 ± 0.04	6.47 ± 0.02	6.45 ± 0.03	6.46 ± 0.01	6.47 ± 0.10
3	6.55 ± 0.04	6.57 ± 0.03	6.62 ± 0.13	6.56 ± 0.03	6.49 ± 0.02	6.49 ± 0.03	6.49 ± 0.08
6	6.65 ± 0.04	6.63 ± 0.06	6.66 ± 0.07	6.66 ± 0.04	6.54 ± 0.03	6.60 ± 0.01	6.60 ± 0.03
9	6.79 ± 0.03	6.64 ± 0.06	6.72 ± 0.09	6.74 ± 0.03	6.57 ± 0.01	6.61 ± 0.01	6.65 ± 0.05
12	6.87 ± 0.03	6.76 ± 0.03	6.74 ± 0.08	6.88 ± 0.00	6.65 ± 0.02	6.77 ± 0.12	6.70 ± 0.03
15	6.87 ± 0.20	6.85 ± 0.02	6.75 ± 0.03	6.90 ± 0.03	6.80 ± 0.11	6.82 ± 0.02	6.87 ± 0.01
18	7.19 ± 0.01	7.01 ± 0.01	6.90 ± 0.01	7.01 ± 0.01	6.91 ± 0.01	6.98 ± 0.04	7.00 ± 0.02

* Results are mean of five determinations (n = 5) with s.d.

Values of mean vary significantly ($p < 0.05$) between the treatments and storage days

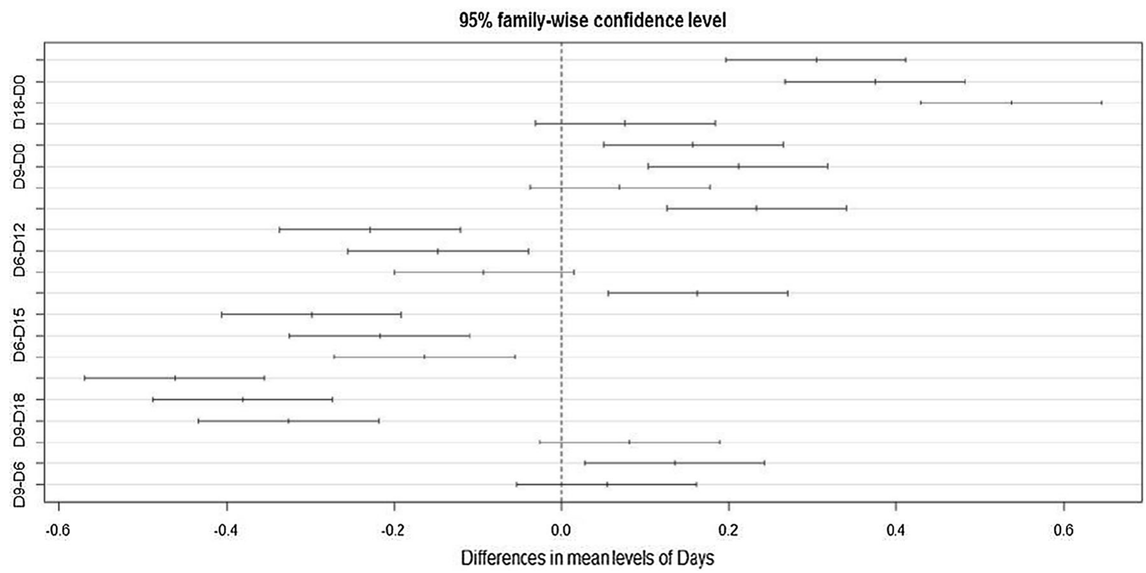


Fig. 3 Plot of Tukey HSD of changes in pH in Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

the range of 6–6.5 (Buchtova and Jezek 2011).A significant increase ($p < 0.05$) in pH was also observed in all samples among the storage days (Fig. 4) which is in accordance with Poli et al (2005), who opined that storage time tends to increase the pH values which can be associated with the production of basic components such as ammonia, volatile alkali and trimethyl amine due to internal enzymatic activity and the growth of bacteria (Chamarana et al. 2012). Additionally, Sikorski et al. (1990) reported that the enzymatic degradation of ATP caused the liberation of inorganic phosphate and ammonia, leading to the changes in pH value.

