



Original Article

Protecting the Quality of Chilled Minced Chicken Thigh Meat Against Oxidation Using Contemporary Preservative-Free Approaches as Opposed to Conventional Methods

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ABSTRACT

The study demonstrated the effect of including the extract of rosemary leaves directly into broiler chicken feed and adding natural extracts to minced chicken thigh meat to prevent their oxidation during chilled storage. The experiment comprised 6 treatments of three replicates each. The first treatment, or control, involved minced meat from birds fed on a diet free of any additives. The second treatment included vitamin E, the third and fourth had different levels of rosemary powder without rosemary extract, while the fifth and sixth treatments involved thigh meat taken from chickens fed on a diet free of any additives, with rosemary leaf extract added later. Adding rosemary leaf, whether in the diet or in extract form to minced chicken thigh meat taken from the control, significantly improved its sensory characteristics, as well as its pH and water-holding capacity. In addition, the rosemary-leaf treatment recorded the lowest values for oxidation indicators, and had significant fluid loss from exudation during and after cooking. The treatment with rosemary leaves added to the feed, whether in powder or extract form, also showed significantly lower oxidation indicators of the meat compared to the control. This is clear evidence of their effect in protecting chilled meat from oxidation by reducing microorganisms and free radical activity.

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Keywords: Rosemary leaf, Chicken thigh meat, Refrigerated storage, Oxidation, Water holding capacity, Malondialdehyde.

1 Introduction

Food processing, involving the washing, drying, cooling, heating, preservation, extraction, concentration, irradiation, and microwaving of food products, has been an important aspect of human life since prehistoric times. These processes have become more complex over time in meeting the expectations of consumers and society, especially in regarding to safety, freshness, and quality concerns. Several natural and chemical additives have been approved as additives for protecting food quality during storage periods ^[1]. However, chemical additives used in food preservation pose health and environmental risks leading consumers to seek alternatives such as natural preservatives that are “generally recognized as safe” (GRAS) ^[2]. The high occurrence of food-borne pathogens makes food a

common disease vector, especially raw meat and its products ^[3]. *Staphylococcus aureus*, a form of bacterium, can spread through human contact during food preparation, processing, and cooking. Good food handling and preservation are crucial since the heat-stable toxins in *S. aureus*- infected meat can cause widespread food poisoning ^[4].

Cleanliness in meat processing is essential as *Enterobacteriaceae* including *Salmonella*, *Escherichia coli*, *Proteus*, and *Klebsiella* are found in meat and poultry products. Meat processing safety indicators include total coliform, *Enterobacteriaceae*, and aerobic plate counts ^[5]. In response to meat quality and safety concerns, many preservation methods have been developed, though no single approach provides comprehensive protection for all kinds of foods. Synthetic preservatives, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone, are routinely utilised to suppress microbial proliferation and prolong the shelf life of meat ^[6]. However, these artificial preservatives have been linked to

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negative health outcomes, such as respiratory conditions including bronchitis and pneumonia, hyperactivity in children, and possible damage to heart cells, particularly in older people^[7], and even cancer. Natural alternatives have grown in popularity as a result of consumers' increased desire for healthier products devoid of traditional chemical preservatives.

Dietary herbs and spices have long been used to improve the sensory aspects of food and prolong its shelf life^[8]. These natural components, derived from tropical plants with antibacterial qualities, include garlic, basil, mint, cumin, and thyme. Extracts of essential oils from several spices, including oregano, rosemary leaf, and *Thymus vulgaris*, contain potent antioxidant and antibacterial properties^[9,10]. Due to the increased interest in safe and effective natural food preservation, spices and herbs are becoming more important as preservatives. The quantity of phenolic compounds they contain affects their antibacterial activities, although several intrinsic and extrinsic parameters must be determined before using them. In addition to their clear role in reducing fat oxidation and the formation of free radicals during storage^[11], a synergistic interaction between natural additives and physical treatments of meat enhances its antibacterial activity at low concentrations and maintains the quality of meat during refrigerated storage^[12,13].

