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# *Research article*

**Chitosan coating for extending postharvest quality of tomatoes (***Lycopersicon esculentum* **Mill.) maintained at different storage temperatures**

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**Abstract:** The growing consumer demand for produces without chemical residues has focused efforts on the assessment of innovative natural antimicrobials. In this context, chitosan, derived from abundantly available chitin sources such as crab, shrimp and insects, has been reported to possess an excellent film-forming ability and inherent antimicrobial properties suitable for development of edible antimicrobial films. Thus, the present study was established to study the effect of chitosan coating on extending postharvest quality of fresh tomatoes (*Lycopersicon esculentum* Mill. cv. 'Diamentino') maintained at two different storage temperatures (5 °C with 90% relative humidity and 21 °C with 65% relative humidity). Coating the tomatoes with chitosan solutions reduced the weight loss, with greater effect at 1% than 0.5 or 2% concentrations. Chitosan-coated tomatoes were firmer, higher in titratable acidity, and exhibited less biochemical changes than the control fruit at the end of storage. The loss in visual quality was significantly reduced by coating the fruits with chitosan solutions of 0.5, 1.0 and 2.0% as compared to the control. Among the applied concentrations, chitosan at 1% can be recommended as it was pioneering for most of the parameters analyzed during cold storage at both 5 °C for 20 d and at 21 °C for 10 d. Due to its lower cost and convenience to human health, chitosan may be one of the attractive and effective biopolymers for achieving adequate conservation of fresh tomatoes.

**Keywords:** chitosan; edible coating; *Lycopersicon esculentum* Mill.; storage

#### **1. Introduction**

Tomatoes are frequently consumed as they represent the predominant source of antioxidants which possess pivotal role in inhibiting oxidative stress, improving vascular function, and preventing cardiovascular disease in humans [1]. Carotenoids (lycopene, β-carotene, and lutein) and flavonoids in tomatoes have been confirmed as essential polyphenols in conferring antioxidant benefits [2,3]. However, relatively short shelf life of tomatoes limits the long distance commercial transport and availability of this produce around the year. As stated by Benhabiles et al. [4], postharvest losses of tomatoes may drastically reach up to 50% of total production in countries where harvest amount peaks in short period.

The prevalent method of maintaining postharvest quality of horticultural commodities is the use of moderately low temperatures around  $0-1$  °C. For certain horticultural produces such as tomatoes, however, low temperatures induce chilling injury [5]. Therefore, such produces are inevitably stored at higher temperatures which, on the other hand, accelerates the senescence and postharvest quality loss. General results of previous studies revealed that optimum temperatures for storage of red and mature green tomatoes are 5 °C [6] and 13 °C [7], respectively. Besides low temperature, packaging materials and edible coatings provide a means to protect and distribute foods. They play a significant role in how these products reach the consumers in a safe and wholesome form without compromising quality. There is a worldwide trend to explore innovative alternatives that control postharvest quality loses, giving priority to methods that reduce decay incidence and avoid side effects on human health resulting from excessive application of synthetic fungicides. Recent studies focused on biodegradable feature of natural compounds derived from plants and animals. Chitosan, as high molecular polymer, nontoxic, bioactive agent, has become a useful appreciated compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissues [8]. Chitosan-based edible coating has been studied for efficacy in inhibiting decay and extending shelf life of perishable produces such as strawberry [9], cucumber [10], plum [11], peach [12] and fresh cut melon [13]. A chitosan coating retarded the decrease in ascorbic acid content of sweet cherry [14]) and strawberry fruits [9] during cold storage. Previous studies also have shown that chitosan reduces decay incidence, mainly caused by *Botrytis cinerea* in tomato fruit [15], and is effective for controlling P. *expansum* in apple fruit during storage [16]. These reports indicate that chitosan offers a great potential as a biodegradable substance that has anti-microbial and eliciting activities.

The objective of the present study was to evaluate the effect of different concentrations of chitosan coatings (0.5%, 1.0% and 2.0%) and storage temperatures (21 and 5 °C) on extending the postharvest quality attributes of tomatoes (*Lycopersicon esculentum* Mill.) during storage.

