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REGULAR ARTICLE

Effects of edible coatings on quality maintenance of fresh-cut nectarines

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ABSTRACT

The study was conducted to investigate the behavior of three different edible coatings formulations on the overall postharvest quality of ready to eat Orion nectarines under fresh-cut commercial storage conditions. Three different coatings were used: 1.5% (w/v) sodium alginate coating, 2% (w/v) chitosan coating and a solution of 1.5% (w/v) of chitosan and 1% (w/v) sodium alginate. Fresh-cut nectarines variations in color, flesh firmness, total soluble solids content (TSSC), pH, titratable acidity (TA) and polyphenol oxidase (PPO) activity were measured. Moreover microorganism counts of molds and yeasts were analyzed. The results indicated that treatments with alginate inhibited decrease in firmness, titratable acidity and delayed flesh browning. Furthermore alginate inhibited the PPO activity throughout the storage period considered. Chitosan coating reduced microorganism proliferation of molds and yeasts compared to control treatment. Our study recommends that alginate edible coating treatment may be a desirable method to maintain fresh-cut nectarines quality and to improve nectarines postharvest life.

Keywords: Alginate; Chitosan; Orion; Polyphenol oxidase activity

INTRODUCTION

The consumer request of ready to eat vegetables and fruit has increased due to changes of lifestyle based on the demand for hale and healthy foods, together with the busy lifestyle. However, mechanical operations like washing, sorting, peeling and slicing or chopping necessary to produce fresh-cut fruits products, damage fruit soft tissues and consequently limit their postharvest life (Chiabrando and Giacalone, 2013; Oms-Oliu et al., 2010). Then fresh-cut fruits are more delicate than the whole. The main aspects that affect the acceptance or not of the consumer being discoloration of the tissues, flesh browning and texture, dehydration and water losses. For these reasons, fruit processing companies needs the advance of different postharvest practices able to maintaining safety, shelf-life and to preserve the visual and organoleptic fresh-like characteristics of fruits.

A current method to extend and improved the shelf-life of minimally processed fruit and vegetables is the use of edible coatings. Edible coatings makes a semipermeable barrier to O₂, CO₂ and water, with the consequent reduction

of weight losses, respiration rate and enzymatic browning (Correa-Betanzo et al., 2011). The basic constituents of coatings for fruit and vegetables are lipids, proteins and polysaccharides. These coatings are directly applied on the superficial part of minimally processed or whole fruit or vegetables. There is a lot of polymers that have been used as coatings for fruit and vegetables, like sodium alginate, gellan, carboxymethyl cellulose, chitosan and whey and soy proteins (Chiabrando and Giacalone, 2013; Navarro-Tarazaga et al., 2008; Rojas-Grau et al., 2009; Reinoso et al., 2008). Maintenance of quality has been reached using chitosan coating in peaches and nectarines (Chiabrando and Giacalone, 2013; Li and Yu, 2001), pectin coating in melons (Ferrari et al., 2013), sodium alginate in apples and blueberries (Chiabrando and Giacalone, 2015; Maftoonazad et al., 2008; Olivas et al., 2007), hydroxypropylmethyl cellulose and proteins in plums (Navarro-Tarazaga et al. 2008; Reinoso et al., 2008).

Nectarines have high functional and qualitative parameters, but the realization of products based on minimally processed nectarines has limited from their very short shelf-life due to browning of the cut surface and to an

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excessive flesh softening after the process. For this reason, edible coating during storage could be used as a treatment to extend and maintain visual and organoleptic quality of fresh-cut nectarines.

Limited information are accessible on organoleptic changes of fresh-cut nectarines in response to edible coatings treatments, therefore, the objective of this research is to evaluate the effects of three coatings on the overall quality of nectarine slices cv Orion, under fresh-cut commercial storage conditions.

MATERIALS AND METHODS

Fruits

Orion Nectarines (*Prunus persica* (L.) Batsch.) were harvested at commercial maturity, and stored (0°C and 95% relative humidity, RH) before processing. To obtain ready to eat nectarines, eight slices were cut around the stone from each fruit.

