

Original Research Article

Quality of Fresh pork packed in biodegradable material in comparison with conventional packaging material under frozen storage

ABSTRACT

Aims: To develop and evaluate biodegradable polylactic acid (PLA) packaging films incorporated with zinc oxide nanoparticles (ZnONPs) and assess their effects on the physico-chemical characteristics as well as the shelf life of pork chops under frozen condition

Study design: It is a controlled experiment where different treatments (types of packaging) are applied to the pork chops, and their effects are measured at multiple time points.

Place and Duration of Study: Sample: Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Wayanad District, Kerala State, India between June 2024 and December 2024.

Methodology: Plain PLA pouches and PLA pouches incorporated with ZnO were prepared. Pork hind leg chops were used as samples and were procured from a local butcher, cut into 200-250g and then packaged into Low Density Poly Ethylene (LDPE), plain PLA and PLA pouches incorporated with 2.5 per cent and 5 per cent concentrations of ZnO nanoparticles. Samples packed in LDPE were labelled as control while samples packed in PLA, 2.5 per cent PLA/ZnO pouches and 5 per cent PLA/ZnO pouches were labelled as treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3) respectively. All samples were kept under frozen storage (-18 ± 2 °C) and analysis was done on the 0th, 30th, 60th, 90th and 120th day of storage. The samples were analysed for their physico-chemical properties such as pH, cooking loss, water holding capacity, thiobarbituric acid reactive substances (TBARS) number and tyrosine value. Four trials of the experiment were conducted.

Results: A significant decrease in pH was observed in all the samples across the storage period except in T2 and the water holding capacity of all samples significantly ($P \leq 0.01$) reduced with time. Corresponding to the trend in pH values over storage, was a significant decrease ($P < 0.01$) could be observed in the cooking loss as well as water holding capacity. The TBARS ($P < 0.01$) and tyrosine values ($P < 0.05$) increased significantly with time. However, there was no significant difference observed between the samples in any day of analysis indicating that PLA is equally effective as LDPE for the frozen storage of pork. The incorporation of nanoparticles, however did not exert a significant change in the quality of frozen pork.

Conclusion: PLA is equally effective as LDPE as a packaging material for the frozen storage of pork and can offer a sustainable solution to the packaging of muscle foods. The incorporation of nanoparticles, however did not exert a significant change in the quality of frozen pork.

Keywords: Polylactic Acid, ZnO nanoparticles, Pork, Frozen.

1. INTRODUCTION

Pork is one of the most widely consumed meat in the world constituting about 36 per cent of all the meat eaten globally. Due to their high breeding rate, quick maturity and short time between generations, pigs are well-suited for intensive farming. Moreover, the upfront investment and upkeep required for pig farming are relatively low. Pork is a rich source of minerals like zinc, selenium and vitamins B₁, B₆ and B₁₂. Due to their relatively high content of unsaturated fatty acids, pork is prone to lipid oxidation and requires proper packaging and storage conditions. Freezing is one of the most common methods of storage for pork as it slows down the oxidation of lipids. Packaging offers an additional safeguard for preserving the qualities of pork and extending its shelf life. Synthetic polymers or plastics have been used for their versatility and excellent physical properties in food packaging. More than 42% of the total plastics produces are used for packaging, most of which are single-use (Geyer *et al.*, 2017). Most of these plastics are petroleum-based and are not biodegradable requiring hundreds of years to break down (Hussain, *et al.*, 2024)

Concerns about the environmental problems and exhaust of petrochemical resources caused by non-biodegradable plastics stimulated the production of eco-friendly renewable, biodegradable biopolymeric materials. Among them, polylactic acid (PLA) is considered a promising material due to its biodegradability, biocompatibility, high transparency, good mechanical properties, moderate water resistance and commercial availability at a reasonable price. Furthermore, innovative nanomaterial-based active agents are being integrated into packaging to enhance the effectiveness of eco-friendly biopolymer films in shielding food products from factors that contribute to food deterioration (Omerović *et al.*, 2021). Zinc oxide (ZnO) nanomaterials have applications across various industries and are also utilised in food packaging and medical fields for their antimicrobial, antitumour and anti-oxidant properties.