Microbial analysis during storage

International Commission on Microbiological Specifications for Foods (ICMSF 1988) recommended that an increase of TPC up to levels exceeding the value of 6 log cfu g^{-1} is regarded as microbiological spoiled of fish muscle, which is not fit for human consumption. The shelf life of fish mince is strongly influenced by the initial microbial quality. The changes in total plate count (TPC) observed in treated tilapia mince during refrigerated storage (4 ± 1 °C) are specified in the Fig. 5. The initial TPC on the 0 day was 4.23 ± 0.04 log cfu/ml for all samples

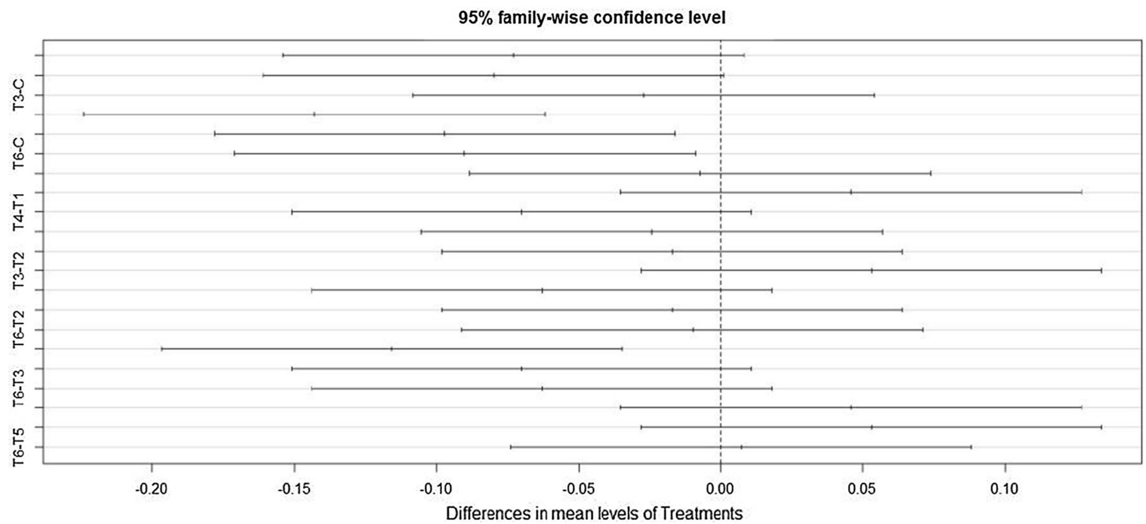
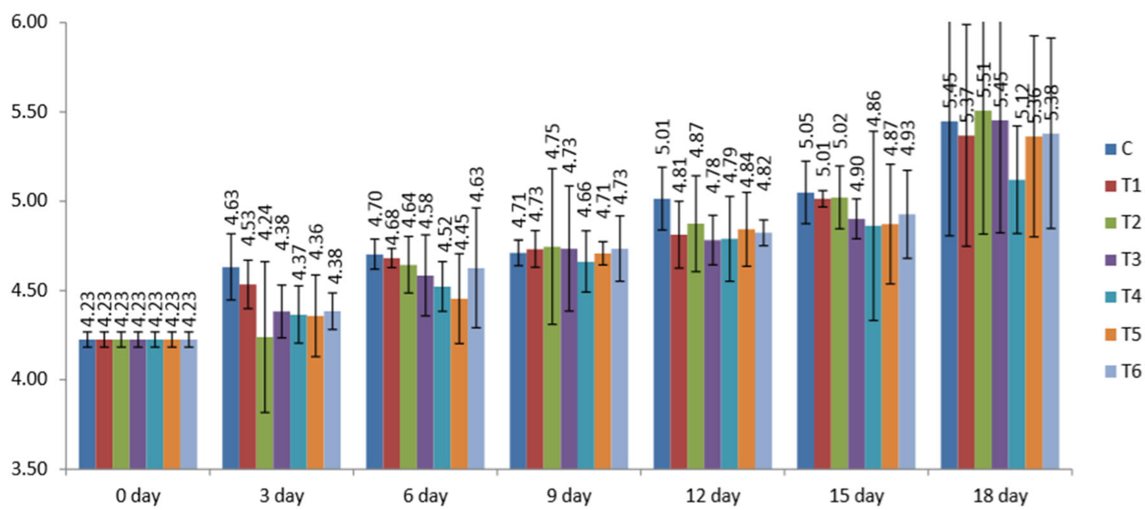


Fig. 4 Plot of Tukey HSD of changes in pH in Tilapia fish mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ± 1 °C)



**Results are mean of three determinations (n=3) with s.d.*

 # Values of mean vary significantly (p<0.05) between the treatments and storage days

Fig. 5 Changes in Total plate count (log cfu g^{-1}) of tilapia mince treated with different concentration of nano zinc under refrigerated storage (4 ± 1 °C)

which increased significantly ($p < 0.05$) over the storage period reaching values of 5.45 ± 0.64, 5.37 ± 0.62, 5.51 ± 0.69, 5.45 ± 0.63, 5.12 ± 0.30, 5.36 ± 0.56 and 5.38 ± 0.53 log cfu g^{-1} for C, T_1, T_2, T_3, T_4, T_5 and T_6 correspondingly (Fig. 6). Samples T_4 and T_5 were recorded to have the lowest ($p < 0.05$) TPC values among all the treatments suggesting a significant influence of ZnO at 60 and 80 ppm level of fortification (Fig. 7). Espitia et al. (2013), reported that ZnO has antimicrobial properties against food borne pathogens and spoilage bacteria which resulted in decreased TPC in tilapia mince which treated with nano zinc oxide compared to control sample at