Coating solutions

Three coatings solutions were prepared. A 2% (w/v) acid-soluble chitosan (Sigma-Aldrich Co., Steinheim, Germany) solution was equipped by dissolving acid-soluble chitosan in 1% acetic acid aqueous added with 50% glycerol and 0.15% Tween 20 (w/v) according to Duan et al. (2011). The coating was homogenized for 90 s and then stored for 12 hours at room temperature. Slices were then dipped for three minutes in the coating and then dried in air at room temperature for about thirty minutes.

A 1.5% (w/v) sodium alginate (Sigma-Aldrich Co., Steinheim, Germany) coating was prepared dissolving the alginate in deionized water upon moving at 70°C for two hours. Then the solution was cooled to 25°C according to Poverenov et al. (2014). After dipping the slices in alginate coating for two minutes, samples were immersed in 5% aqueous solution of CaCl₂ for three minutes (Sigma-Aldrich Co., Steinheim, Germany) to help gelation by cross-linking of sodium alginate particles. The samples then were dried in air at room temperature for about thirty minutes.

A 1.5% (w/v) chitosan and 1% (w/v) sodium alginate solution was set with 3% chitosan solution and 2% sodium alginate solution at a 1:1 ratio with 25% glycerol and 0.15% Tween 20 (w/v) according to the method of Duan et al. (2011). Slices were dipped in this coating solution for three minutes and then dried in air at room temperature for about thirty minutes.

Control samples were dipped in water and then air dried.

For each coating treatment (alginate, chitosan, alginate+chitosan and control) nine packages were set.

Polyethylene (PE) bags (20 cm x 30 cm size and 39 μ thickness) with 50 cm³ O₂/m²/bar/day and 15 g/m²/bar/day water vapor transmission rate (Sealed, Italy) were used. Each package contained 15 slices (150 g). The packages were sealed (UNIMEC packaging systems, Italy) and stored in darkness in a low temperature storage room for 8 days (4°C, 95% RH). 4°C is the temperature usually used in Italian supply chain, although 0°C was establish to be the best temperature for the shelf-life of minimally processed nectarines (Gorny et al., 1999).

Atmosphere composition

Headspace concentrations of O₂ and CO₂ of the packages were observed at day 3, 6 and 8 during storage and analyzed using a Check- Point gas analyzer (PBI Dansensor, Italy). At each storage time and for each treatment were analyzed three bags. Samples of headspace atmospheres were taken with a syringe through silicone septa positioned to the film. The headspace atmosphere of the bags were determined with a paramagnetic sensor for O₂ concentration and an infrared sensor for CO₂ concentration. The instrument has been calibrated towards atmosphere. Results has expressed as kPa of O₂ and CO₂ inside the packages.

Quality evaluations

Physicochemical quality attributes of nectarines slices were measured at the beginning of the testing (time 0) and at the end (8 days).

TSSC (°Brix), pH and TA (meq/l) were analyzed using juice from five slices blended at high speed in a homogenizer. TSSC was determined by a digital refractometer (Atago refractometer, PR-32, Co., Ltd, Japan) and the concentrations expressed as °Brix. TA and pH were analyzed by the titration of the juice, using 0.1 N NaOH and an automatic titrator (Compact 44-00, Crison Instruments SA, Barcelona, Spain). Three replicates were made (five slices each) for each coating treatment.

Textural measurements were carried out individually on 15 slices for each coating treatment at the beginning of the experiment (time 0) and after 3 and 8 days of storage. Nectarines samples were cooled at about 20° C for 3 hours before the analysis. Bourne (1980) explained that in most fruit and vegetables firmness decrease with the increasing of the temperature. Slices firmness was determined by a penetration test using a Texture Analyzer TaxT2i® (Stable Micro System, UK). Measurements were performed in the equatorial part of the slice, at a crosshead speed of 3 mm/s and with a 3 mm diameter probe (Chiabrando et al., 2009). A 5-Kg load cell has been used for firmness determination and the probe was programmed to penetrate 3 mm into the slice. The maximum penetration force (N), which has

been related to the firmness, was the parameter selected for the statistical analysis of data.