This study was proposed to evaluate the quality of pork chops packaged in polylactic acid pouches incorporated with nanoparticles by analysing the physico-chemical attributes on 0, 30, 60, 90, and 120 days of storage.

2. MATERIALS AND METHODS

2.1 Preparation of Polylactic Acid (PLA) films

Biodegradable films were prepared using PLA granules (Banka Bioloo Ltd, Hyderabad, India) with glycerol as a plasticiser and chloroform as solvent. The concentrations of PLA and glycerol were set at 2 per cent and 0.1 per cent, respectively, based on the preliminary trials. The film-forming solution with nanoparticles was prepared by dispersing the necessary amount of zinc oxide nanoparticles in chloroform, followed by sonication for 15 min using a probe sonicator (Hielscher Ultrasonics, Germany). The PLA granules were then added to the chloroform mixture along with glycerol, and the mixture was continuously stirred for 5 h at 1000 rpm until it fully dissolved using a magnetic stirrer (Neuation magnetic stirrer, India). Plain PLA solution was prepared by dissolving PLA granules and glycerol in chloroform using a magnetic stirrer by stirring for 5 h at 1000 rpm. The concentrations of zinc oxide nanoparticles were determined based on previous studies and initial trials. The film-forming solutions were poured onto glass plates (40 x 18 cm), levelled by a four-sided film applicator (Raj Scientific, India) and dried in an incubator at 60 °C for 10-15 min to form the films. The resulting films were peeled off, made into pouches of size (15 x 13 cm) by sealing and stored under dry and hygienic conditions.

2.2 Packaging of Pork Chops

Pork chops were cut into 200-250 g pieces, divided into four groups and then aerobically packed into low density polyethylene (LDPE), plain PLA pouches, pouches, PLA incorporated with 2.5% (w/w of PLA) ZnO and PLA incorporated with 5% (w/w of PLA) ZnO were used for packaging the pork chops. The packed control and treatment chops were stored under freezer (-18±2° C) condition.

2.3 Measurement of pH

The sample pH was measured using a digital bench model pH meter (EUTECH instruments pH 510, Singapore) as per the method described by AOAC (2016). Ten grams of the sample was blended with 50ml distilled water for one minute using a tissue homogeniser (Kinematica, Switzerland) at the speed of 4000 rpm. The pH of the homogenate was recorded by immersing the combined glass electrode of the digital pH meter.

2.4 Cooking Loss

Cooking loss was calculated as per Boccard *et al.* (1981). Eighty grams of meat sample was placed in an HDPE pouch, remove the trapped air between the sample and the wall of the pouch and then aerobically packed. The pouch was kept in water bath at 80°C for 50 min. Then it was cooled under running tap water for 40 min, after which the cooked meat was taken out, mopped dried and weighed. The per cent of cooking loss was assessed as follows,

$$\text{Cooking loss (per cent)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.5 Thiobarbituric Acid Reactive substances (TBARS) number

TBARS directly indicate the presence of lipid oxidation in meat and meat products. Estimation of TBARS is done by measuring the concentration of secondary lipid oxidised products like malondialdehyde.

The TBARS number of the samples was determined as per Witte *et al.* (1970) with modifications. Accurately weighed 20 g sample was blended with 50 ml chilled extracting solution containing 20 per cent trichloroacetic acid in 2 M orthophosphoric acid for 2 minutes. The resultant slurry was transferred to a 100 ml volumetric flask and made up to 100 ml with deionised distilled water. This solution was filtered through Whatman No.1 filter paper. From the filtrate, 5 ml was transferred to a screw-capped vial followed by the addition of 5 ml of 2-thiobarbituric acid solution (0.005M in distilled water). The solution was mixed by inverting the vial and kept for 15 h in darkness at room temperature. The absorbance was determined at a wavelength of 530 nm against a blank containing 5 ml of distilled water and 5 ml of 2-thiobarbituric acid solution in a colourimeter (Systronics photoelectric colourimeter, India). The absorbance was determined at a wavelength of 530 nm against a blank containing 5 ml of distilled water and 5 ml of 2-thiobarbituric acid solution. The absorbance was expressed as TBARS number of samples.

