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Characterization of the Protective Effect of Ginger against Frequent Freeze–thaw Cycles on Meat Products

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Abstract

Repeated freezing and thawing of meat products, which might occur during transport or in the consumers' kitchens, have deteriorative effects on their quality, including lipid oxidation and protein decomposition. This study evaluated three meat products (meat pie (burger), kofta, and sausage) to estimate the protective role of ginger (natural antioxidant) against lipid oxidation and protein decomposition during freeze–thaw cycles. These meat products were subjected to six freeze–thaw cycles and were tested after the first, third, and sixth weeks. This study measured the thiobarbituric acid and total volatile nitrogen levels to estimate lipid oxidation and protein decomposition, respectively. Further, the three meat products were examined histologically. The muscle diameters and shrinkage of muscle fibers was observed in all groups, even after the addition of ginger. Results indicated that repeated freezing and thawing increased both lipid oxidation and protein decomposition, which were reduced after the addition of ginger. Histologically, intact muscle fibers with obvious muscle striation were seen after the first freeze–thaw cycle. However, in the third and sixth cycles, the striations were absent even after adding ginger. Therefore, ginger could be used during meat products manufacture for reduction of lipid oxidation and protein decomposition during meat products preservation.

KEYWORDS
Lipid oxidation, Protein decomposition, Histology, TBA, TVBN.

INTRODUCTION

Freezing is an efficient method for preserving meat for an unlimited period as it retains most of its original properties (Babić *et al*., 2009). However, repeated freezing and thawing of meat products affect their quality (Wachirasiri *et al*., 2019). Freeze-thaw cycles cause protein oxidation, as confirmed by a 36.46% reduction in the sulfhydryl content. They also cause lipid oxidation, indicated by 209.06% and 338.46% higher carbonyl and malondialdehyde contents, respectively. Therefore, more than three freeze-thaw cycles are not recommended (Cao *et al*., 2022). It decreases the immobilized water, resulting in meat hardness (Cheng *et al*., 2019). It also damages the myofibrillar protein of muscles (Du *et al*., 2021). Decreased myowater and lipid oxidation was observed after six freeze-thaw cycles, resulting in lighter-colored flesh (Ali *et al*., 2016).

Thiobarbituric acid (TBA) test is valuable for the detection of lipid oxidation via measuring malondialdehyde contents (Grotta *et al*., 2017). The total volatile basic nitrogen (TVB-N) test is used for the assessment of meat freshness. TVB-N increases with inappropriate storage, and it is usually associated with biomarkers of meat spoilage (Bekhit *et al*., 2021). The TVB-N contents are related to changes in meat colour, moisture content, microbial load, and tenderness (Holman *et al*., 2021). Meat spoilage leads to protein decomposition due to the growth of microorganisms (Li *et al*., 2019; Ajaykumar and Mandal, 2020).

Sen and Sharma (2004) reported that the signs of muscle damage increased with increasing the number of freeze–thaw cycles, Via histology, no obvious histological changes were observed after the first freeze-thaw cycle. However, during the 2nd cycle, the muscle fibers were damaged with numerous interfibrillar gaps between them. Moreover, double freezing of fish muscles damaged the perimysium and the muscle fibers. It also caused water loss and increased the total volatile nitrogen concentration (Popelka *et al*., 2014). Repeated freeze–thaw cycles caused protein oxidation, which decreases the water-holding capacity of the muscle fibers (Ali *et al*., 2015). Additionally, Cao *et al*. (2022) confirmed that repeated freeze-thaw cycles increased the relaxation time, transformed immobile water to free water, and therefore water loss.

Several trials have evaluated the use of antioxidants for meat preservation (Falowo *et al*., 2014; Ribeiro *et al*., 2019). Synthetic antioxidants, including butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate, cause health hazards to humans when consumed in high quantities for a long time. They have several side effects, including carcinogenic, mutagenic, cytotoxic, and genotoxic effects (Sarafian *et al*., 2002; Faine *et al*., 2006). Therefore, it is important to use natural antioxidants to preserve meat products (Carocho *et al*., 2018). Ginger is a natural antioxidant that is also anti-inflammatory and prevents oxidative stress when consumed (Kota *et al*., 2008; Tinello and Lante, 2019). Ginger rhizomes could preserve canned meat and

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improve its color, taste, springiness, and chewiness (Draszanowska *et al*., 2020).

This study aimed to estimate the deteriorative effect of repeated freeze–thaw cycles on meat products (burger patties, kofta, and sausage) and evaluate the protective effect of ginger as a natural antioxidant against lipid oxidation and protein decomposition during freeze–thaw cycles.