Mediterranean rosemary leaf (*Rosmarinus officinalis*) is a fragrant shrub of the Lamiaceae family^[14]. It is used in flavouring food, and its leaf extract (RE) or oil (RO) possesses antioxidant, anti-inflammatory, and anti-cancer properties^[15,16], while several investigations have highlighted its antifungal, antibacterial, insecticide, and hepatoprotective attributes^[17]. The chemical ingredients of rosmanol, carnosol, carnosic acid, ursolic acid, rosemariquinone, caffeic acid, and rosmaridiphenol in rosemary leaf cause these traits^[18-20]. Research indicates that 90% of RE's antioxidant properties are linked to carnosic acid and carnosol,^[21] and contain flavonoids, diterpenes, steroids, and triterpenes, among other bioactive ingredients, while the antimicrobial properties of RO are mostly due to α -pinene and 1,8-cineole. The different chemical compositions of RE and RO affect their antibacterial and antioxidant properties with climate, genotype, extraction time and method affecting their chemical composition^[22].

The shelf life of lamb meat has been extended by rosemary leaf diets such as distilled leaves^[23] and extracts^[24]. Dietary rosemary leaves inhibit lipid oxidation, flavor deterioration, rancidity, and, to a lesser extent, colour deterioration and microbial spoilage, though the authors found that the levels of meat preservation were directly related to the leaf source and feeding stage. Supplementing ewe diets with distilled leaf extract improves the preservation of their stored meat^[25]. However, the heterogeneity of plant materials limits the effectiveness of adjusting polyphenol levels in rosemary leaf diets^[26].

2 Materials and Methods

2.1 Extract preparation

Rosemary minced leaf samples were obtained from local marketplaces in Ramadi, Iraq. They were pulverized using a laboratory grinder and the uniform powder was extracted using ethanol, following the methodology outlined by^[27]. Aqueous extraction was conducted based on^[28].

2.2 Experimental parameters

1st treatment: minced chicken thigh meat from broilers fed an additive-free diet (control).

2nd treatment: minced chicken thigh meat from broilers fed diet supplemented with 300 mg/kg of vitamin E.

3rd treatment: minced chicken thigh meat from birds fed a diet supplemented with 5g/kg rosemary leaf powder.

4th treatment: minced chicken thigh meat from birds fed a diet supplemented with 10g/kg rosemary leaf powder.

5th treatment: Minced chicken thigh meat from birds, sprayed with 5g/kg of dried aqueous rosemary leaf extract.

6th treatment: Minced chicken thigh meat from birds, sprayed with 5g/kg of dried alcoholic rosemary leaf extract.

The minced meat in the fifth and sixth treatments was from chickens in the control, which had no feed additives.

* The weight of all meat samples was 100 g, with three replicates.

2.3 pH determination

The pH of the meat samples was determined using the procedure described by^[29], which involved adding 100 ml of distilled water to 10g of minced chicken thigh meat, homogenizing the mixture well, and thereafter measuring the pH with a pH meter.

2.4 Water holding capacity

The water-holding capacity of meat was estimated according to the method described by^[30]. It involved homogenizing 50g of the sample with 25 ml distilled water for one minute and centrifuging the mixture for ten minutes. The water-holding capacity of the meat was calculated as a percentage.

2.5 Drip loss percentage

Following the method described by^[31], the drip loss percentage was estimated using 5g of meat placed in polyethene bags and refrigerated at 4 °C for 24 hours. It was then re-weighed after drying and removing the water collected on the surface of the sample using filter paper. The percentage of liquid loss was calculated according to the following equation:

Drip loss % =

$$\frac{\text{Weight of the initial model} - \text{Weight of the final model}}{\text{the initial model weight}} \times 100$$

2.6 Cooking Loss

To determine the percentage of loss during cooking, 5g of the sample was put in polyethene bags and sealed. The bags were then cooked in a water bath at 70 °C for 90 minutes. The liquid was then removed from the bags and stored at -4 °C, and the samples weighed later. The filter paper was used to wipe off the liquid that formed on the screen^[32]. The following method was used for calculating the cooking-loss percentage:

Cooking loss % =

$$\frac{\text{Sample weight before cooking} - \text{Sample weight after cooking}}{\text{Sample weight before cooking}} \times 100$$

2.7 Estimating the level of malondialdehyde (MDA)

Fat oxidation was estimated according to the method described by Al-Azzami et al^[33].