Color of coated slices was measured individually in 15 slices for each coating treatment at the beginning of the experiment and then at day 3, 6 and 8 of storage. Surface color was analyzed with a Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) with the illuminating D75 and an observation angle of 10° and calibrated with a standard white plate. Color was expressed as changes in L* (lightness), h° (hue angle), b* (yellow) and a* (green) coordinates during cold storage.

Browning potential (BP) and polyphenol oxidase (PPO) activity

BP was determined as described by Arias et al. (2008), with some modification at day 3 and 8 of storage. The extract was obtained as follows: 10 g of nectarines was homogenized for 2 min at 13,500 rpm with an Ultra-Turrax T25 (IKAs WERKE, Germany), centrifuged (Centrifuge AVANTIM J-25, Beckman Instruments Inc., Fullerton, CA, USA) for 10 min at 4000 rpm and filtered through Whatman 4 paper (Whatman Intl., UK). The absorbance of the clear juice was measured spectrophotometrically (Hitachi, U-5100, Japan) at 440 nm. Three replicates were made (five slices each) for each coating treatment.

The determination of the PPO activity was performed at day 3 and 8 of cold storage at 4°C as described by Soliva-Fortuny et al. (2001).

Enzyme extraction. 50 g of nectarines was mixed with a buffer solution (1:1) at pH=6.5 contained NaCl 1M (Sigma-Aldrich Chemie, Steinheim, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The sample was blended, homogenized with an Ultra Turrax T25 (IKAs WERKE, Germany) and centrifuged for 30 min at 12000 rpm at 4°C (Centrifuge AVANTIM J-25, Beckman Instruments Inc., U.S.A.). The supernatant was filtered through Whatman 1 paper (Whatman Intl., U.K.) to obtain the enzymatic extract, which was used for the analysis.

PPO activity measurement. PPO activity was analyzed spectrophotometrically with the addition of 3 mL of 0.05 M catechol (Sigma-Aldrich Chemie, Steinheim, Germany) and 75 µL of the enzymatic extract to a 4.5 mL quartz cuvette (1 cm path length). The absorbance (Beckman Du®530 spectrophotometer) were recorded at 400 nm every 5 s up to 3 minutes from the time that the enzyme extract was added to the catechol solution. One unit of PPO activity was defined as a change in absorbance of 0.0010/min*mL of the extract. The initial reaction rate was estimated from the linear portion of the plotted curve.

For each coating treatment, three replicates of samples were made.

Microbiological analysis

To estimate the microbiological efficiency of the coatings, microbiological analyses of yeasts and molds were carried out at the end of cold storage (8 days), as described by the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant and Splittstoesser, 1992). Molds and yeast counts were performed using a chloramphenicol glucose agar (CGA) (ISO 21527, 2008). All the plates were incubated at room temperature for 3-5 days. Data were obtained for each measurement in three replicates for each treatment. Microbiological counts were expressed as colony forming units (CFU) g⁻¹ of sample.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using statistical procedures of the STATISTICA ver. 6.0 (Statsoft Inc., Tulsa, OK, USA) and the means were compared by Tukey's HSP test (honest significant differences). Source of variation was coating treatments. Mean values were considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Atmosphere composition

Gas composition inside the samples bags is an important parameter which reflects the fruits respiration, transpiration and decay grade of samples. Tapia et al. (2008) described that the rate of respiration and transpiration increases by 1–7 times in processing fruits. Changes in O₂ and CO₂ concentrations in the samples packages during the 8 days of storage were showed in (Figs. 1 and 2). Significant ($p < 0.05$) changes in O₂ and CO₂ concentration were detected between coated and control samples during cold storage period. After 3 days of storage, the O₂ concentration was lower ($p < 0.05$) in uncoated samples compared with coated nectarines, but not at the end of storage, where the O₂ values were not significantly different amongst all samples. The O₂ concentration in the headspace of the samples bags was: alginate > alginate+chitosan > chitosan > control. The trend and order was reversed for CO₂ concentration. These results suggest that sodium alginate practices a barrier for the exchanges of gases, isolating the coated product from the environment. Comparable results have been available for coatings based on sodium alginate and chitosan in fresh-cut papaya, mango or apples (Chien et al., 2007; Roja-Grau, 2007; Tapia et al., 2008).