2.6 Tyrosine Value

Tyrosine value indicates the proteolysis in meat and meat products as tyrosine is the first formed amino acid during proteolysis.

The tyrosine value of the samples was estimated as per the method described by Pearson (1968). To accurately weigh 2 g of sample, 40 ml of 5 percent trichloroacetic acid solution (TCA) was added. After homogenisation for 2 minutes in a tissue homogeniser (Kinematica, Switzerland) at the speed of 4000 rpm, the sample was filtered through Whatman filter paper no. 1. The filtrate was used in the estimation of

tyrosine value. To 2.5 ml of TCA extract in a test tube, an equal quantity of distilled water and 10 ml of 0.5 N sodium hydroxide were added and shaken. Three millilitres of diluted Folin-Ciocalteu's phenol (FC) reagent (1 ml of concentrated FC reagent and 2 ml of distilled water) was added, mixed and the contents were allowed to stand for 5 minutes at room temperature. The absorbances were measured at a wavelength of 660 nm in a colourimeter (Systronics photoelectric colourimeter, India) using a blank containing 2.5 ml of 5 per cent TCA, an equal quantity of distilled water, 10 ml of 0.5 N sodium hydroxide and 3ml of diluted FC reagent for comparison. By referring to the standard graph of tyrosine, tyrosine values of samples were calculated and expressed as mg of tyrosine/100 g of sample.

2.5.1 Standard graph for Tyrosine

Accurately weighed 0.10 g of tyrosine was dissolved in five per cent TCA in a 500 ml volumetric flask and made up to the mark with double distilled water. The following volumes of tyrosine solution were added to a series of 100 ml volumetric flasks: 0, 1, 3, 5, 7, 10, 12, 15, and 20 ml. Each was made up to the mark with double distilled water and mixed thoroughly. Five millilitres of each solution were mixed thoroughly with 10 ml of 0.5 N sodium hydroxide solution and 3 ml of diluted Folin-Ciocalteu's (F-C) reagent and then treated as described above. The standard graph (Fig. 1) was prepared by plotting optical density against milligrams of tyrosine dissolved in trichloroacetic acid solution.