#### **2. Materials and method**

#### *2.2. Plant material and postharvest treatments*

Tomatoes (*Lycopersicon esculentum* Mill.) cv. 'Diamentino' were harvested from commercial field in Cumra, Turkey at light red stage using the tomato ripeness color classification chart of United States Department of Agriculture [17] and immediately transferred to the laboratory of the Department of Horticulture at Selcuk University. The tomatoes at a mean  $50.17 \pm 1.8$  Hue angle value were selected according to their uniformity in color, size and absence of damages. They were

randomly divided into four equal groups, in which three were assigned to different concentrations of chitosan (0.5%, 1.0% and 2.0%) treatments while the fourth group was non-treated control. Each group further divided into two lots for different storage temperatures (5 and 21 °C). Sixty tomatoes per treatment were used considering four analysis dates with three replications consisted five tomatoes each.

The chitosan solutions were prepared by dissolving 5.0, 10.0 and 20.0 g chitosan [low molecular weight (50.000–190.000 Da), 75–85% deacetylated and viscosity 20–300 cP, 1 wt. % in 1% acetic acid (25 °C, Brookfield) Sigma-Aldrich] in 1000 ml distilled water containing 10 ml (v/v) acetic acid [18]. Fruits of three of the groups were dipped into different concentrations of chitosan for 5 min while control fruits immersed into distilled water contain 10 ml acetic acid for same duration. After treatments, fruits were dried for 2 hours at room temperature (22 °C). Treated and untreated fruits were stored at  $21 \pm 1$  °C (ambient temperature with 65% relative humidity) for 10 d or 5 °C (cold storage with 90% relative humidity) for 20 d in open boxes. Fruit quality attributes was evaluated after 0, 3, 5, 7 or 10 d at ambient temperature and 0, 5, 10, 15 or 20 d at cold storage by measuring weight loss, fruit firmness, ascorbic acid, total phenol, antioxidant capacity (FRAP), lycopene content and visual quality (9-1 scale).

# *2.2. Determination of postharvest fruit quality changes*

Weight loss was determined as percent loss from initial weight. Five fruits in each replication for each treatment were weighed before storage and at each analysis date. Fruit firmness was evaluated by using digital penetrometer (Fruit pressure tester, model 53205; TR, Forlì, Italy) with 8 mm probe on three different regions of samples and expressed as Newton (N). Visual quality was assessed as described by Azadanlou [19]. Briefly, semi-trained panelists evaluated fruit quality feature such as firmness, color, juiciness and overall visual appreciation using a 1–9 hedonic scale  $(1 =$  extremely bad, unusable;  $3 =$  unsalable;  $5 =$  fair;  $7 =$  good;  $9 =$  extremely good). The number of fruits receiving a rating of 5 and above was evaluated as marketable fruits.

### *2.3. Determination of total lycopene*

Total lycopene was determined as previously described by Sharma and Maguer [20] and Rao et al. [21] with slight modifications. For lycopene analysis, pericarp tissue of tomatoes was blended with a warring blender for 1 min. One gram homogeneous tissue and 50 mL hexane: ethanol: acetone (2:1:1, v/v) mixture were shaken in a 100-mL flask wrapped with aluminum foil on an orbital shaker at 150 rpm for 30 min. After shaking, 10 mL of distilled water were added and shaken for 5 min again. The solution was then placed to a separatory funnel and after phase separation, the lower phase was discarded. Extract was filtered via Whatman 42 (Sigma-Aldrich Co., St. Louis, MO) and lycopene concent was determined by measuring the absorbance of solution in UV-vis spectrophotometer (U-5100, Hitachi, Tokyo, Japan) at 503 nm against hexane:ethanol:acetone blank. Results were expressed as mg  $kg^{-1}$  fresh weight.

### *2.4. Determination of ascorbic acid*

Tomatoes were ground with a warring blender and 5 g sample was mixed with 45 mL 0.4% oxalic

### *2.5. Extraction and determination of total phenol and antioxidant activity*

Fruit extracts for total phenol and antioxidant activity were prepared using method described by Thaipong et al. [23], with some modifications. Five grams tomato tissue was homogenized in methanol using the Ultra-Turrax homogenizer (IKA, T18 digital, Staufen, Germany) for 5 min. The homogenates were kept at 4 °C for 14–16 h and then centrifuged at 8000 x g for 15 min at 5 °C. The supernatants were recovered and stored at  $-20$  °C in dark color bottles until analysis.