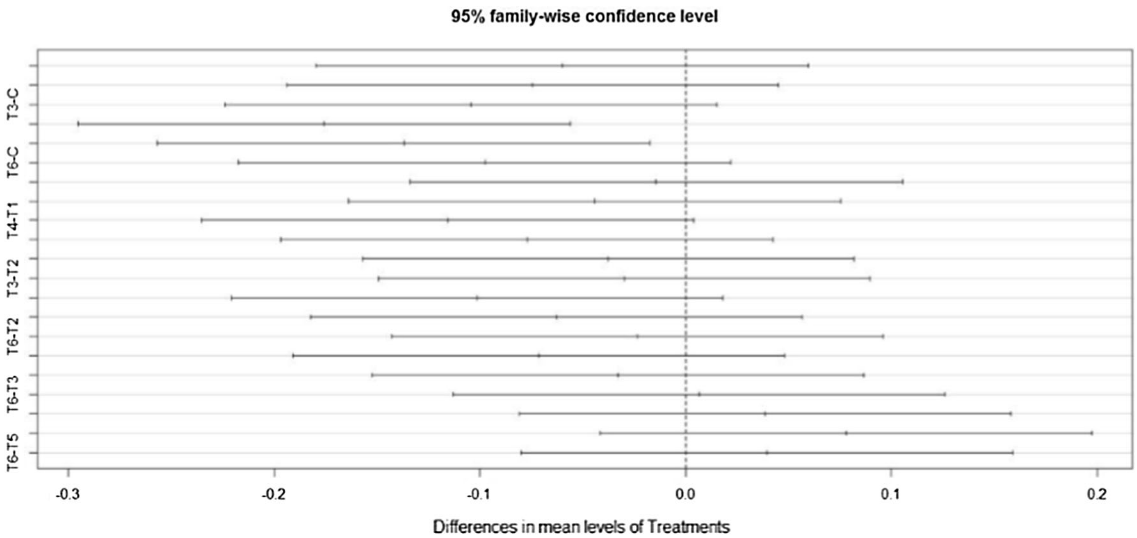


Fig. 6 Plot of Tukey HSD of changes in TPC (log cfu g^{-1}) in Tilapia fish mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ± 1 °C)