MATERIALS AND METHODS

Samples preparation

The present study used kofta, beef burger, and oriental sausage from the same brand from a commercial hypermarket. Dry-ground ginger (normal dried ginger from trusted supermarket) was used as a natural antioxidant. The meat products and ginger were obtained from a local market (Giza, Egypt). The experiments were performed on sixty meat products (twenty of each kind). The experiments were repeated three times. The meat product samples (approximately 350 g) were selected randomly, packed in moisture-proof polyethylene bags, sealed, and stored at −10°C (air freezing) for each cycle. The samples manipulation was done at histology lab, faculty of veterinary medicine, Benha University. Ginger powder was added once at the surface of the meat pie, kofta, and sausage before the initiation of freezing.

The frozen samples were subjected to 0-6 freeze-thaw cycles. Every week, a set of frozen samples were thawed at room temperature (25°C) for 17 h and analyzed on the first, third, and sixth weeks. The samples were divided into three groups. The first is the control group, in which the samples were preserved in the freezer for 1, 3, or 6 weeks without thawing weekly. In the second group, the samples were subjected to weekly freeze-thaw cycles. In the third group, ginger (13 g/350 g of meat product) was added to the samples once directly before freezing, and then, they were subjected to weekly freeze-thaw cycles. The samples were used for measuring Thiobarbituric Acid (TBA) and Total Volatile Basic Nitrogen (TVB-N) at animal health research institute.

Chemical analysis

Determination of Thiobarbituric Acid (TBA) (mg/kg) (EOS 63/10-2006)

TBA was determined according to the method described by Ulu (2004) and Grotta *et al*. (2017) with some modifications and measured in mg of malondialdehyde equivalents per kg of sample.

10 grams of sample were blended with 50 mL of trichloroacetic acid (TCA) in water distilled 7% (ω/υ) to bring the pH to 1.5. The blended samples were distilled by the Kjeldahl distillation apparatus. The flask containing samples was heated to 50°C and a few glass beads were added to prevent foaming. 50 ml distillate was collected 10 minutes from the time of boiling commences. 5 mL of the distilled solution was mixed with 5 mL of a 20 mM TBA solution prepared in 90% acetic acid (0.2883g/100 ml of 90% glacial acetic acid) in a glass- Stoppard tube. The tube was then shacked and immersed in a boiling water bath for 35 minutes. A blank was similarly prepared using 5 ml distilled water with 5ml TBA reagent without the addition of meat product samples. After heating, the tube was cooled under tape water for 10 minutes.

The optical density (OD) was read in the tube containing 5 ml of prepared meat product samples reacted with TBA reagent (0.2883 g/100 ml of 90% glacial acetic acid) against the blank (5 ml distilled water with 5 ml TBA reagent) using a spectrophotometer (Perkin Elmer, 2380, USA) at 538 nm wavelength. The TBA values were calculated as follows:

TBA (mg malondialdehyde/kg of the sample) = D × 7.8, where D: OD of the sample against the blank.

Determination of Total Volatile Basic Nitrogen (TVB-N) (mg/100 gm) (EOS 63/10-2006)

TVB-N values were determined based on the method by Holman *et al*. (2021) with some modifications. Ten grams of each meat product sample was homogenized using a chopper for 1–2 min. The minced samples were placed in a distillation flask, and 2 grams of magnesium oxide with 300 ml of distilled water were added. 100 ml of the distillate was placed in a beaker containing 25 ml of 2% boric acid after 30 min of distillation. Then, the samples were titrated against 0.1 M sulfuric acid until the solution turned faint pink. TVB-N was calculated using the following equation.

TVBN (mg/100 g) = R × 14, where R is the volume of sulfuric acid used for titration.

Histological examination

Three random samples were collected from each meat product in the first, third, and sixth weeks and divided into three experimental groups. The samples were thawed and preserved in 10% neutral buffered formalin for 1 week. Then, they were dehydrated, cleared, and embedded in paraffin. A microtome was used to cut 5 µm thick sections for hematoxylin and eosin (H and E) staining. The photos were capturedusing the Leica light microscope and performed the histological procedures according to Bancroft and Gamble (2008). The Image J software (Schindelin *et al*. 2012) was used to quantify the diameter of the muscle fibers using the sections from the first, third, and sixth weeks samples (7 images/section) at x 200 from all groups.

Statistical analysis

The data were analyzed using the IBM® SPSS® software version 26 (SPSS, Richmond, VA, USA) as described by Heck *et al*. (2013). Multivariable analysis, a two-way analysis of variance, was used in data analysis. Duncan's multiple range test was utilized for post hoc comparison of the differences between means. Differences were considered significant at $P < 0.05$. The normal distribution of the data was evaluated using the Kolmogorov-Smirnov (K-S) test. The statistical results demonstrating the effect of the weekly freeze-thaw cycles on MDA, TVBN, and muscle thickness of the burger, kofta, and sausage samples are shown in Tables 1–9.