Quality evaluations

Sugar in fruits play an important role with organic acids in the organoleptic properties of fruits, besides having a metabolic function. Considering the TSSC, decreases

in this quality parameters of coated and uncoated slices were observed with storage (Table 1). A corresponding progressive decrease is probably due to the ordinary metabolism of the cells. The decrease was higher in chitosan and sodium alginate samples and lower in control and in alginate+chitosan coated slices. This changes were also reported by Maftoonazad et al. (2008) in peaches coated with alginate and by Valero et al. (2013) in plums.

Organic acid content of the fruits decreases during ripening due to the respiratory metabolism. Therefore, the change in organic acid concentration is a good indicator for observing ripening phases because the higher the metabolic respiration, the higher would be the decay of acidity content. In this work, titratable acidity of nectarine slices declined significantly ($p < 0.05$) during storage period (Table 1). The decay in acidity was fewer in sodium alginate coated samples related to the other samples. In particular, the edible coating with sodium alginate delayed the changes in acidity content probably due to a lower respiration rate. In fact, the tendencies of changes in the acid contents were similar to those of O_2 concentration (Fig.1). The same result was obtained also in plums with edible coating (Valero et al., 2013).

The pH values remained quite stable in the range of 5.06-4.76 in all the treatments, with no significant differences amongst all the treatments (Table 1).

Variations in fruit firmness values have been associated with ripening processes and is one of the most significant quality parameters of fresh-cut postharvest fruit quality

Table 1: Effects of edible coating on overall quality (TSSC, TA and pH) of nectarine slices at the end of storage period (8 days)

Treatment	TSSC (°Brix)	TA (meq/l)	pH
Fruits before the treatment	9.45±0.13	138.05±0.02	4.19±0.01
Control	8.90±0.25 a	98.18±6.32 b	5.01±0.24
Sodium alginate	8.00±0.32 b	114.54±4.52 a	4.76±0.71
Chitosan	7.83±0.57 b	104.40±5.55 b	5.06±0.50
Sodium alginate+chitosan	9.25±0.25 a	105.70±7.45 b	4.93±0.32

TSSC: Total soluble solids content; TA: Titratable acidity. Each value is the mean of three replicate measurements±standard deviation (±SD). Different letters in the same column means significantly different ($p \leq 0.05$). Column without letters means no significant differences

Table 2: Effects of edible coating on texture (N) of nectarine slices during post-harvest storage period

Texture (N)	Days of storage		
	0	3	8
Control	4.63±0.71	6.92±0.91 b	7.42±0.82 a
Sodium alginate	4.63±0.71	7.21±0.97 ab	7.59±0.63 a
Chitosan	4.63±0.71	7.51±0.81 a	6.91±0.59 b
Sodium alginate+chitosan	4.63±0.71	6.91±0.49 b	6.87±0.75 b

Each value is the mean of 15 replicate measurements±standard deviation (±SD). Different letters in the same column means significantly different ($p \leq 0.05$). Column without letters means no significant differences

(Dhall, 2013). Preservation of fruit firmness is important to determine acceptability of minimally processed nectarines and to maintain the shelf-life of the product. Mean comparison revealed that the control slices and coated with 1.5% sodium alginate registered, after 8 days of storage, the maximum firmness values with value of about 7.59 N (Table 2). After 8 days of storage, neither chitosan nor chitosan+alginate coatings preserved the original (time 0) firmness values. Respiration is the main reason for these changes. Decrease in respiration rate reduces ripening and, for this reason, limits the detrimental reduction in fruit firmness according to the results in fresh-cut apples (Rojas-Grau et al., 2007), in strawberry and in papaya pieces (Fan et al., 2009; Narsaiah et al., 2015). Moreover, sodium alginate acts as an obstacle to water transference, delaying dehydration and, consequently, improving the fruit firmness of the coated fruits.