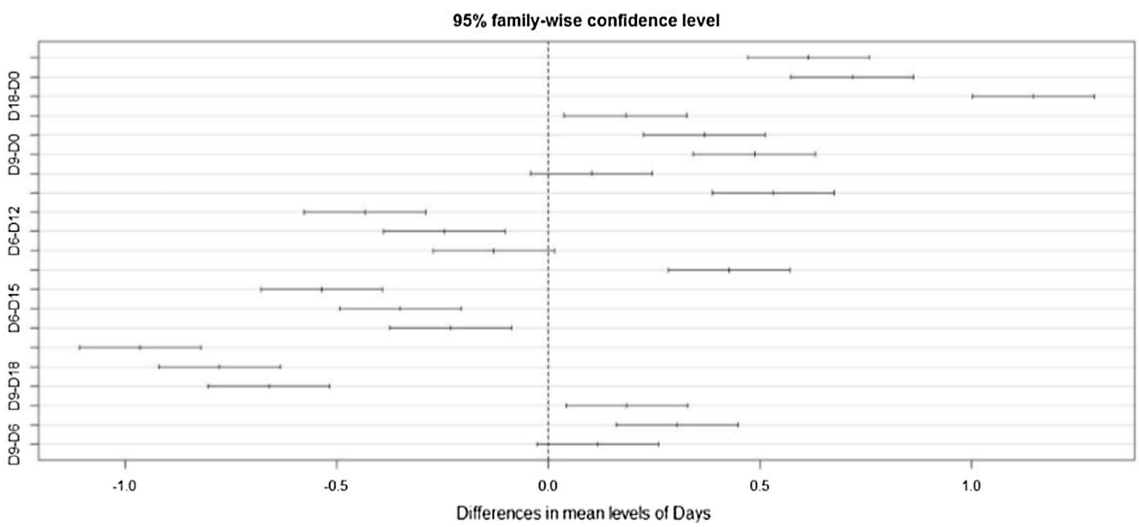


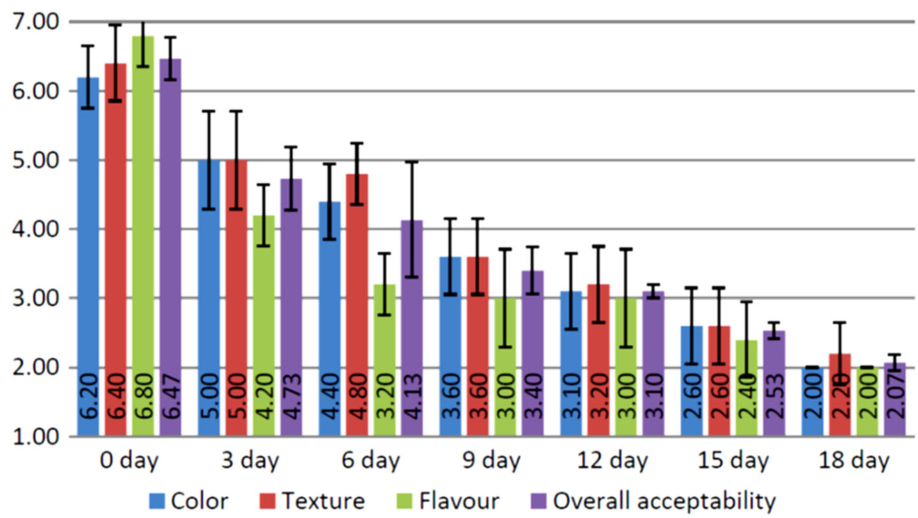
Fig. 7 Plot of Tukey HSD of changes in TPC (log cfu g^{-1}) in Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

60–80 ppm level of fortification. Nano-sized particles of ZnO have more pronounced antimicrobial activities than large particles due to the small size (< 100 nm). According to Morones et al. (2005); Kimet al. (2007), nano-scale materials have emerged up as novel antimicrobial agents due to their high surface area to volume ratio and also the unique chemical and physical properties which allow for better interaction with bacteria. Seil et al. (2012) opined that when particle size is reduced to the nanometer range, the nano-sized ZnO can interact with bacterial surface and/or with the bacterial core and subsequently exhibits distinct bactericidal mechanisms. Although in the present study application ZnO nano particle appeared to be bacteriostatic in nature rather than bactericidal as the TPC figures were always on an increasing trend with reduced growth rate in case of 60–80 ppm level of fortification.

Sensory analysis during storage

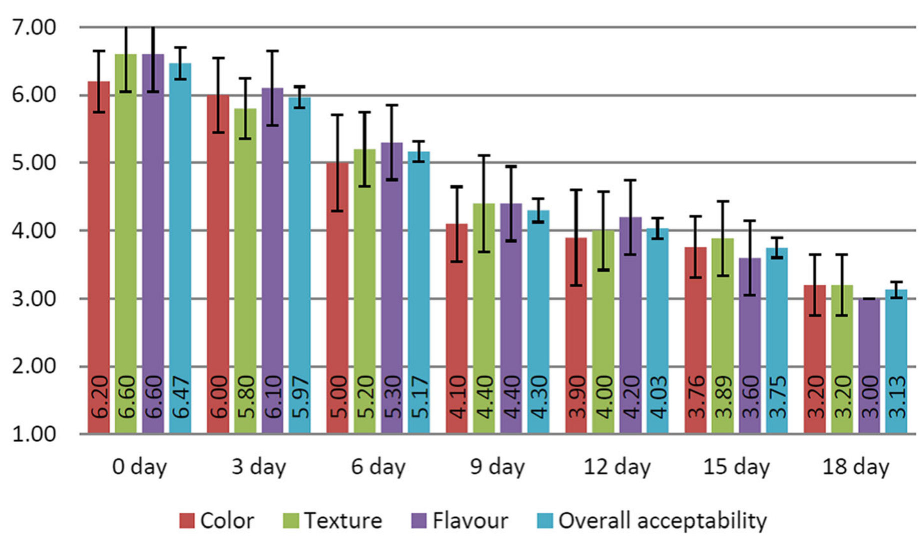
Sensory evaluation of fillets was performed during storage by a sensory panel composed of 15 experienced members and the results are given in the Figs. 8, 9, 10, 11, 12, 13 and 14. In case of control samples (C) the score for overall acceptability reached a value of 3.40 ± 0.35 on day 9, which was below the limit of "neither like nor dislike" (Fig. 8). For tilapia mince fortified with inorganic ZnO (T1) the overall acceptability score was 4.03 ± 0.15 on day 12 and was below "neither like nor dislike" on day 15 (Fig. 9). ZnO nano particle fortification resulted in better sensory scores with overall acceptability of the tilapia mince evaluated at 4.20 ± 0.20 on day 12 for T2 sample (Fig. 10); 4.40 ± 0.10 on day 15 for T3 sample (Fig. 11); 4.20 ± 0.10 on day 18 for T4 sample (Fig. 12); 4.07 ± 0.12 on day 15 for T5 sample (Fig. 13) and 4.10 ± 0.10 on day 12 for T6 sample (Fig. 14). The results of sensory scores indicated that fortification of tilapia mince with ZnO nano particle resulted in higher sensory scores as compared to the control with T4 samples having 60 ppm of fortification level having scores above 4 ("neither like nor dislike") till the end of the storage study at 4 ± 1 °C. The antimicrobial property of ZnO nano particle (Espitia et al. 2013) may have resulted in lowering the activity of the spoilage microorganisms and hence the spoilage.