Total phenols were determined according to the method of Singleton et al. [24] with slight modifications. The 0.1 mL extract, 6.0 ml distilled water and 0.5 ml Folin-Coiocalteu were mixed and then were vortexed. The mixture were incubate 3 min and then 1.5 ml 20% sodium carbonate solution supplemented and volume was made up 10 ml distilled water. The solution was incubated at 25 °C for 2 h and the absorbance was measured at 760 nm. The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as mg gallic acid 100  $g^{-1}$  FW.

Antioxidant activity was determined by ferric reducing ability antioxidant power (FRAP) according to the procedure described by Benzie and Strain [25]. 150 µL of extract and 2.85 mL of the FRAP reagent [0.3 M acetate buffer (pH 3.6) containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 40 mM FeCl<sub>3</sub>.6H<sub>2</sub>O] was incubated at 30  $^{\circ}$ C for 30 min. after incubation, reaction mixture was measured at 593 nm on a UV-vis spectrophotometer. Standard curve was prepared using different concentrations of 1 mM trolox and expressed as  $\mu$ mol kg<sup>-1</sup>.

### *2.6. Statistical analysis*

The experiment was carried out in a completely randomized design with three replications. For each storage temperature, data from analyzed parameters were subjected to analysis of variance separately. Sources of variation were treatment, storage time and their interaction. Means were compared by Student's t-test at  $P \le 0.05$ , using JMP statistical software version 5.1 (SAS Institute Inc., Cary, NC, USA).

# **3. Results and discussion**

#### *3.1. Weight loss*

As illustrated in Figure 1A, weight loss progressively increased during the cold storage of tomatoes and the magnitude of such increment was more pronounced after 10th d. In cold stored products, treatment effects were quite little up to 5th d, although significant effects of the treatment become apparent later. After 5 d storage at 5 °C, all the treatments significantly delayed the loss in weight with varying degrees with a persistent maximum effects observed in 1.0% chitosan coating during the prolonged cold storage. At the end of the storage, the greatest loss in weight occurred in non-treated control tomatoes (7.4%), while the lowest value was obtained from 1.0% chitosan (3.7%). As for the tomatoes stored at 21 °C (Figure 1B), similar weight loss course was seen with that of the cold storage findings. Accordingly, all the treatments significantly restricted the loss in weight during the storage at 21 °C, with more pronounced effect following 5th d. Among them, chitosan coating at 1.0% resulted in the lowest loss in weight with the value 4.5%, which was followed by 0.5% chitosan (5.2%). On the other hand, the weight loss in control tomatoes was as high as 9.3%, resulting from a progressive increment in moisture loss from produces along with the storage at 21 °C. The weight loss is known to be the major determinant of storage life and quality of fresh commodities [26]. The slower rate of moisture loss from the chitosan coated tomatoes in both of two storage temperatures may be attributed to the additional barrier against diffusion through stomata as previously indicated by Paull and Chen [27]. It is evident from the present and previous studies that coating tomatoes with chitosan reduced the loss in weight compared with the control fruit, probably as a result of covering the cuticles with chitosan on the fruit surfaces. These findings are in well concordance with those of Pérez-Gago et al. [28], where effectiveness of covering with a plastic film or coating on water loss was emphasized. Further, El-Eleryan [29] demonstrated that dipping Washington Navel orange fruits in chitosan alone was markedly effective in decreasing weight loss percentage. The mentioned researchers indicated that the chitosan formed a film on the fruit skin, reducing the weight loss.



Figure 1. Effects of chitosan coating on weight loss (%) of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each bar represents the mean of three replicates of 5 fruits each. Vertical bars represent the standard deviation of that mean.