To evaluate color changes during storage of nectarine slices, colorimetric parameters of hue angle (h°) and lightness (L^*) were evaluated and showed on Table 3. H° is a good indicator of color variations during shelf-life of fruit

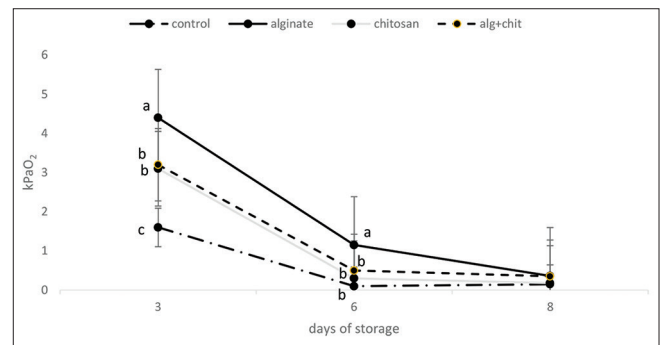


Fig 1. O_2 concentration (kPa $O_2 \pm SD$) inside fresh-cut nectarine slices packages during storage. Each symbol is the mean of three replicate measurements; vertical lines represent standard deviation (±SD). Different letters in the same storage time means significantly different ($p \leq 0.05$).

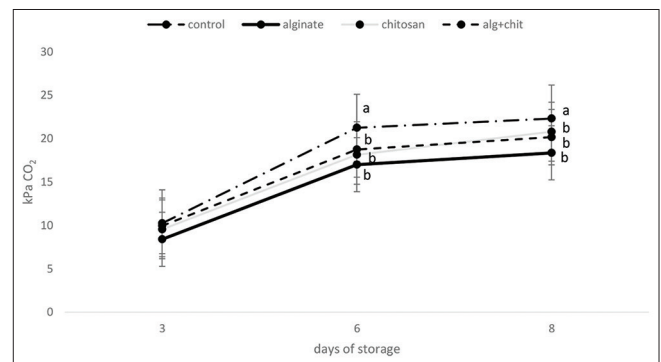


Fig 2. CO_2 concentration (kPa $CO_2 \pm SD$) inside fresh-cut nectarine slices packages during storage. Each symbol is the mean of three replicate measurements; vertical lines represent standard deviation (±SD). Different letters in the same storage time means significantly different ($p \leq 0.05$).

because it incorporates two color parameters (a^* and b^*) (Greer, 2005). During storage period, the decrease in L^* and h° values reflects the increase of tissue browning. In this study, L^* decrease slowly during storage, in particular in chitosan coated slices and control (Table 3). At the end of storage period, samples coated with alginate and alginate + chitosan showed significant ($p > 0.05$) lower L^* values. These lower values can be explained by the film opacity of sodium alginate coating that probably changes the surface reflection properties. In this sense, is probable that alginate film turned opaque during film formation, resulting in lower L^* values.

At harvest, the h° was 97.9 and decrease very slowly during storage. After 8 days of storage, h° remained quite stable in the range of 95.02-93.03, with no significant differences between treatments (Table 3).

Browning potential and polyphenol oxidase activity

Tissues browning of fruit and vegetables decreases visual quality and is often the factor that limit the shelf-life and then the merchantability of minimally processed products. BP increased or remained quite stable over the 8 days of cold storage. The lowest BP values were observed in chitosan samples and the highest in control nectarine slices, after 3 days of storage (Table 4). After 8 days of storage, sodium alginate coated slices showed the lowest browning values related to other nectarines samples.

The primary enzyme responsible of the browning reaction is the polyphenol oxidase. This enzyme catalyze the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to their equivalent o-quinones (Zhou et al., 2016). In the present work, PPO activity of minimally processed nectarines samples with edible coatings decreased throughout storage period (Table 4).

Table 3: Effects of edible coating on colorimetric parameters lightness (L^*) and hue angle (h°) of nectarine slices during post-harvest storage period

	Days of storage			
	0	3	6	8
L^*				
Control	69.97±4.52	70.08±6.43	67.92±5.84	65.51±5.26 a
Sodium alginate	69.97±4.52	63.24±3.89	63.35±2.25	62.67±4.59 b
Chitosan	69.97±4.52	68.47±3.55	67.65±3.32	67.56±4.59 a
Sodium alginate+chitosan	69.97±4.52	65.39±4.76	62.11±3.43	60.06±3.29 b
h°				
Control	97.9±1.69	96.08±2.87	95.85±1.95	95.02±1.87
Sodium alginate	97.9±1.69	97.54±1.85	97.51±2.39	95.78±3.64
Chitosan	97.9±1.69	93.38±1.85	93.16±2.46	93.03±4.59
Sodium alginate+chitosan	97.9±1.69	97.41±2.52	94.81±1.81	94.74±2.30