Fig. 8 Changes in sensory scores of Control (C) Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)



Results are mean of fifteen determinations (n=15) with s.d.

Fig. 9 Changes in sensory scores of T1 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)



Results are mean of fifteen determinations (n=15) with s.d.

Fig. 10 Changes in sensory scores of T2 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

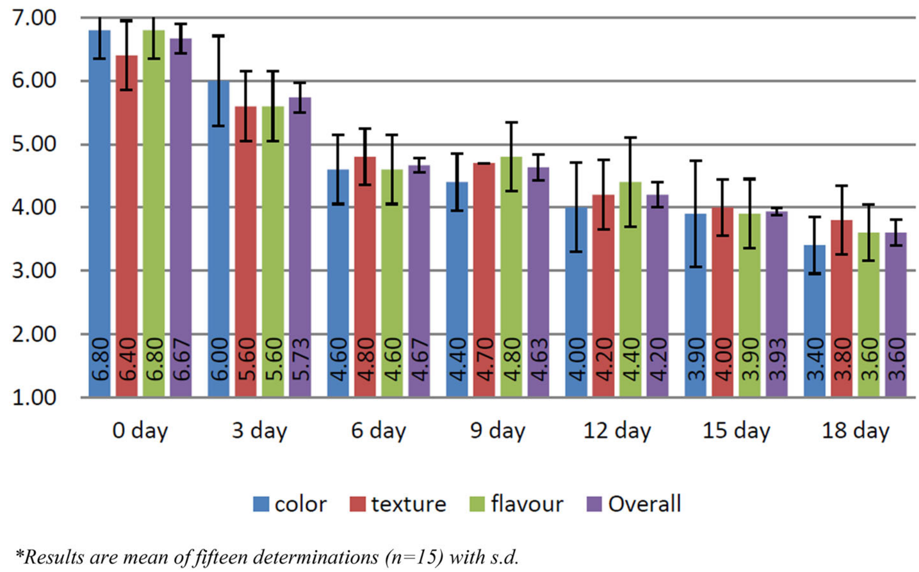
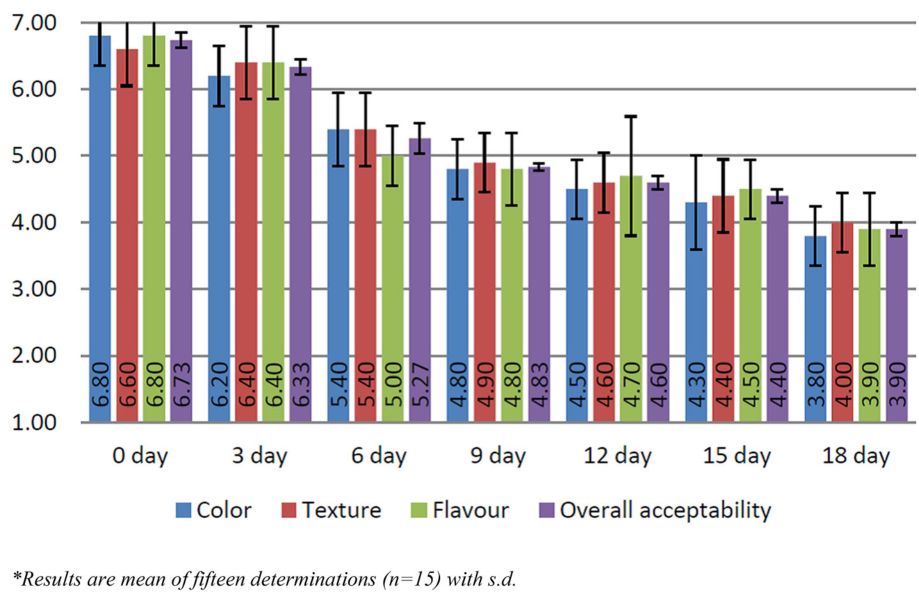


Fig. 11 Changes in sensory scores of T3 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)