RESULTS

Properties of burger

TBA evaluation

The TBA value was estimated in the three groups to evaluate the protective effect of ginger as shown in (Table 1). MDA content of the control samples obtained on the 1st, 3rd, and 6th weeks were 0.22±0.02, 0.22±0.01, and 0.23±0.02 mg/kg, respectively. The MDA content in the freeze–thaw samples obtained on the 1st, 3rd, and 6th week was 0.37±0.02, 0.80±0.07, and 1.23±0.07 mg/kg, respectively. However, the MDA content of the freeze-thaw + ginger samples taken on the 1st, 3rd, and 6th weeks were 0.25±0.05,

0.43±0.07, and 0.59±0.03 mg /kg, respectively.

The MDA content was significantly higher (P<0.05) in the freeze-thaw group than in the control group. However, in the freeze-thaw + ginger group, the MDA content was slightly higher (P<0.05) than the control group and lower than the freeze-thaw group. Therefore, results confirmed that ginger could reduce lipid oxidation during freeze-thaw cycles but it can't prevent it completely.

TVB-N evaluation

The results obtained in the control group on the 1st, 3rd, and 6th weeks were 10.24±0.15, 11.17±2.29, and 11.64±0.41 mg/100 g, respectively. The TVB-N contents in the freeze–thaw group were 11.56±0.23, 16.3±1.40, and 20.96±0.65 mg/100 g, respectively, while those in the freeze-thaw + ginger group were 10.84±0.13, 11.42±0.30, and 17.33±2.25 mg/100 g, respectively. The TVB-N content in the freeze-thaw + ginger group was (P < 0.05) slightly higher than the control group. However, the TVB-N content of the freeze–thaw group was higher (P < 0.05) than both the freeze-thaw + ginger and control groups. The TVB-N test results indicate that protein decomposition was decreased during freeze-thaw cycles after the addition of ginger (Table 2).

Histological examination

In the control group (Fig. 1A, B, and C), the skeletal muscle fibers displayed distinct striations and peripherally located nuclei in the 1st week (Fig. 1A). In the third week, the striations disappeared from the muscle fibers with the peripheral nuclei still in-

Table 1. Average of thiobarbituric acid number (mg MDA/kg) in the examined beef burger samples. Malondialdehyde(MDA) is a lipid peroxidation marker.

Time (week)	Groups			SEM	P values		
	Control	Beef burger (F-T)	Beef burger + Ginger (F-T)		Treatment	Week	T*W
1	0.22 ± 0.02^{bB}	0.37 ± 0.02^{aC}	0.25 ± 0.05^{bC}	0.02	0.00	0	0
3	0.22 ± 0.01^{cA}	0.80 ± 0.07^{aB}	0.43 ± 0.07^{bB}	0.03	0		
6	0.23 ± 0.02^{bcA}	1.23 ± 0.07^{aA}	0.59 ± 0.03^{bA}	0.02	0		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

Table 2. Average of total volatile nitrogen (TVN) (mg/100 g) in the examined beef burger samples.

Time (week)	Groups			SEM	P values		
	Control	Beef burger (F-T)	Beef burger + Ginger (F-T)		Treatment	Week	T*W
1	10.24 ± 0.15^{cC}	11.56 ± 0.23^{aC}	10.84 ± 0.13^{bB}	0.10	0	0	0.06
3	11.17 ± 2.29^{abB}	16.30 ± 1.40^{aB}	11.42 ± 0.30^{bB}	0.77	0.02		
6	11.64 ± 0.41^{abA}	20.96 ± 0.65^{aA}	17.33 ± 2.25^{bA}	0.64	0.05		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