### *3.2. Firmness*

Changes in firmness of the tomatoes during the cold storage at 5 °C is shown in Figure 2A. The lowest firmness values were always determined in control while treatments significantly maintained the fruit firmness along with the 20 d storage duration. The greatest diminish in firmness was observed at 20th d with the lowest value of 6.3 N for control, while the highest value was obtained from 1.0% chitosan coating (12.6 N). At 15th d, the firmness value of tomatoes subjected to 1.0% chitosan was almost the same as that of its 20th d value, indicating its good protective effect. Chitosan coatings at 0.5% (9.0 N) and 2.0% (8.8 N) concentrations had also significantly positive effects on firmness in comparison with the control (6.3 N). Firmness of the tomatoes also underwent a significant decrease during their storage at 21 °C (Figure 2B). A sharp decrease in firmness of tomatoes occurred just before 3rd d of storage at 21 °C and the effects of chitosan treatments at lower doses were insignificant, while its higher dose (2.0%) was significantly higher. Afterwards, control fruits displayed significant decreases during the storage at 21 °C up to 10 d, reaching the lowest value of 6.5 N. After 10 d storage, all doses of chitosan had significantly positive effects compared with control and the highest firmness value was obtained from 1.0% chitosan, followed by its 0.5 and 2.0% doses with similar effects. Firmness is a major attribute that dictates the postharvest life and quality of tomatoes [30]. In the current study, some loss of firmness was observed during the storage of tomatoes, most probably due to the action of endogenous enzymes linked to cell wall degradation [31], since no microbial growth was observed during storage at 5 or 21 °C (data not shown). The retention of firmness with chitosan coating in the present study is in agreement with the results of Benhabiles et al. [4], where tomatoes treated with chitosan coating were firmer than the control during 29 days storage at ambient temperature. Mango fruits have also been reported to be firmer when coated with chitosan [32]. The control fruit lost their textural integrity faster than the higher concentration coatings, particularly 1% which largely maintained the fruit appearance and quality until the end of storage. When the effects of treatments on weight loss and firmness are considered together, it is clear that moisture loss is the main cause of firmness change because the change course of fruit firmness is just opposite of that of weight loss as already revealed by Paniagua et al. [33].



Figure 2. Effects of chitosan coating on firmness (N) of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each data point represents the mean of three replicate samples. Vertical bars represent the standard deviation of that mean.

### *3.3. Visual quality*

As can be seen in Figure 3A, visual quality of tomatoes determined with 1–9 scale, in cold storage at 5 °C displayed gradual decrease during the storage. Changes in visual quality become more apparent after 10 d storage. According to the investigations performed at 20th d, chitosan coatings at 1% concentrations with panelist score 6.5 had significant positive effects on the maintenance of visual quality of the products. The greatest decrease with a statistical significance in

visual quality occurred in control fruits (4.6) that received the lowest panelist score below the acceptability level in markets. Visual qualities of tomatoes during the storage at 21 °C displayed no significant change up to 10th d (Figure 3B). At the end of the storage at 21 °C, the greatest decrease with a statistical significance in visual quality occurred in control fruits that received the lowest panelist score (6.7). Among the chitosan treatments, the highest value was obtained from 1.0% doses (8.2), followed by 2.0% (7.8) and 0.5% (7.7). Similar to our findings, previous studies demonstrated that the external appearance or visual quality of fruits and vegetables is generally improved by chitosan coating. This is most probably due to the fact that anthocyanin degradation on chitosan-treated fruit is generally retarded. Such beneficial impacts have been reported by [34] studying on strawberries and raspberries, although there was certain contradictory knowledge on synthesis of anthocyanins on strawberries treated with chitosan which possibly be associated with cultivar, source of chitosan and doses applied [35].



Figure 3. Effects of chitosan coating on visual quality of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each bar represents the mean of three replicates of 5 fruits each. Vertical bars represent the standard deviation of that mean.

### *3.4. Total lycopene*

Changes in lycopene content of tomatoes during the cold storage at 5 °C were presented in Table 1. Initial lycopene content of tomatoes was 18.96 mg kg<sup>-1</sup> and underwent a remarkable increase due to ripening advancement, with the greatest change in control along with the prolonged storage. Chitosan coatings significantly maintained the postharvest lycopene content of the commodities with almost similar effects between the applied concentrations. At the end of the storage, the greatest lycopene content was found in control fruits  $(32.7 \text{ mg kg}^{-1})$ , while the chitosan coatings had remarkable lower values, ranging from 23.26 (1% dose) to 24.98 mg kg<sup>-1</sup> (0.5%). As can be seen in Table 2, lycopene content of the tomatoes markedly increased along with the storage at 21 °C similar to cold storage findings. But, the effects of the treatments were insignificant though the chitosan coatings slightly delayed the lycopene changes. Overall lycopene content of tomatoes stored at 21 °C was markedly greater than those stored at 5 °C. Such an inducing effect of high temperature storage on lycopene biosynthesis was also determined by Javanmardi and Kuboto [36] studying on variation

of certain biochemical features of tomatoes during storage.