2.8 Estimation of free fatty acids and peroxide values

The percentages of free fatty acids (FFA) and peroxide values (PV) of the minced and cold-stored chicken thigh samples were estimated based on the method mentioned by Egan et al^[34].

3 Results and Discussion

3.1 Effect of rosemary extract on the pH value of chicken meat during refrigerated storage

As seen in Table 1, the pH values in the thighs of minced chicken meat were significant ($p < 0.05$) on the first day of storage, most likely influenced by storage and additive variables. The minimal meaningful value was observed after 14 days of the experiment's duration during the evaluation of storage effects. The addition of rosemary leaf in the sixth treatment demonstrated statistically significant superiority ($p < 0.05$) over all other treatments and the control, while the other treatments had no significant differences among themselves. A comparison of the interaction between the storage and addition factors showed the most significant value ($p < 0.05$) occurring in all treatments on the first day of storage, while the sixth treatment exhibited notable enhancement after seven days of storage.

Table 1: Effect of refrigerated storage and adding rosemary leaves on the pH of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	6.713 a	6.096 f	6.266 e	6.358 b	0.0183
2nd	6.700 a	6.233 e	6.113 f	6.348 b	
3rd	6.720 a	6.316 d	6.020 g	6.352 b	
4th	6.713 a	6.400 c	6.026 g	6.344 b	
5th	6.700 a	6.323 d	5.920 i	6.350 b	
6th	6.720 a	6.450 b	5.963 h	6.377 a	
Average	6.71 a	6.30 b	6.05 c	Significance level of interaction	<.0001
Storage period. The significance level storage period	<.0001			SEM*	0.0395
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations (P≤0.05). Significant coefficient differences (P≤0.05) are indicated by different letters in the average coefficient column. A significant difference (P≤0.05) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

This suggests that the optimal interaction occurred between the treatments of including rosemary leaves directly into the chicken

feed and keeping it for 14 days. This may result from the diminished efficacy of the extract over prolonged storage and the

rationale for sustaining pH values during chilled storage at a temperature of 4 °C to capacity. Rosemary leaf powder retains water, occupying the interstitial spaces between peptide chains with extra water while also facilitating the water's binding with protein^[35]. As seen, the addition of rosemary leaf powder retained the pH value of the meat samples, thereby extending storage duration by inhibiting bacterial proliferation that influences acidity development, which, in turn, helps retain the meat's qualitative and sensory attributes for consumer acceptability^[36].

3.2 Effect of adding rosemary leaves on the water holding capacity of chilled thigh meat

Table 2 presents the findings on the water-holding capacity of minced chicken thigh meat. On the first day, the chill-stored

treatments exhibited a major advantage ($p < 0.05$) when evaluating the storage factor independently, but the water-holding capacity showed the least significant superiority ($p < 0.05$) when stored at 4 °C on the fourteenth day. The direct incorporation of rosemary leaf either in the chicken feed or as an extract in their thigh meat, significantly enhanced ($P < 0.05$) water-holding capacity across all treatments. However, no significant differences were observed when assessing the interaction between this incorporation and chilled storage in any treatment. This is due to the natural antioxidants found in rosemary leaf which improves the qualitative characteristics of the thigh meat, including its ability to hold water by preserving the proteins in the meat that have the property of binding water and protecting it from damage. This results in improving the meat's water-holding capacity^[37].