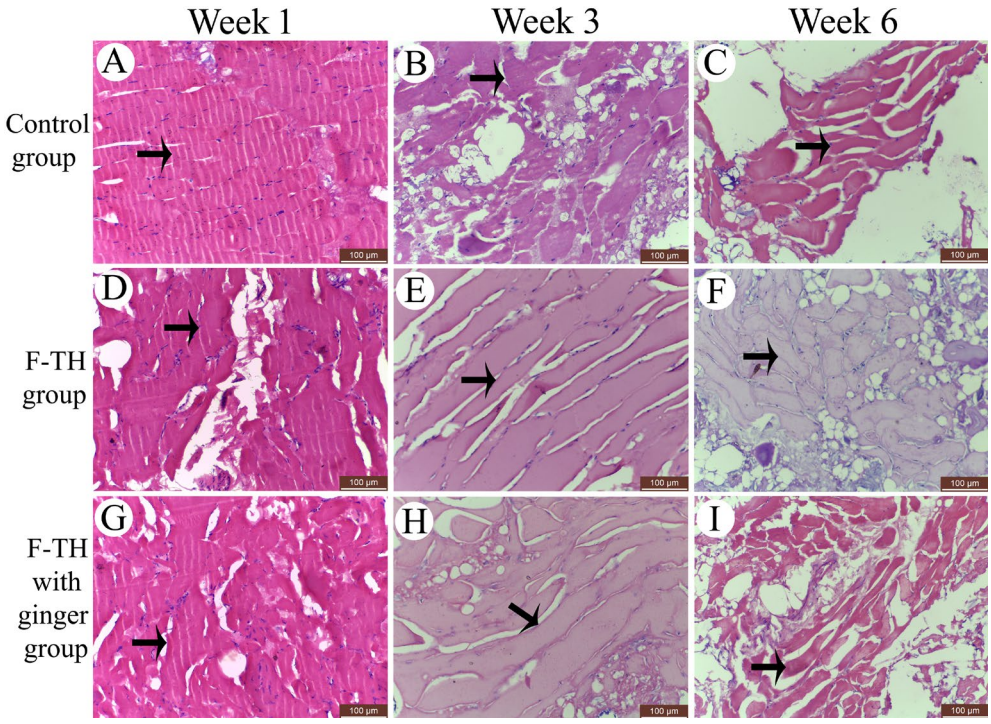

Fig. 1. Effect of freeze–thaw cycles on burgers. The control group (A, B, and C) were frozen for 6 weeks without thawing. The freeze–thaw group (F–TH) were exposed to weekly freeze–thaw cycles (D, E, and F). The third group (F–TH with ginger) was exposed to weekly freeze–thaw cycles after adding ginger to the samples (G, H, and I). The arrows show the longitudinal and cross sections of the skeletal muscle fibers with peripheral elongated nuclei that were striated in the firstweek but lost their striations in the third and sixth weeks of freeze–thaw.

tact (Fig. 1B). The histological structure of the samples obtained in the 6th week resembled the 3rd week samples but with apparent shrinkage of muscles fibers (Fig. 1C).

The freeze-thaw group (Fig. 1D, E, and F) samples displayed similar features as the control group in the 1st week (Fig. 1D). However, muscle striations were lost in the third and sixth weeks (Fig. 1E and F, respectively). In the freeze-thaw + ginger group (Fig. 1G, H, and I), we observed distinct striations in the first week (Fig. 1G), which were lost by the third and sixth weeks (Fig. 1H and I, respectively).

This result showed that denaturation of myofibrillar occurred in all groups at third week of freezing even in a continuous freezing group (control group). This result indicates that the freezing period would affect the quality of meat.

The muscle fiber diameters of the control group in the 1st, 3rd, and 6th weeks were 0.54±0.08, 0.40±0.12, and 0.27±0.09, respectively. In the freeze–thaw group, they were 0.67±0.11, 0.48±0.13, and 0.35±0.06 in the 1st, 3rd, and 6th weeks, respectively. However, the muscle fibers' diameters of the freeze–thaw + ginger group were 0.66±0.10, 0.45±0.14, and 0.29±0.05 in the 1st, 3rd, and 6th weeks, respectively. Results indicated that continuous freezing and freeze–thaw cycles significantly (P < 0.05) decreased the diameter of muscle fibers in the 3rd and 6th weeks in all groups (Table 3). The decrease in the diameter of muscle fibers is closely related to water loss (dripping). Therefore, water loss increases with the increase freezing period of meat products even without freeze-thaw cycles. Also, the addition of ginger will not prevent water loss during freezing.

Properties of kofta

TBA evaluation

The TBA contents of the control group for kofta samples were 0.25±0.07, 0.26±0.02, and 0.26±0.10 mg/kg in the 1st, 3rd, and 6th weeks, respectively, while those in the freeze–thaw groups were 0.32±0.02, 0.72±0.04, and 0.74±0.07 mg/kg, respectively. However, the values in the freeze–thaw + ginger group were 0.41±0.21, 0.42±0.06, and 0.45±0.06 mg/kg in the 1st, 3rd, and 6th weeks, respectively. These results indicate lipid oxidation via measuring MDA levels as shown in (Table 4). These data indicate that adding ginger to the kofta samples significantly (P < 0.05) decreased lipid oxidation during the freeze–thaw with ginger addition in comparison to the freeze–thaw group without ginger addition.

TVB-N evaluation

The TVB-N values in the control group were 9.27±0.15, 9.81±1.15, and 10.60±1.01 mg/100 g in the 1st, 3rd, and 6th weeks, respectively, while those in the freeze-thaw group were 9.87±0.78, 16.27±0.55, and 20.93±0.21 mg/100 g, respectively. However, in the freeze–thaw + ginger group, they were 9.73±1.18, 11.39±0.96, and 14.4±0.89 mg/100 g in the 1st, 3rd, and 6th weeks, respectively. Therefore, adding ginger to the kofta samples before freeze-thaw cycles significantly (P < 0.05) decreased the TVB-N content. The TVB-N values are shown in (Table 5).