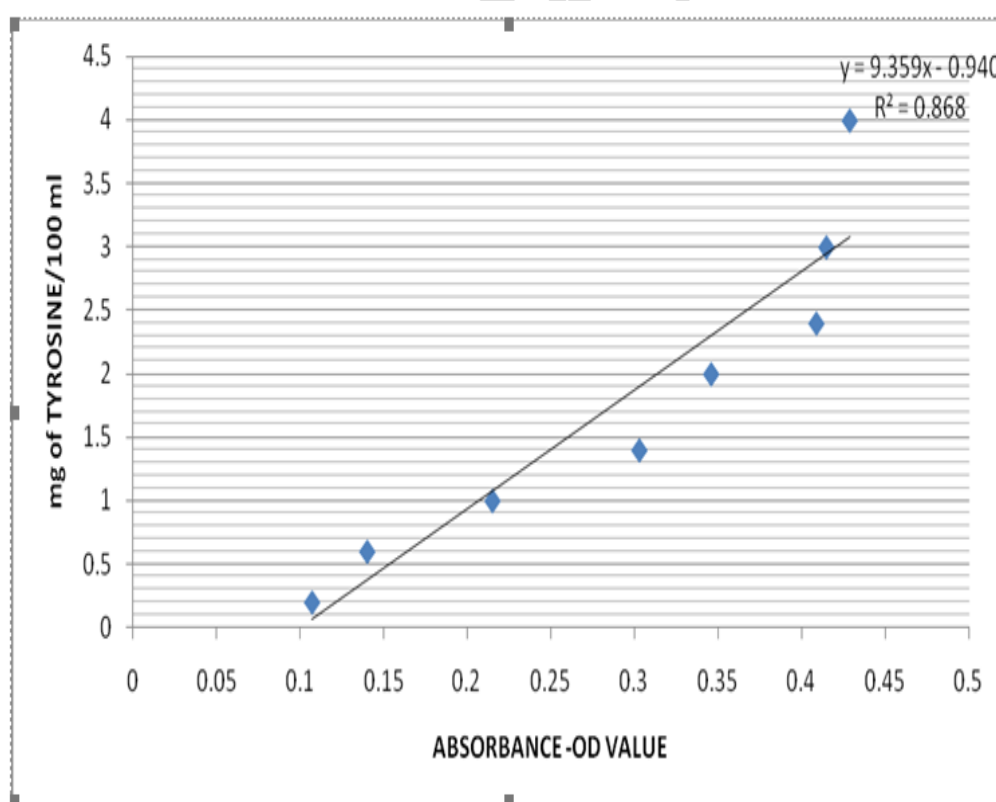


Figure 1. Standard graph for tyrosine value

3. RESULTS AND DISCUSSION

3.1 pH

pH was found to decrease significantly ($P \leq 0.001$) in all the samples across the storage period except in T2. A significant decrease in pH was observed from the 60th day in T1, 90th day in C and 120th day in T3 (Table 1). Similarly, Kim *et al.* (2018) also observed that the pH value of frozen and thawed pork loins decreased with the storage period. Leygonie *et al.* (2021) stated that the pH of meat tended to decrease after freezing and thawing compared to its initial state which could be caused by the denaturation of buffer proteins during freezing, accompanied by exudate production, which released hydrogen ions and lowered the pH. Another possible explanation is that the loss of fluid from the meat tissue increased solute concentration, leading to a pH drop. In another study, Muela *et al.* (2015) found significantly lower pH values in lamb meat frozen for up to 21 months, compared to unfrozen samples. On the contrary, Medić *et al.* (2018) observed that the pH of pork was significantly higher in 6, 12 and 18 months of frozen storage than in fresh meat or in samples with 3 months of frozen storage. However, Teuteberg *et al.* (2021) reported that there was no difference in observed pH values between fresh pork and pork stored frozen for 24 weeks. There was no significant difference in pH between the pork packaged in plain PLA films and pork packaged in ZnO nanoparticle-incorporated films on any day of the analysis. In contrast, Suo *et al.* (2017) observed that the pH of pork meat wrapped in ZnO-nanoparticle-coated film increased to 6.12 after 14 days of refrigeration while the control group samples reached the same pH level within 6 days, eventually rising to 8.85.

Table 1. Results of comparison of pH between treatments and between days

Day	C	T1	T2	T3	F-value (P-value)
Day 0	5.68 ^a ± 0.08	5.68 ^a ± 0.08	5.68 ± 0.08	5.68 ^a ± 0.08	0.00 ^{ns} (1.00)
Day 30	5.68 ^a ± 0.08	5.67 ^{ab} ± 0.07	5.67 ± 0.08	5.67 ^a ± 0.08	0.002 ^{ns} (1.00)
Day 60	5.66 ^{ab} ± 0.06	5.65 ^{bc} ± 0.08	5.65 ± 0.08	5.64 ^{ab} ± 0.08	0.020 ^{ns} (0.996)
Day 90	5.63 ^b ± 0.07	5.64 ^c ± 0.08	5.65 ± 0.06	5.64 ^{ab} ± 0.08	0.007 ^{ns} (0.999)
Day 120	5.62 ^b ± 0.08	5.62 ^d ± 0.08	5.64 ± 0.06	5.61 ^b ± 0.07	0.041 ^{ns} (0.988)
F-value (P-value)	9.182 ^{**} (0.001)	24.391 ^{**} (<0.001)	1.444 ^{ns} (0.279)	14.627 ^{**} (<0.001)	