Chitosan concentration	Storage time (days)				
$(\%)$	$\boldsymbol{0}$	5	10	15	20
	Lycopene				
Control	$18.96 \pm 2.64$ <sup>f-i</sup>	$19.66 \pm 0.71$ <sup>d-i</sup>	$22.29 \pm 1.79^{b-e}$	$31.62 \pm 1.76^a$	$32.70 \pm 1.42^a$
0.5		$19.93 \pm 1.27$ <sup>d-i</sup>	$20.20 \pm 0.58$ <sup>d-h</sup>	$22.51 \pm 2.69^{bcd}$	$24.98 \pm 2.59^b$
$1.0\,$		$17.27 \pm 0.43^{\mathrm{i}}$	$18.14 \pm 0.71^{ghi}$	$21.63 \pm 2.06^{\text{c-f}}$	$23.26 \pm 0.51$ <sup>bc</sup>
2.0		$17.45 \pm 0.76^{\rm hi}$	$19.45 \pm 0.18^{e-i}$	$20.94 \pm 1.04^{\text{c-g}}$	$23.72 \pm 1.48^{\rm bc}$
	Ascorbic acid				
Control	$11.26 \pm 1.34^a$	$9.15 \pm 0.88^a$	$6.03 \pm 0.58^a$	$5.28 \pm 0.77^{\rm a}$	$5.98 \pm 1.29^{\rm a}$
0.5		$10.33 \pm 1.14^a$	$10.13 \pm 0.50^a$	$9.17 \pm 0.88^a$	$8.19 \pm 0.40^a$
$1.0\,$		$10.24 \pm 0.20^a$	$9.02 \pm 0.22^{\text{a}}$	$7.99 \pm 0.69^{\rm a}$	$7.85 \pm 0.26^a$
2.0		$10.31\pm0.63^{\textrm{a}}$	$9.99\pm0.98^{\rm a}$	$6.69\pm0.36^{\mathrm{a}}$	$7.44 \pm 1.10^a$
	Total phenol				
Control	$41.85 \pm 2.67$ <sup>f</sup>	$47.04 \pm 1.61^{\text{de}}$	$47.65 \pm 2.78^{\text{cde}}$	$52.10 \pm 0.77$ <sup>c</sup>	$68.27 \pm 1.30^a$
0.5		$45.31 \pm 1.67^{\text{def}}$	$44.32 \pm 0.57$ <sup>ef</sup>	$47.41 \pm 2.25^{\text{cde}}$	$59.01 \pm 1.30^b$
$1.0\,$		$47.53 \pm 5.69^{\text{cde}}$	$47.28 \pm 5.67$ <sup>de</sup>	$46.05 \pm 2.73$ <sup>def</sup>	$59.14 \pm 0.77^b$
2.0		$43.21 \pm 1.19$ <sup>ef</sup>	$36.42 \pm 3.78$ <sup>g</sup>	$49.88 \pm 3.19^{cd}$	$60.74 \pm 3.87^b$
	Antioxidant activity				
Control	$0.83 \pm 0.11^{\rm i}$	$1.70 \pm 0.34$ <sup>hi</sup>	$3.45\pm0.84^{\text{def}}$	$4.16 \pm 1.20^{bcd}$	$6.64 \pm 0.44^a$
0.5		$1.48 \pm 0.21$ <sup>hi</sup>	$2.85 \pm 1.02^{\text{fg}}$	$3.76 \pm 0.38$ <sup>cde</sup>	$4.65 \pm 0.20^b$
$1.0\,$		$1.13 \pm 0.72$ <sup>hi</sup>	$2.70 \pm 0.12^{\text{fg}}$	$3.29 \pm 0.91^{\text{def}}$	$4.07 \pm 0.49^{bcd}$
2.0		$2.00\pm0.49^{\text{gh}}$	$2.91 \pm 0.32$ <sup>ef</sup>	$3.93\pm0.09^{bcd}$	$4.54 \pm 0.14^{\rm bc}$