Table 3. Measurements of muscle fibers diameters in burger samples at first, third, and sixth weeks of freeze thaw cycles.

Time (week)	Groups			SEM	P values		
	Control	Beef burger (F-T)	Beef burger + Ginger (F-T)		Treatment	Week	T*W
1	0.54 ± 0.08^{A}	0.67 ± 0.11^{A}	0.66 ± 0.10^{A}	0.06	0.25	0	0.93
3	0.40 ± 0.12^{AB}	0.48 ± 0.13^{AB}	0.45 ± 0.14^{AB}	0.08	0.76		
6	0.27 ± 0.09^{B}	0.35 ± 0.06^{B}	0.29 ± 0.05^{C}	0.04	0.33		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

Table 4. Average of thiobarbituric acid number (mg malondialdehyde /kg) in the examined Kofta samples.

Time (week)	Groups				P values		
	Control	Kofta (F-T)	Kofta + Ginger (F-T)	SEM	Treatment	Week	T*W
1	0.25±0.07	0.32 ± 0.02^{B}	0.41±0.21	0.06	0.21	0.00	0.01
3	0.26 ± 0.02^{b}	0.72 ± 0.04^{aA}	0.42 ± 0.06^{b}	0.02	0		
6	0.26 ± 0.10^{b}	0.74 ± 0.07^{aA}	0.45 ± 0.06^{b}	0.04	0.00		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

Table 5. Average of total volatile nitrogen (mg/100 g) in the examined Kofta samples.

Time (week)	Groups				P values		
	Control	Kofta (F-T)	Kofta + Ginger (F-T)	SEM	Treatment	Week	T*W
1	9.27 ± 0.15^{B}	9.87 ± 0.78^{C}	9.73 ± 1.18^{B}	0.44	0.64	0	0
3	9.81 ± 1.15^{aA}	16.27 ± 0.55^{aB}	11.39 ± 0.96^{bB}	0.51	0.00		
6	10.60 ± 1.01^{bA}	20.93 ± 0.21^{aA}	14.40 ± 0.89^{bA}	0.41	0		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

Histological examination

The microscopic observations of the kofta samples in the control group (Fig. 2A, B, and C) showed distinct striations in the skeletal muscles till the 3rd week. These striations were not observed in the 6th week. In the freeze–thaw (Fig. 2D, E, and F) and freeze–thaw + ginger groups (Fig. 2G, H, and I), the muscle striations were lost in the 3rd week.

The muscle fiber diameters of the control group samples were 0.66±0.04, 0.49±0.07, and 0.23±0.04 in the 1st, 3rd, and 6th weeks, respectively, while in the freeze–thaw group, they were 0.52±0.14, 0.4±0.07, and 0.26±0.07, respectively. However, in the freeze–thaw + ginger group, they were 0.53±0.10, 0.38±0.03, and 0.27±0.03 in the 1st, 3rd, and 6th weeks, respectively. Hence, repeated freeze-thaw cycles significantly ($P < 0.05$) decreased the muscle fiber diameter in the 3rd and 6th weeks in all groups (Table 6).

Properties of sausages

TBA evaluation

The TBA levels in the control group were 0.25±0.02, 0.25±0.02, and 0.26±0.03 mg MDA/kg in the 1st, 3rd, and 6th weeks, respectively. In the freeze–thaw group, they were 0.27±0.04, 0.51±0.18, and 1.13±0.22 mg MDA/kg, respectively, while in the freeze–thaw + ginger group, they were 0.24±0.05, 0.37±0.04, and 0.44±0.09 mg MDA/kg in the 1st, 3rd, and 6th weeks, respectively. The TBA results are shown in (Table 7). Adding ginger before the freeze–thaw cycles significantly ($P < 0.05$) lowered lipid oxidation.

TVB-N evaluation

The TVBN values in the control group were 9.70±0.26, 9.80±0.66, and 10.23±1.01 mg/100 g in the 1st, 3rd, and 6th weeks, respectively, while those in the freeze–thaw group were 10.07±0.25, 14.63±0.50, and 17.80±0.26 mg/100 g, respectively. However, in the freeze–thaw + ginger group, they were 9.60±0.44, 13.10±0.71, and 16.73±0.97 mg/100 g in the 1st, 3rd, and 6th weeks, respectively. Adding ginger to the sausage samples before the freeze–thaw cycles significantly ($P < 0.05$) decreased the TVB-N content (Table 8).