^{**} Significant at 0.01 level; ns non-significant

Means having different small letter as super script differ significantly within a column

3.2 Water Holding Capacity

A significant ($P \leq 0.01$) reduction in water holding capacity was observed in all the samples across the storage period. The decrease was noted from day 30 in T1, T2 and T3, whereas in control samples the same was observed on day 60 only (Table 2). Lee *et al.* (2021) also found out that the water holding capacity of pork loin was significantly reduced by freezing and thawing processes. Similarly, Kim *et al.* (2015) observed that the water holding capacity of pork loins significantly reduced across a storage period of 6 months. According to Huff-Lonergan and Sosnicki (2003), the decrease in water-holding capacity was associated with the degradation of muscle fibre structures and the denaturation or modification of proteins, resulting in the contraction of myofibrils. Similar observations were cited by Lebret and Čandek-Potokar (2022) who stated that the WHC of meat was mainly influenced by the rate, the extent of pH decline and the ultimate pH. Packaging was not seen to influence the WHC of pork throughout the period of storage. Saeed *et al.* (2022) observed that there were no significant differences in chiller-stored chicken meat dipped in ZnO nanoparticle solutions (0.5 mg/ml, 1 mg/ml and 1.5 mg/ml in deionised water) compared to untreated chicken meat on any day of

analysis, although the water-holding capacity (WHC) value significantly decreased across all groups as the storage period progressed. However, Suo *et al.* (2017) observed a significantly higher WHC in chiller-stored pork for 14 days in carboxymethyl cellulose (CMC-Na) incorporated with ZnO nanoparticles films as compared to pork stored in plain CMC-Na films.

Table 2. Results of comparison of WHC between treatments and between days

Day	C	T1	T2	T3	F-value (P-value)
Day 0	15.80 ^A ± 0.11	15.80 ^A ± 0.11	15.80 ^A ± 0.11	15.80 ^A ± 0.11	0.00 ^{ns} (1.00)
Day 30	15.60 ^{AB} ± 0.11	15.43 ^B ± 0.09	15.44 ^B ± 0.16	15.49 ^B ± 0.13	0.403 ^{ns} (0.753)
Day 60	15.53 ^{BC} ± 0.11	15.28 ^{CD} ± 0.11	15.50 ^{AB} ± 0.04	15.33 ^{BC} ± 0.09	1.857 ^{ns} (0.191)
Day 90	15.43 ^{CD} ± 0.08	15.33 ^{BC} ± 0.12	15.40 ^B ± 0.07	15.23 ^C ± 0.12	0.838 ^{ns} (0.499)
Day 120	15.33 ^D ± 0.08	15.15 ^D ± 0.09	15.35 ^B ± 0.06	15.08 ^C ± 0.09	2.915 ^{ns} (0.078)
F-value (P-value)	47.545** (<0.001)	33.690** (<0.001)	4.589** (0.018)	26.883** (<0.001)	

** Significant at 0.01 level; ns non-significant

Means having different capital letter as super script differ significantly within a column

3.3 Cooking Loss

The changes in cooking loss of the samples during storage are depicted in Table 3. There was a significant ($P < 0.01$) difference in the cooking loss of all the samples across the storage period. All the samples showed a significantly higher cooking loss on day 0 with a significant decrease observed on day 30th for all the samples with the values maintained up to day 120 for T1 and T2. However, in C and T3, the values further reduced up to day 120. A similar trend in the reduction of cooking loss was also observed by Kim *et al.* (2020) who reported that pork necks frozen at -18°C showed a significant decrease from the second month of storage. Medić *et al.* (2018) reported that fresh pork samples tended to exhibit the highest cooking loss while lower cooking loss could be observed in pork stored frozen for 3 and 6 months. According to Dawood (1995), certain initial weight losses, such as thawing loss, may account for reduced cooking loss. No significant difference was observed in the cooking loss between the samples on any day of the analysis. However, Suo *et al.* (2017) who analysed cooking loss of pork meat stored in different films over 14 days in cold storage found that pork in plain sodium carboxymethyl cellulose (CMC-Na) films increased by 63% while CMC-Na films with zinc oxide nanoparticles showed an increase of 31% only.