Table 2: Effect of refrigerated storage and adding rosemary leaves on the water- holding capacity of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	36.6 a	28.1 a	20.4 a	28.4 b	<.0001
2nd	28.2 a	30.1 a	22.3 a	26.8 b	
3rd	44.5 a	36.6 a	27.3 a	36.1 a	
4th	47.7 a	40.0 a	32.2 a	40.0 a	
5th	45.3 a	38.5 a	28.1 a	37.3 a	
6th	48.1 a	41.0 a	33.0 a	40.7 a	
Average	41.7 a	35.7 b	27.2 c	Significance level of interaction	**NS
Storage period. The significance level storage period	<.0001			SEM*	1.257
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations ($P\leq0.05$). Significant coefficient differences ($P\leq0.05$) are indicated by different letters in the average coefficient column. A significant difference ($P\leq0.05$) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

3.3 Effect of adding rosemary leaves on the drip loss percentage of chilled thigh meat

Table 3 presents the percentage of drip loss from minced chicken thigh meat stored at 4 °C. The 14-day storage period exhibited a statistically significant ($p < 0.05$) increase in the percentage of drip loss compared to other treatments, while the lowest significant value was observed on the first day of storage ($P > 0.05$). The control had the highest significant loss value when only rosemary

leaf was added, whereas the fourth treatment recorded the lowest significant value ($p < 0.05$). The interaction between storage and rosemary leaf addition produced a significant increase ($p < 0.05$) in drip loss in the control group after 14 days of storage, while the least significant loss ($p < 0.05$) occurred in the fourth treatment stored for one day, with subsequent progression noted.

For storage duration, the fourth treatment exhibited the lowest drip loss compared to the others. Including rosemary leaf powder

in the chicken feed enhances meat quality and reduces drip loss in chilled-stored minced chicken thigh meat. Rosemary leaves have natural antioxidants, including rosmarinic and carnosic acids, which protect the fatty tissue in the minced chicken thigh meat from oxidation, hence minimizing loss. The drip loss from these tissues, which retains water, enhances the economic

viability of the final product [38]. Additionally, incorporating rosemary leaf into feed bolsters the immune system of broilers, promotes overall avian health, and diminishes antibiotic usage. This enhances the quality attributes of the meat, including reduced drip loss, and its nutritional and health value [39].

Table 3: Effect of refrigerated storage and adding rosemary leaves on drip loss percentage of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	1.133 m	3.133 f	5.023 a	3.096 a	<.0001
2nd	1.030 n	2.883 h	4.870 b	2.927 b	
3rd	0.930 op	2.343 i	3.763 d	2.345 c	
4th	0.853 q	1.766 l	3.040 g	1.886 e	
5th	0.976 o	2.083 j	4.023 c	2.361 c	
6th	0.923 p	1.943 k	3.353 e	2.073 d	
Average	0.974 c	2.358 b	4.012 a	Significance level of interaction	<.0001
Storage period. The significance level storage period	<.0001			SEM*	0.1845
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations ($P\leq0.05$). Significant coefficient differences ($P\leq0.05$) are indicated by different letters in the average coefficient column. A significant difference ($P\leq0.05$) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

3.4 Effect of adding rosemary leaves and refrigerated storage on chicken meat cooking loss

Table 4 presents the impact of cold storage and rosemary leaf addition on cooking loss in the meat samples. A notable decrease in cooking loss is evident on the first day of storage ($p < 0.05$) with the lowest significant value occurring in meat stored for 14 days. The control demonstrated significant superiority ($p < 0.05$) over the other treatments with the addition of rosemary leaf while the fourth treatment had the lowest significant value in cooking loss.

For the interaction between rosemary leaf addition and refrigerated storage, the first treatment of first day storage yielded the highest significant ($p < 0.05$) value while the fourth had the most significant improvement ($p < 0.05$). The findings indicate that optimal interaction between the two factors, rosemary leaf addition and refrigerated storage, occurred in all treatments. Extended storage periods correlated with notable enhancements in this treatment for cooking loss. The active compounds in rosemary leaf may protect the minced meat and mitigate the effects of oxidation on fats and proteins, thereby enhancing its qualitative and sensory characteristics and minimizing losses during cooking [40, 41].