Measurement of Zn concentration of Fish tissue during storage

The Zinc oxide nano particle (NPs) has a wide area of applications in various fields such as active laser medium and luminescence for fluorescent bulbs as well as in antimicrobial activities (Maryanti et al. 2014). It also has a potential application in preparation of functional food to tackle Zn deficiency in undernourished populations. Addition of Zinc oxide at 60 ppm and 80 ppm level to tilapia mince was found to have antimicrobial activity resulting in lowest ($p < 0.05$) TPC. Further, the retention of the ZnO nano particles in the mince can help in providing individuals with Zinc supply along with their diet. Hence, the changes in Zinc concentration in the fortified tilapia mince were assessed to ascertain the elemental Zn available after storage under 4 ± 1 °C.

The changes in Zn concentration was given in Table 3 and Fig. 15. The control samples of tilapia mince inherently had 8.91 ± 0.92 ppm of Zn which over the period of

Fig. 12 Changes in sensory scores of T4 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

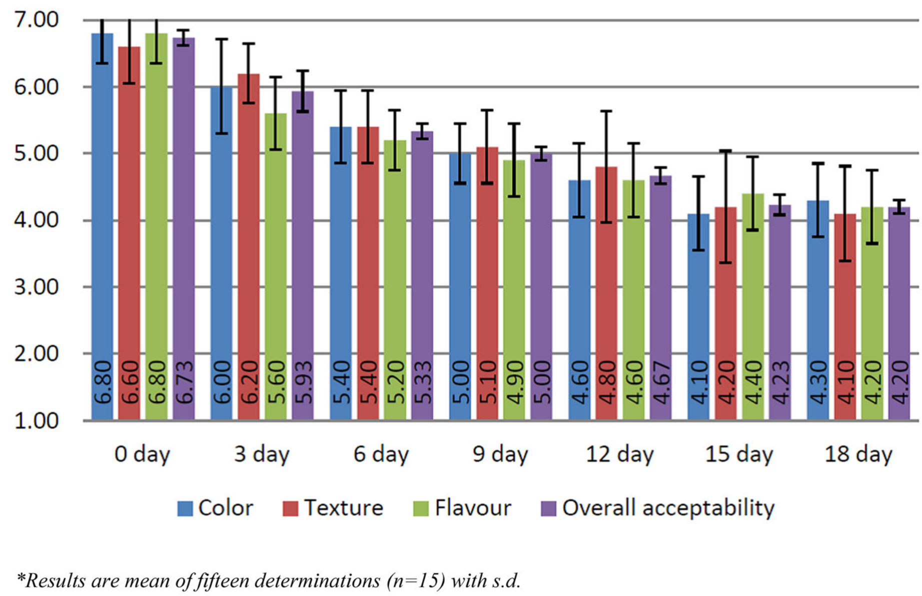
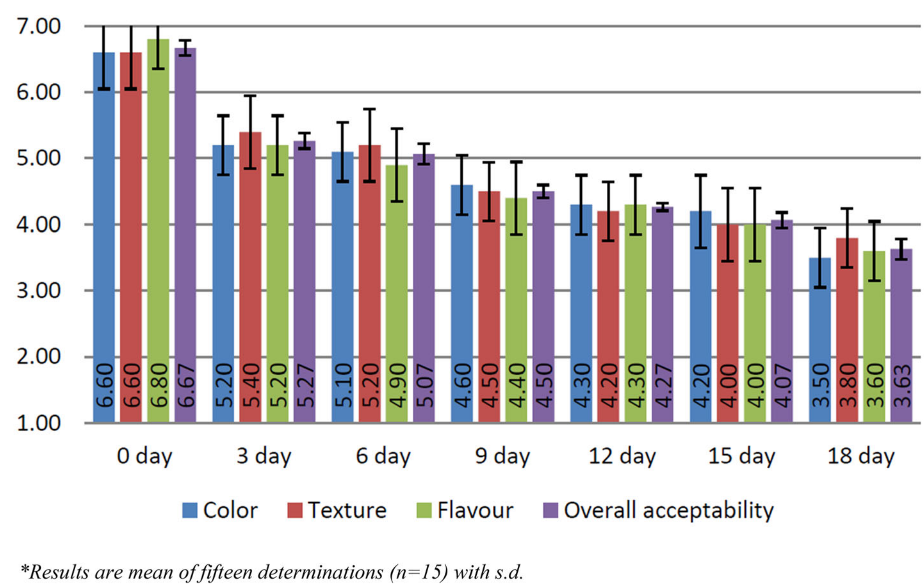