Histological examination

Microscopically, the sausage samples showed distinct muscular striations in the first week in all the groups. However, these striations were lost from the 3rd week onwards (Fig. 3A–I). The muscle fiber diameters in the control group were 0.42±0.07, 0.35±0.06, and 0.28±0.03 in the 1st, 3rd, and 6th weeks, respective-

Table 6. Measurements of muscle fibers diameters in kofta samples at first, third, and sixth weeks of freeze thaw cycles.

Time (week)	Groups			SEM	P values		
	Control	Kofta (F-T)	Kofta + Ginger (F-T)		Treatment	Week	T*W
1	$0.66±0.04^{A}$	$0.52±0.14^{A}$	$0.53±0.10^{A}$	0.05	0.32	0	0.13
3	$0.49±0.07^{B}$	$0.4±0.07^{AB}$	$0.38±0.03^{B}$	0.03	0.12		
6	$0.23±0.04^{C}$	$0.26±0.07^{B}$	$0.27±0.03^{B}$	0.03	0.59		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

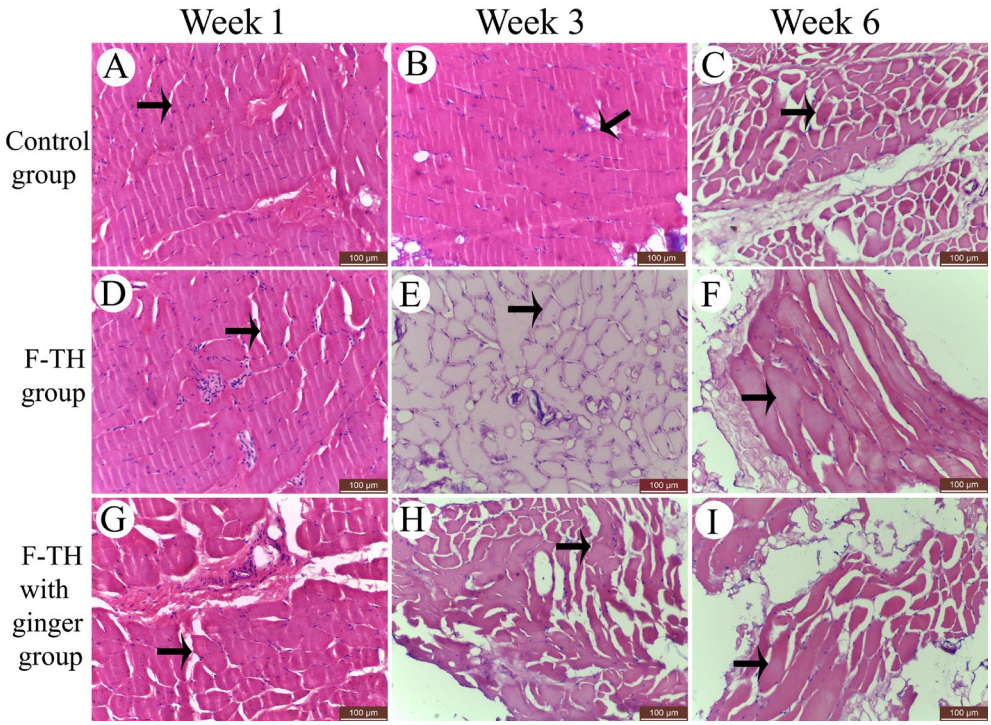

Fig. 2. Effect of freeze–thaw cycles on koftas. The control group (A, B, and C) were frozen for 6 weeks without thawing. The freeze–thaw group (F–TH) were exposed to weekly freeze–thaw cycles (D, E, and F). The third group (F–TH with ginger) were exposed to weekly freeze–thaw cycles after adding ginger to the samples (G, H, and I). The arrows show the longitudinal and cross sections of the skeletal muscle fibers with peripheral elongated nuclei that were striated in the first and third week (for the control) but lost their striations in the third and sixth week of the freeze–thaw cycle for the other groups.

ly. For the freeze-thaw group, they were 0.49±0.10, 0.29±0.04, and 0.22±0.03, and for the freeze–thaw + ginger group, they were 0.49, 0.31, and 0.28 in the 1st, 3rd, and 6th weeks, respectively. Significant (P < 0.05) decrease in the muscle fiber diameter was noticed in all the groups (Table 9).