Table 4: Effect of refrigerated storage and adding rosemary leaves on cooking loss percentage of minced chicken thigh meat.

storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	33.7 a	28.3 e	22.3 k	28.1 a	<.0001
2nd	33.2 b	26.7 f	20.1 l	26.7 b	
3rd	32.4 c	24.4 h	18.0 o	24.9 d	
4th	32.0 d	22.7 j	17.3 p	24.0 f	
5th	33.2 b	24.9 g	19.7 m	25.9 c	
6th	32.1 d	23.1 i	19.1 n	24.8 e	
Average	32.8 a	25.0 b	19.4 c	Significance level of interaction	<.0001
Storage period. The significance level storage period	<.0001			SEM*	0.7795
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations ($P\leq0.05$). Significant coefficient differences ($P\leq0.05$) are indicated by different letters in the average coefficient column. A significant difference ($P\leq0.05$) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

3.5 Effect of adding rosemary leaves and chilled storage on malondialdehyde (MDA) in the chicken meat

Table 5 presents the malondialdehyde (MDA) values for minced chicken thigh meat subjected to different storage periods and the incorporation of rosemary leaf. The 14-day refrigerated storage period exhibited significant ($p < 0.05$) superiority in MDA levels compared to the other treatments, with the first day of storage registering the lowest value. When accounting for the impact of adding rosemary leaf, the control exhibited significant superiority ($p < 0.05$) over the other treatments, with the fourth recording the lowest value.

For the interaction between storage and rosemary addition factors, significant enhancement ($p < 0.05$) was observed in the fourth treatment during the initial storage period, with the most substantial improvement ($p < 0.05$) noted in the first treatment after 14 days of refrigerated storage. The optimum values for this interaction occurred for the fourth and sixth treatments. At 14 days' storage, the fourth treatment had significantly higher MDA levels compared to the other treatments. Rosemary leaves tend to lower MDA levels by diminishing the activity of free radicals that impact the proteins and fats in chicken meat [42]. MDA is an indicator of fat oxidation and helps in assessing poultry meat

quality; elevated MDA levels can adversely impact the sensory attributes of the product [43].

3.6 Effect of adding rosemary leaves and chilled storage on peroxide values in chicken meat

Table 6 presents the peroxide values (PV) in minced chicken thigh meat due to storage and rosemary leaf addition factors. A significant increase ($p < 0.05$) in PV was observed after 14 days' storage at 4 °C. The least significant peroxide value occurred at 1-day storage with the addition of rosemary leaf. The control had a significant increment in PV ($p < 0.05$) compared to the other treatments, while the lowest PV was noted in the fourth and sixth treatments.

For the interaction between rosemary leaf addition and refrigerated storage, significant superiority ($p < 0.05$) occurred for the initial treatment after 14 days of storage. Conversely, the lowest significant PV was observed in the fourth and sixth treatments for the same duration, relative to the other treatments. The low PV may be due to the antioxidant compounds in rosemary leaf powder or extracts, which inhibited or prevented lipid peroxide formation in chicken meat [44].

Table 5: Effect of refrigerated storage and adding rosemary leaves on MDA (mg/kg) of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	0.316 k	0.753 f	2.053 a	1.04 a	<.0001
2nd	0.293 k	0.716 g	1.870 b	0.960 b	
3rd	0.226 l	0.553 i	1.243 d	0.674 d	
4th	0.183 m	0.436 j	1.043 e	0.554 f	
5th	0.226 l	0.650 h	1.360 c	0.745 c	
6th	0.193 lm	0.520 i	1.020 e	0.577 e	
Average	0.240 c	0.605 b	1.43 a	Significance level of interaction	<.0001
Storage period. The significance level storage period	<.0001			SEM*	0.0759
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations (P≤0.05). Significant coefficient differences (P≤0.05) are indicated by different letters in the average coefficient column. A significant difference (P≤0.05) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