Fig. 13 Changes in sensory scores of T5 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)



storage decreased significantly ($p < 0.05$) to 2.14 ± 0.08 ppm. The Zn concentration in tilapia mince fortified with inorganic ZnO (T_1) decreased from an initial level of 24.76 ± 0.82 ppm to 6.99 ± 0.84 ppm after 18 days. The higher initial values in all treated samples were attributed to the ZnO added to the tilapia at different levels. In samples T_2, T_3, T_4, T_5 and T_6 the initial Zn concentration recorded was 18.80 ± 1.14, 31.06 ± 0.56, 38.70 ± 1.02, 40.80 ± 0.84 and 40.41 ± 0.18 ppm respectively. The values in all samples decreased significantly reaching figures of 4.84 ± 0.36, 8.60 ± 0.22, 7.78 ± 0.24, 7.38 ± 0.22 and 6.98 ± 0.32 ppm for T_2, T_3, T_4, T_5 and T_6 respectively. The reduction in Zn might be attributed to leaching action that resulted in loss of fortified ZnO nano particles.

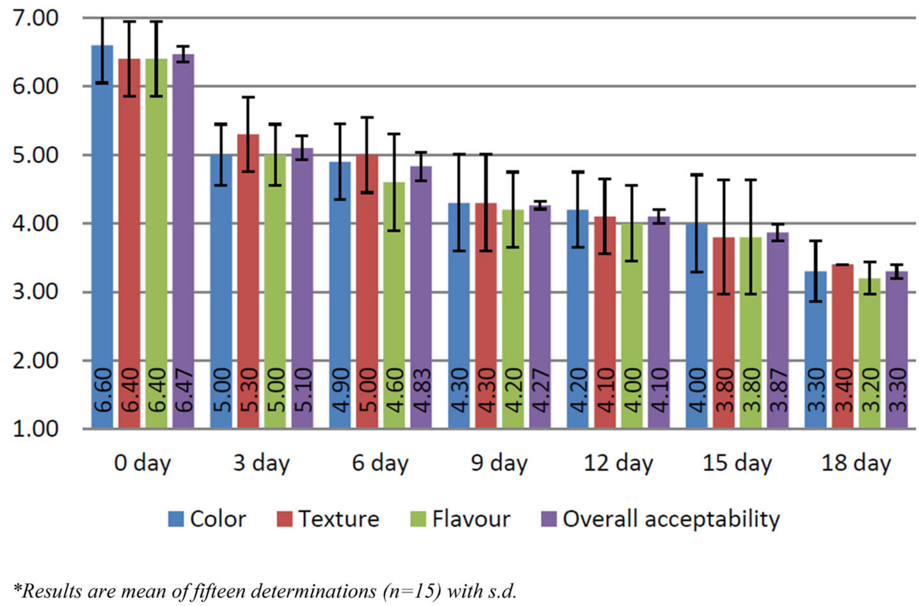


Fig. 14 Changes in sensory scores of T6 samples of Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

Table 3 Changes in Zinc concentration (ppm) of tilapia mince treated with different concentration of nano zinc oxide under refrigerated storage (4 ± 1 °C)

Days	Zinc Concentration (ppm)						
	C	T_1	T_2	T_3	T_4	T_5	T_6
0 day	8.91 ± 0.92	24.76 ± 0.82	18.80 ± 1.14	31.06 ± 0.56	38.70 ± 1.02	40.80 ± 0.84	40.41 ± 0.18
3 day	7.37 ± 0.26	20.68 ± 1.12	16.45 ± 0.42	27.34 ± 0.16	28.96 ± 1.26	31.38 ± 0.28	31.15 ± 0.42
6 day	6.74 ± 0.24	14.24 ± 0.16	12.83 ± 0.48	19.02 ± 0.82	20.58 ± 0.84	19.79 ± 0.34	22.51 ± 0.52
9 day	4.98 ± 0.47	8.40 ± 0.38	6.04 ± 0.28	10.54 ± 0.25	10.26 ± 0.68	12.55 ± 0.62	13.71 ± 0.28
12 day	3.52 ± 0.12	8.25 ± 0.48	6.05 ± 0.14	10.47 ± 0.38	9.74 ± 0.16	11.52 ± 0.14	9.76 ± 0.32
15 day	2.50 ± 0.15	7.12 ± 0.10	5.42 ± 0.32	10.66 ± 0.42	9.33 ± 0.22	8.74 ± 0.18	8.16 ± 0.26
18 day	2.14 ± 0.08	6.99 ± 0.84	4.84 ± 0.36	8.60 ± 0.22	7.78 ± 0.24	7.38 ± 0.22	6.98 ± 0.32

**Results are mean of 5 determinations with s.d*

 # Values of mean vary significantly ($p < 0.05$) between the storage days

Researcher affirmed that the daily requirement of zinc for adult is 10–15 mg/day, 10 mg/day for children and 3–5 mg/day for infants (Ociepa and Ociepa 2012). From Table 4; it was evident that the control sample without fortification (C) did not meet the daily requirement of Zn for an adult even if 100 g of the same was served. 100 g of the T_1 samples meet the dietary requirements till 3 days of storage. T_3, T_4, T_5 and T_6 samples however provided enough Zn till 6 days under storage at 4 ± 1 °C and in certain cases it was more than the dietary requirement.