DISCUSSION

Lipid oxidation occurs due to the reaction of polyunsaturated fatty acids with reactive oxygen species, resulting in rancidity (Amaral *et al.*, 2018). Results from this study indicated that repeated freeze–thaw cycles significantly increased lipid oxidation and protein decomposition. This was consistent with Ali *et al.* (2016), who showed that six freeze-thaw cycles altered the meat color, decreased the myowater, and increased lipid oxidation in chicken meat. Lipid oxidation decreases the quality and shelf life of meat, thus making it less acceptable to consumers. Therefore, it is imperative to reduce lipid oxidation during meat storage (Pérez-Palacios and Estévez, 2020). Multiple freeze–thaw cycles cause protein oxidation that is accompanied by reduced sulfhydryl content and increased carbonyl groups (Hu and Xie, 2021). This has been previously seen in cuttlefish (Lv and Xie, 2021), rabbit meat (Wang *et al.*, 2021b), and pork (Tippala *et al.*, 2021). TVB-N is a biomarker of meat freshness through evaluation of protein and amine degradation (Bekhit *et al.*, 2021).

Several novel methods have been used to mitigate the effects of repeated freeze–thaw cycles, such as vitamin E supplementa-

Table 7. Average of thiobarbituric acid number (mg malondialdehyde /kg) in the examined Sausage samples.

Time (week)	Groups			SEM	P values		
	Control	Sausage (F-T)	Sausage + Ginger (F-T)		Treatment	Week	T*W
1	0.25±0.02^{C}	0.27±0.04^{B}	0.24±0.05	0.02	0.66	0	0
3	0.25±0.02^{B}	0.51±0.18^{B}	0.37±0.04	0.05	0.21		
6	0.26±0.03^{bA}	1.13±0.22^{aA}	0.44±0.09^{b}	0.06	<0.001		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

Table 8. Average of total volatile nitrogen (mg/100 g) in the examined Sausage samples.

Time (week)	Groups			SEM	P values		
	Control	Sausage (F-T)	Sausage + Ginger (F-T)		Treatment	Week	T*W
1	9.70±0.26^{B}	10.07±0.25^{C}	9.60±0.44^{C}	0.18	0.26	0	0.01
3	9.80±0.66^{A}	14.63±0.50^{B}	13.10±0.71^{B}	0.39	0.12		
6	10.23±1.01^{bA}	17.80±0.26^{aA}	16.73±0.97^{abA}	0.43	0.02		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

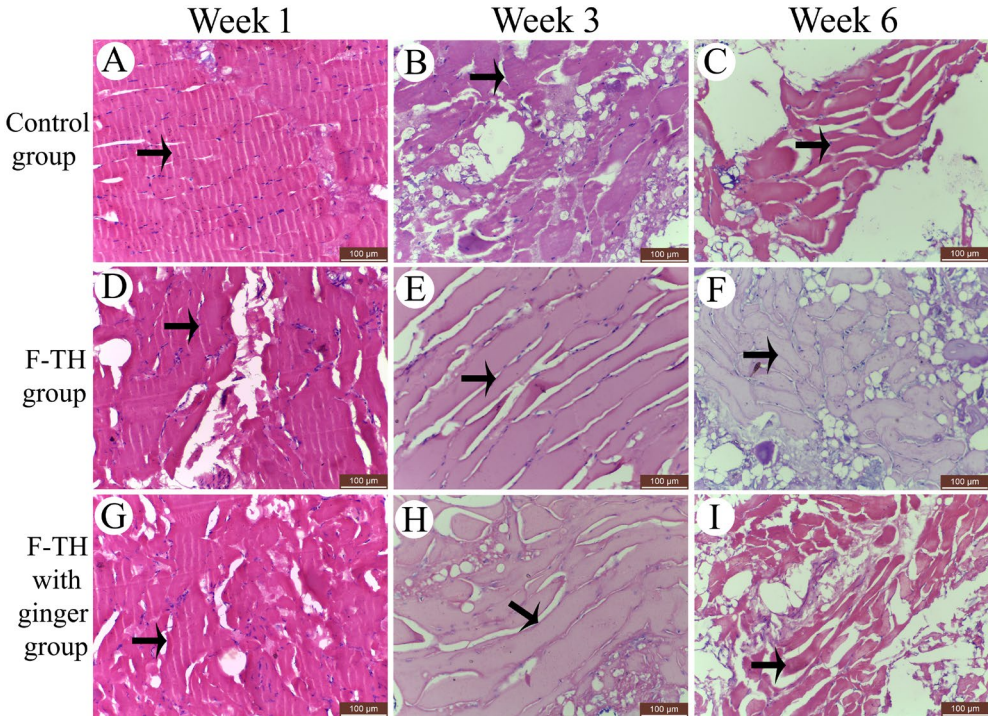

Fig. 3. Effect of freeze–thaw cycles on sausages. The control group (A, B, and C) were frozen for 6 weeks without thawing. The freeze–thaw group (F–TH) were exposed to weekly freeze–thaw cycles (D, E, and F). The third group (F–TH with ginger) was exposed to weekly freeze–thaw cycles after adding ginger to the samples (G, H, and I). The arrows show the longitudinal and cross sections of the skeletal muscle fibers with peripheral elongated nuclei that were striated on the first week but lost their striations on the third and sixth week of the freeze–thaw cycle.