Table 6: Effect of refrigerated storage and adding rosemary leaves on PV (milliequivalent/kg) of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	1.34 k	2.92 g	4.88 a	3.04 a	<.0001
2nd	1.14 l	2.65 h	4.42 b	2.73 b	
3rd	0.910 o	2.22 i	3.87 c	2.33 c	
4th	0.730 q	1.15 l	3.14 e	1.67 e	
5th	0.983 n	2.11 j	3.46 d	2.18 d	
6th	0.783 p	1.04 m	2.97 f	1.59 f	
Average	0.981 c	2.01 b	3.79 a	Significance level of interaction	<.0001
Storage period. The significance level storage	<.0001			SEM*	0.1781
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations (P≤0.05). Significant coefficient differences (P≤0.05) are indicated by different letters in the average coefficient column. A significant difference (P≤0.05) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

3.7 Effect of adding rosemary leaves on free fatty acids of chicken thigh meat under different cold storage conditions

Table 7 indicates the significant superiority ($p < 0.05$) of the 14-day storage treatment in the percentage of free fatty acids (FFA) compared to the other treatments. Furthermore, the evaluation of treatment effects indicates a significant superiority ($p < 0.05$) over the initial control treatment across all interventions. The lowest significant value for FFAs was observed in the sixth treatment. The interaction between storage factors and additives

demonstrates the fourth and sixth treatments yielding the most significant improvement ($p < 0.05$). Additionally, the highest significant value ($p < 0.05$) was observed in the interaction between the control treatment and the 14-day storage period. The incorporation of rosemary leaf powder or extract effectively alleviated oxidation during storage by decreasing microbial activity and inhibiting the oxidation of fats in the meat, attributable to the enzymes produced by these microbes, as evidenced in the first treatment^[45].

Table 7: Effect of refrigerated storage and adding rosemary leaves on free fatty acids percentage of minced chicken thigh meat.

Storage Time Treatment	Day 1	Day 7	Day 14	Average storage period	Significance level of transactions
1st	1.34 k	2.92 g	4.88 a	3.04 a	<.0001
2nd	1.14 l	2.65 h	4.42 b	2.73 b	
3rd	0.910 o	2.22 i	3.87 c	2.33 c	
4th	0.730 q	1.15 l	3.14 e	1.67 e	
5th	0.983 n	2.11 j	3.46 d	2.18 d	
6th	0.783 p	1.04 m	2.97 f	1.59 f	
Average	0.981 c	2.01 b	3.79 a	Significance level of interaction	<.0001
Storage period. The significance level storage period	<.0001			SEM*	0.1781
*SEM: Standard Error Mean **NS: not a significant. Variations in average storage time (a, b, c) indicate significant variations (P≤0.05). Significant coefficient differences (P≤0.05) are indicated by different letters in the average coefficient column. A significant difference (P≤0.05) exists between the averages of storage and addition factors within the intersection of storage duration columns and transaction rows.					

Conclusion

This study examined the effect on some physical and chemical characteristics of adding rosemary leaves as a natural additive to the diet and compared it with a direct addition to minced chicken thigh meat. Adding rosemary leaves directly to the feed at a 10-gm concentration, in addition to adding a dry alcoholic extract of the leaves, improved the studied qualitative traits.

Authors contribution

A.A. Al-Azzami, Hayder jumaah AL-Kaabi, N. A. Mirzan, Th. T. Mohammed, Hassan M. Alnori, A. A. Yaseen, S. M. Abdulateef, Mohammed A. Al-Bayar and Osama A. Saeed prepared the original text, reviewed it, and made edits. The published version of the manuscript has been read and approved by all authors.

Conflict of interests

The authors declare no conflict of interest in conducting this research.

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