Hence, the intake of tilapia mince of these treatments might be adjusted accordingly to provide dietary Zn. Fortification of tilapia with Zn was not only improving the shelf life of the mince but also prove to be a good food fortification strategy to eradicate Zn deficiency.

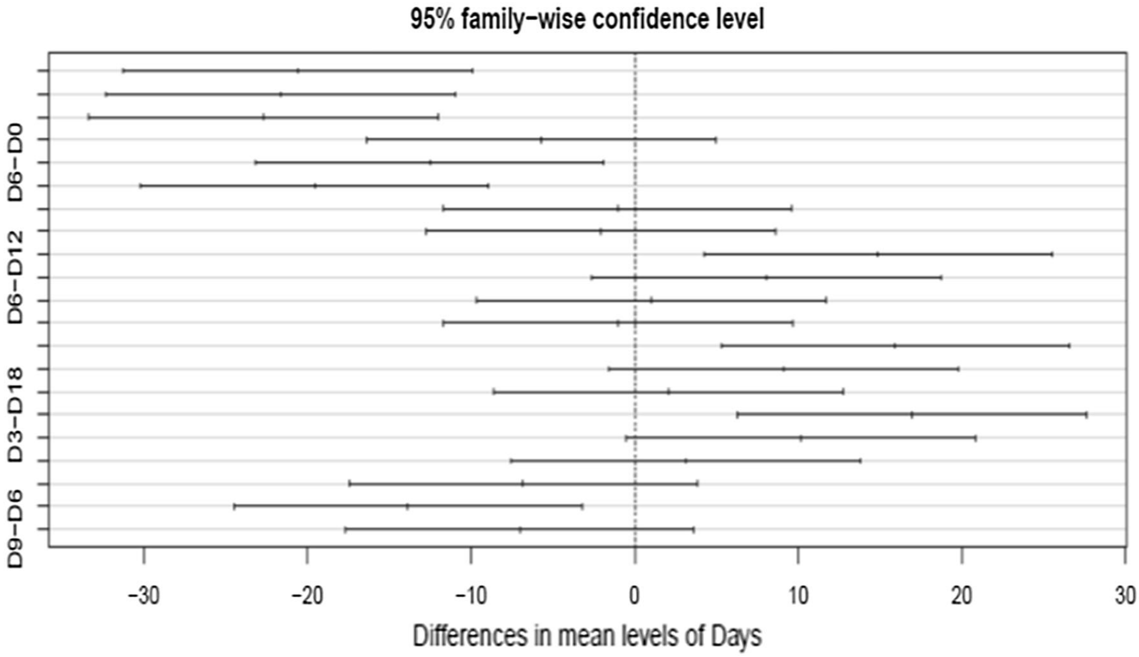


Fig. 15 Plot of Tukey HSD of changes in Zinc concentration (ppm) in Tilapia fish mince with storage days under refrigerated storage (4 ± 1 °C)

Table 4 Availability of Zn (mg/100 g of tilapia mince) treated with different concentration of nano zinc under refrigerated storage (4 ± 1 °C)

Days	Zinc (mg/100 g of tilapia mince)						
	C	T_1	T_2	T_3	T_4	T_5	T_6
0	5.35	14.86	11.28	18.64	23.22	24.48	24.25
3	4.42	12.41	9.87	16.40	17.38	18.83	18.69
6	4.04	8.54	7.70	11.41	12.35	11.87	13.51
9	2.99	5.04	3.62	6.32	6.16	7.53	8.23
12	2.11	4.95	3.63	6.28	5.84	6.91	5.86
15	1.50	4.27	3.25	6.40	5.60	5.24	4.90
18	1.28	4.19	2.90	5.16	4.67	4.43	4.19

[*]Results are obtained by multiplication of the mean Zn concentration in Table 02 with a factor 0.6

Conclusions

ZnO nano particles shows great interests in the application in fields of food additives, packing and agriculture, and biomedicine, due to the high antibacterial activity, chemical stability and solubility. The present research is an attempt to achieve fortification of tilapia mince with Zn and to improve the shelf life of the mince. Application of ZnO nano particle appears to be bacteriostatic in nature rather than bactericidal as the TPC figures were always on an increasing trend with reduced growth rate in case of fortification level 60–80 ppm.

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Declarations

Conflict of interest There are no conflict of interests and declaration is accurate.

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