Table 3. Results of comparison of cooking loss between treatments and between days

Day	C	T1	T2	T3	F-value (P-value)
Day 0	25.70 ^A ± 0.87	25.70 ^A ± 0.87	25.70 ^A ± 0.87	25.70 ^A ± 0.87	0.00 ^{ns} (1.00)
Day 30	23.66 ^B ± 1.14	22.77 ^B ± 0.60	22.65 ^B ± 0.63	23.41 ^B ± 1.22	0.272 ^{ns} (0.844)
Day 60	22.75 ^{BC} ± 1.41	21.82 ^B ± 0.72	22.50 ^B ± 1.03	22.00 ^C ± 1.34	0.140 ^{ns} (0.934)
Day 90	22.13 ^C ± 1.33	21.17 ^B ± 0.37	23.08 ^{AB} ± 1.15	21.92 ^C ± 1.25	0.515 ^{ns} (0.68)

Day 120	21.79 ^C ± 1.68	21.06 ^B ± 0.25	22.03 ^B ± 0.54	20.99 ^C ± 0.90	0.271 ^{ns} (0.845)
F-value (P-value)	15.183** (<0.001)	32.727** (<0.001)	6.383** (0.005)	30.695** (<0.001)	

** Significant at 0.01 level; ns non-significant

Means having different capital letter as super script differ significantly within a column

3.4 Thiobarbituric Acid Reactive Substance (TBARS) Number

The TBARS method is generally used to measure the quality of secondary products of lipid oxidation in foods which are responsible for rancid, fatty, pungent and other off-flavours. The TBARS number of control and treatments are depicted in the table 4. On the day of preparation, all the samples of pork hind leg chops had a TBARS value of 0. The samples did not differ among themselves on any day of analysis. A significant ($P < 0.01$) increase in TBARS values was observed across the storage period in all the samples with the highest value recorded on day 120 of storage in all samples. On the contrary, Alonso *et al.* (2016) observed no significant differences in lipid oxidation values among fresh pork, and pork stored under freezer storage for one -year and two years concluding that lipid oxidation remained stable throughout frozen storage for 24 months. Similarly, Hansen *et al.* (2004) observed that TBARS values of pork showed no significant increase after 30 months of frozen storage. During frozen storage at -20°C , a fraction of water, referred to as unfrozen water, remains in a liquid state and facilitates chemical reactions. Typically, lipid oxidation accelerates after thawing, as peroxidation (the primary stage of lipid oxidation) takes place during storage. This leads to rapid and intense secondary lipid oxidation characterised by thiobarbituric acid formation, which subsequently increases TBARS values (Owen and Lawrie, 1975). Packaging was not found to influence the lipid peroxidation as no significant difference was observed between the samples on any day of analysis. On the contrary, according to Souza *et al.* (2020), although lipid oxidation increased statistically with time in all chilled poultry meat samples, samples wrapped in bio-nanocomposites containing ZnO nanoparticles exhibited minimal changes in MDA levels from day zero to day 11, highlighting the material's effectiveness in slowing oxidative processes. In contrast, unwrapped meat showed MDA values of 0.5 mg/kg or higher from day 7 onward, a threshold commonly recognised as an indicator of rancidity.