**Table 1.** Effects of chitosan coating on lycopene (mg  $kg^{-1}$ ), ascorbic acid (mg 100 g<sup>-1</sup>), total phenol (mg 100 g<sup>-1</sup>) and antioxidant activity (µmol kg<sup>-1</sup>) of light red tomato during cold storage  $(5 \degree C)$ .

\*Note: For each quality feature, the values significantly different at  $P \le 0.05$  are indicated by different letters according to Student's t-test.

# *3.5. Ascorbic acid*

In contrast to lycopene, ascorbic acid underwent a constant decrease during the cold storage period (Table 1). But the differences between the treatments were statistically insignificant although the lowest and the highest ascorbic acidcontents were always determined in control and 0.5% chitosan treatment. Ascorbic acid decreased with significant differences in response the chitosan doses along with the prolonged storage time at 21 °C. The greatest decrease, from 11.26 to 7.59, was determined in control fruits, while the lowest change was found in the tomatoes treated with 1.0% chitosan (Table 2). Previous studies revealed that the coating with chitosan inhibited ascorbic acid synthesis in strawberries and promotes vitamin C synthesis in cherries [37]. A 0.5% chitosan coating delayed the changes in ascorbic acid content of three sweet cherry cultivars [14] and strawberries [38]. It has been also reported that ascorbic acid content decreased during storage particularly in coated with chitosan carrot sticks [39].

#### *3.6. Total phenol and antioxidant activity*

Total phenol and antioxidant activity of the tomatoes increased gradually during the cold storage (Table 1). There were significant differences between the treatments for all the sampling dates regarding total phenol and antioxidant activity. At the end of storage period, the highest values for both parameters were found in control fruits. Chitosan coating markedly delayed phenol changes regardless of application dose. Treatments also restricted the increase in antioxidant activity with a maximum effect of 1.0% concentration. Total phenol content of the tomatoes slightly increased during storage at 21 °C. Prolonged storage also led to gradual increases in antioxidant activity with significant differences resulting from the treatments. From the beginning of the storage to the final date, chitosan coatings help to maintain the antioxidant activity with the greatest effect of 1.0% dose. Antioxidative activities of chitosan in food have also been reported in a number of reviews [40,41]. To illustrate, Petriccione et al. [42], evaluated changes in certain biochemical content and antioxidant activity of three strawberry cultivars stored at 2 °C after coating with 1% and 2% chitosan. In accordance with our results, they detected different effect of chitosan doses on antioxidant response of three strawberry cultivars.



**Table 2.** Effects of chitosan coating on lycopene (mg kg<sup>-1</sup>), ascorbic acid (mg 100 g<sup>-1</sup>), total phenol (mg 100 g<sup>-1</sup>) and antioxidant activity ( $\mu$ mol kg<sup>-1</sup>) of light red tomato during ambient temperature storage (21 °C).

\*Note: For each quality feature, the values significantly different at  $P \le 0.05$  are indicated by different letters according to Student's t-test.

# **4. Conclusion**

Overall investigations indicated that coating with chitosan extended the postharvest quality of tomatoes during storage at by reducing weight loss, retaining fruit firmness, maintaining visual quality and delaying the changes in biochemical compounds such as lycopene, ascorbic acid, phenols and antioxidant activity. Among the application doses, chitosan concentration at 1% can be recommended as it was pioneering for most of the parameters analyzed during cold storage at 5 °C for 20 d or at 21 °C for 10 d. Low temperature storage at 5 °C in comparison to 21 °C inhibited weight loss and certain changes regarding ripening process such as lycopene accumulation and loss in fimness. Finally, due to its lower cost and convenience to human health, chitosan may probably be one of the most attractive and effective biopolymer for achieving conservation of fresh tomatoes.

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# **Conflict of interest**

The authors declare no conflicts of interest in this paper.

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