Table 9. Measurements of muscle fibers diameters in sausage samples at first, third, and sixth weeks of freeze thaw cycles.

Time (week)	Groups			SEM	P values		
	Control	Sausage (F-T)	Sausage + Ginger (F-T)		Treatment	Week	T*W
1	0.42±0.07^{A}	0.49±0.10^{A}	0.49±0.10^{A}	0.05	0.59	0	0.39
3	0.35±0.06^{AB}	0.29±0.04^{B}	0.31±0.07^{B}	0.03	0.42		
6	0.28±0.03^{B}	0.22±0.03^{B}	0.28±0.06^{B}	0.02	0.22		

Significant differences between groups are shown by distinct superscript small letters within rows, whereas significant differences between sampling times (weeks) are indicated by different superscript capital letters within columns. The values used in tables are presented as Mean±SD.

tion, modified atmospheric packaging, antifreeze protein inclusion, and brine injection (Leygonie *et al*., 2012; Amaral *et al*., 2018). Ice structuring proteins (ISP) have been used as food preservatives to enhance meat quality after freeze–thaw cycles depending on their ability to inhibit ice recrystallization (Hassas-Roudsari and Goff, 2012). When preserved pork patties containing 0.2% ISP were subjected to five freeze–thaw cycles, their hardness, and springiness improved. Moreover, ISP also decreased water dripping, carbonyl content, and TBA reactive substance levels (Wang *et al*., 2021a). However, pork patties frozen without ISP become unacceptable after the third freeze-thaw cycle due to increased lipid and protein oxidation (Pan *et al*., 2021). Further, when lysine/sodium bicarbonate was used for freezing shrimp to prevent the damaging effect of repeated freeze–thaw cycles, it decreased the thawing loss, total volatile nitrogen, and oxidation compared to the control group (Wachirasiri *et al*., 2019).

Synthetic antioxidants have detrimental effects on human health. Therefore, alternative natural antioxidants need to be used for meat preservation (Pateiro *et al*., 2018). Natural antioxidants from plants, essential oils, or extracts, are being increasingly studied (Zhang *et al*., 2016; Horbańczuk *et al*., 2019; Pateiro *et al*., 2021; Rathod *et al*., 2021). Natural essential oils are used in the meat industry as a potent natural antioxidant (Pateiro *et al*., 2018; Amiri *et al*., 2019; Ansarian *et al*., 2022). Thyme, green tea, and rosemary extracts have also been used as natural antioxidants to preserve meat products. Green tea is among the best in decreasing lipid oxidation, while rosemary is the most efficient in lowering protein degradation (Hęś and Gramza-Michałowska, 2017). Additionally, thyme and cinnamon oils also displayed antioxidant and antimicrobial effects during the storage of ground meat by extending its shelf life to 6 days (Shaltout and Koura, 2017).

The obtained results indicated the beneficial role of ginger as an antioxidant in lowering lipid oxidation and protein decomposition during meat storage. Consistent with the obtained findings, 5% ginger extract was optimized to improve cooking yield and prevent meat oxidation during storage (Ruitong *et al*., 2010). The ginger essential oil has also been proven to protect large yellow croakers against protein oxidation (Lan *et al*., 2021).

Histological examination results indicated that the muscle striations were lost in the 3rd week of the freeze–thaw cycle, even after adding ginger. Moreover, the shrinkage of muscle fibers also increased after repeated freeze-thaw cycles, despite adding ginger. Another study demonstrated that the space between the muscle fibers increased after the 3rd freeze-thaw cycle. Ultrastructural examination of chicken breast muscle fibers showed shrinkage after repeated freeze–thaw cycles (Ali *et al*., 2022). However, lysine/sodium bicarbonate-treated shrimp displayed swelling of muscle fibers (Wachirasiri *et al*., 2019). Scanning electron microscope results also indicated that repeated freeze–thaw cycles ruptured the endomysia (Wang *et al*., 2021b) and increased the spaces between the muscle fibers (Cheng *et al*., 2019; Wang *et al*., 2021b). Tippala *et al*. (2021) reported that repeated freeze–thaw cycles increased the thickness of the endomysium and perimysium and decreased the muscle fiber diameters. Lipid and protein oxidation exacerbate muscle shrinkage and increase the gap between the muscle fibers (Xia *et al*., 2009; Liu *et al*., 2010).

CONCLUSION

This study confirms the protective role of ginger as an antioxidant against lipid oxidation and protein decomposition by measuring MDA and TVBN contents. Therefore, ginger can be used as a potent antioxidant during food processing to reduce lipid oxidation and protein decomposition during meat products' transportation and storage. It's recommended to lower freezing period of meat before consumption for obtaining better quality meat.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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