Each value is the mean of 15 replicate measurements±standard deviation (±SD). Different letters in the same column means significantly different ($p \leq 0.05$). Column without letters means no significant differences

In particular, we noticed that PPO activity values were dissimilar depending on the type of edible coating used, in particular chitosan coated samples showed significant higher PPO activity. Sodium alginate is effective in controlling PPO activity during storage and delaying flesh browning of nectarine slices during storage, according to the results of BP.

Microbiological analyses

Fresh-cut fruit and vegetables are more susceptible to microbial decay if related to whole fruit, due to lesions caused during minimally processing (Rojas-Grau et al., 2009). In addition, due to the high quantity of humidity and organic sugar present on the substrate of the fruit, minimally processed nectarines are a favorable condition for microorganisms to grow. The counts for yeasts and molds after 8 days of cold storage is showed in Table 5. The chitosan coated samples registered lower microbial spoilage than the other samples. On the contrary, in sodium alginate samples the growth of microorganisms was the highest, with values of 3.28 log CFU g^{-1} for yeasts. Microbiological standards (IFST, 1999) for non-thermal minimally processed fruit specified that a count of 6 log CFU g^{-1} of yeast and molds is tolerable at all the points of the supply chain of fruit products. Our microbiological results showed that yeast and molds counts did not exceed these points in any samples. In particular, chitosan edible coating

Table 4: Effects of edible coating on browning potential and polyphenoxidase activity of nectarine slices during post-harvest storage period

	Days of storage	
	3	8
Browning potential (Abs 440 nm)		
Control	1466 a	1330 a
Sodium alginate	0.601 b	0.561 b
Chitosan	0.316 b	1260 a
Sodium alginate+chitosan	0.666 b	1268 a
Relative PPO activity		
Control	0.101 b	0.061 b
Sodium alginate	0.079 b	0.029 b
Chitosan	0.338 a	0.092 a
Sodium alginate+chitosan	0.047 b	0.028 b

Each value is the mean of three replicate measurements±standard deviation (±SD). Different letters in the same column means significantly different ($p \leq 0.05$)

Table 5: Effects of edible coating on microbial growth (log CFU g^{-1} of fruit) of nectarine slices at the end of storage period

	Yeasts (log CFU/g)	Molds (log CFU/g)
Sodium alginate	3.28 a	<1
Sodium alginate+chitosan	2.52 b	<1
Chitosan	<1 c	<1
Control	1.96 b	<1

Different letters in the same column means significantly different ($p \leq 0.05$). Column without letters means no significant differences

minimized the development of yeasts and effectively inhibited the growth of microorganisms during post-harvest period according to Chien et al. (2007) in mango, Gonzalez-Aguilar et al. (2009) in papaya and Hernandez-Munoz et al. (2006) in strawberry. On the contrary, results indicated also that sodium alginate might stimulate the microbial proliferation, because the microbial counts in these samples were even higher than control. This effect was also found in the study of Benitez et al., (2015) on kiwi fruit slices.

CONCLUSION

In conclusion, sodium alginate coating treatment would provide the best compromise to maintain the quality attributes of fresh-cut nectarines by reducing respiration rate, preventing the tissue softening and maintaining the acidity and the TSSC during storage. These coating is also effective in controlling the activity of browning related enzymes compared to the chitosan coating slices and control. The chitosan coating reduced microorganism counts compared to control, resulting in a better maintenance of safety of the fresh-cut products. Although the sodium alginate coating does not minimize the growth of yeasts, the levels are acceptable also after 8 days of cold storage. Considering our results, the sodium alginate treatment represent a possible alternative for postharvest handling of fresh-cut nectarines with the objective to preserve the organoleptic quality of the fruit during storage and to delay the inevitable ripening process.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Authors' contributions

V.C and G.G. have an equal contribution to the paper. Study concept and management: G.G.. Conducted the experiments: V.C.. Analysis and interpretation of data: V.C., G.G. Drafting of manuscript: V.C.. Critical revision: G.G..

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