Table 4. Results of comparison of TBARS between treatments and between days

Day	C	T1	T2	T3	F-value (P-value)
Day 0	0 ^C ± 0	0 ^B ± 0	0 ^D ± 0	0 ^C ± 0	-
Day 30	0.01 ^C ± 0.01	0.02 ^B ± 0.01	0.01 ^C ± 0	0.01 ^{BC} ± 0.01	0.255 ^{ns} (0.856)
Day 60	0.02 ^B ± 0	0.02 ^{AB} ± 0.01	0.02 ^{BC} ± 0	0.02 ^{AB} ± 0	0.118 ^{ns} (0.948)
Day 90	0.02 ^B ± 0	0.03 ^{AB} ± 0.01	0.03 ^{AB} ± 0.01	0.03 ^{AB} ± 0.01	0.724 ^{ns} (0.557)
Day 120	0.03 ^A ± 0.01	0.06 ^A ± 0.02	0.04 ^A ± 0.01	0.05 ^A ± 0.01	1.876 ^{ns} (0.187)
F-value (P-value)	6.027** (0.007)	8.08** (0.002)	20.94** (<0.001)	13.251** (<0.001)	

** Significant at 0.01 level; ns non-significant

Means having different capital letter as super script differ significantly within a column

3.5 Tyrosine Value

Tyrosine values increased significantly ($P < 0.05$) across the storage period from day 60 in all samples, with the highest values observed on day 120. A significant ($P < 0.05$) increase in tyrosine values with time was observed across the storage period in all samples (Table 5). Similarly, Kandeepan and Biswas (2003) observed that the tyrosine content in frozen buffalo meat increased with extended storage duration. In their study, Ziauddin *et al.* (1993) observed that in plate-frozen buffalo meat cuts and minced buffalo meat, tyrosine values slightly decreased during storage. Strange *et al.* (1977) stated that the tyrosine value, an indicator of proteolysis that measured the amino acids tyrosine and tryptophan in meat and non-protein extracts, increased during storage, with the increase becoming more pronounced during advanced spoilage compared to the early stages of spoilage. They also observed a significant correlation of tyrosine value with ammonia, non-protein nitrogen (NPN) values and bacterial counts. Xiong (2000) explained that the freezing and thawing process damaged the ultrastructure of muscle cells, leading to the release of mitochondrial and lysosomal enzymes, haem iron, and other pro-oxidants. This, in turn, accelerated and intensified protein oxidation. In our study, the samples did not differ among themselves on any days of analysis.

Table 5. Results of comparison of Tyrosine values between treatments and between days

Day	C	T1	T2	T3	F-value (P-value)
Day 0	19.40 ^C ± 0.48	19.40 ^B ± 0.48	19.40 ^C ± 0.48	19.40 ^D ± 0.48	0.00 ^{ns} (1.00)
Day 30	22.60 ^{BC} ± 2.15	22.64 ^{AB} ± 1.32	21.54 ^C ± 2.13	21.04 ^D ± 1.09	0.207 ^{ns} (0.89)
Day 60	28.67 ^B ± 2.55	27.71 ^A ± 0.98	26.07 ^B ± 1.73	26.40 ^C ± 1.04	0.500 ^{ns} (0.689)
Day 90	31.88 ^A ± 2.58	28.94 ^A ± 1.14	31.78 ^B ± 2.90	29.45 ^A ± 0.91	0.548 ^{ns} (0.659)
Day 120	34.01 ^A ± 2.12	38.32 ^A ± 6.67	34.83 ^A ± 2.83	32.92 ^A ± 1.81	0.362 ^{ns} (0.782)
F-value (P-value)	18.45** (<0.001)	5.09* (0.012)	15.755** (<0.001)	36.29** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

Means having different capital letter as super script differ significantly within a column

4 CONCLUSION

It was observed that the quality of pork chops was preserved under 120 days of frozen storage in both LDPE pouches as well plain PLA and PLA / ZnO pouches. The changes in physico-chemical characteristics of frozen pork under frozen storage were within the agreeable limits and were comparable in both conventional as well as biodegradable packaging. PLA was equally effective as LDPE as a packaging material for the frozen storage of pork and can offer a sustainable solution to the packaging of muscle foods. The incorporation of nanoparticles, however did not exert a significant change in the quality of frozen pork, which emphasizes the need for further research on the characterisation of nanoparticles and their interaction with different polymer matrices.

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