International Journal of
Food Science & Technology

Original article

Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while not affecting quality attributes

Lucimeire Pilon,[1]* Poliana C. Spricigo,[2] Marcela Miranda,[2] Márcia R. de Moura,[2] Odilio Benedito G. Assis,[2] Luiz Henrique C. Mattoso[2] & Marcos David Ferreira[2]

1 Embrapa Vegetables, Brasilia, DF, 70351-970, Brazil
2 Embrapa Instrumentation, Sao Carlos, SP, 13560-970, Brazil

(Received 14 April 2014; Accepted in revised form 3 June 2014)

Abstract This study addressed the effects of chitosan-based nanoparticles on microbiological quality, colour, polyphenol oxidase (PPO) and peroxidase (POD) and firmness of fresh-cut 'Gala' apple slices during storage at 5 °C for 10 days. The treatments carried out were as follows: (i) slices pulverised with 110-nm chitosan nanoparticles, (ii) slices pulverised with 300-nm chitosan nanoparticles, (iii) 2 g L^{-1} chitosan dissolved in 2% citric acid and (iv) noncoated samples. There was an increase in chroma and a proportional decrease in hue angle and lightness. Browning of the slices coated with conventional chitosan and control was slightly intense than those coated with chitosan nanoparticles of 110 and 300 nm. The PPO and PDO activities increased with time for all samples, with irrelevant difference among the treatments. Flesh firmness did not change for any treatment and period. Coatings with chitosan nanoparticles of 110 nm showed higher antimicrobial activity against moulds and yeasts, and mesophilic and psychrotrophic bacteria than the other treatments. No *Salmonella*, and total and faecal coliforms were detected. This investigation supports the potential use of chitosan nanoparticles as edible coatings in controlling microbial activity in fresh-cut apples.

Keywords Apple, chitosan, edible coating, fruit, mesophiles, moulds, nanoparticles, physicochemical quality, psychrotrophs.

Introduction

In fresh-cut fruit and vegetables, the wounding associated with the processing accelerates the physical and physiological disorders, and also contributes to microorganism proliferation due to cell exudates. For these, edible coatings have been considered as an effective simple technology to maintain the quality and improve the shelf life of processed fruits and vegetables. Edible coatings create a barrier which inhibits microbial growth, reduces respiration and transpiration rates, moisture loss, regulating the transfer of flavour, aroma and acts positively in maintaining colour and texture quality (Kester & Fennema, 1986; Baldwin, 1994; Park, 1999; Krochta, 2002).

In particular, polysaccharide-based coatings have demonstrated to be effective in preserving fresh-cut products. Among them, the chitosan, a polysaccharide obtained from deacetylation of chitin in alkali medium, is an abundant polymeric product widely accepted as feasible to be included in the edible coating formulations (Hirano, 1999). Chitosan is a versatile polymer with good mechanical properties and film-forming ability, selective permeability to gases (Caner *et al*., 1998; Wiles *et al*., 2000), and presents a broad spectrum of antimicrobial activity (Zhang & Quantick, 1998; Romanazzi *et al*., 2002; Qi *et al*., 2004; Chien *et al*., 2007; Du *et al*., 2009; Rodríguez-Núñez *et al*., 2012).

Nevertheless, the characteristics of films and coatings can be improved by incorporating nanostructures into their matrix. Some studies have shown satisfactory results from the use of edible films and coating based on nanoparticles and composites on fruits and vegetables. Medeiros *et al*. (2012) applied nanolayered coatings of κ-carrageenan and lysozyme on 'Rocha' intact and fresh-cut pears reporting the positive effect on gas barrier properties and antimicrobial action, also maintaining the colour and reducing mass loss. Jiang *et al*. (2013) found that alginate/nano-Ag coating had considerable benefits on the physicochemical and physiological quality of shiitake mushroom stored for 16 days at 4 ± 1 °C comparing to the alginate-based coating and the control. Costa *et al*. (2012) showed

*Correspondent: Fax: +55 (61) 3556-5744;
e-mail: lucimeire.pilon@embrapa.br

doi:10.1111/ijfs.12616

© 2014 Institute of Food Science and Technology

reduction in mesophilic and psychrotrophic bacteria, *Pseudomonas* spp. and yeasts by one or two log cycles in fresh-cut carrots when coated with calcium-alginate coating loaded with silver–montmorillonite (Ag-MMT) nanoparticles during cold storage.

When incorporate in a polymer matrix, nanoparticles can enhance the mechanical and barrier properties, and also act as antimicrobial agent as in the case of chitosan, with benefits for quality and safety of food (Belbekhouche *et al*., 2011; Martelli *et al*., 2013). The addition of unmodified and organically modified montmorillonites, nano-silver and silver-zeolite into chitosan-based coatings, also improved mechanical, water vapour barrier, and antimicrobial properties when compared to conventional chitosan films (Rhim *et al*., 2006).

The chitosan most accepted model of antimicrobial action is the electrostatic interaction between the positively charged amino groups with negative residues present on the cell membranes of microorganisms (Tsai & Su, 1999). This interaction is effective in promoting internal osmotic imbalances and thus inhibiting microorganisms' growth (Shahidi *et al*., 1999). Du *et al*. (2009) evaluated antibacterial activities of chitosan by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Escherichia coli*, *Salmonella choleraesuis* and *S. aureus in vitro* and reported that chitosan in nanoparticles format showed much higher activity than dissolved chitosan in Muller–Hinton (MH) broth. Han *et al*. (2010) also demonstrated that chitosan–montmorillonite nanocomposites were significantly more effective against *S. aureus* and *E. coli* than found to isolated chitosan and Na-montmorillonite. Such efficiency is attributed to the reduction in molecular size of the compounds that increases the surface interaction to microbial cells. In this context, nanotechnology is stated as a promising technique for the development of new materials with properties suitably potentialised for the food industry and foodstuffs, including materials for conservation and fresh-cut fruit treatment.

Therefore, we have hypothesised that chitosan nanoparticles would reduce drastically the microorganisms development in fresh-cut 'Gala' apples (*Malus x domestica* Borkh.). In this paper, we show evidence for the first confirming the efficacy of antimicrobial activity of the chitosan-based nanoparticles coating when compared to conventional chitosan continuous coating on fresh-cut 'Gala' apples during cold storage.

Materials and methods

Material

Chitosan (MW = 71.3 kDa, degree of deacetylation 94%) was purchased from Polymar Ciência e Nutrição S/A (Fortaleza, CE, Brazil). Citric acid (Synth Ltd., Diadema, SP, Brazil) was added to dissolve the chitosan. Sodium tripolyphosphate was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid (Synth Ltd., Diadema, SP, Brazil) was the antibrowning agent used. Sodium dichloroisocyanurate dehydrate (Sumaveg®, JohnsonDiversey Brasil Ltda., São Paulo, SP, Brazil) was used to sanitise apples.

Preparation of conventional chitosan gel coating

The gel for conventional chitosan coating (no nanoparticles) was prepared by dissolving 2 g of chitosan in 1 L of 2% citric acid aqueous solution in distilled water with constant stirring for 12 h. The mixture was heated to 40 °C to facilitate dispersion. The solution pH was 4.16.

Preparation of chitosan–tripolyphosphate (CS-TPP) nanoparticles

Chitosan nanoparticles were obtained from two concentrations of citric acid aqueous solution, following procedure described by de Moura *et al*., 2009. For the synthesis of nanoparticles, the citric acid concentrations were 2.0 and 4.0 mg mL^{-1}. Under mechanical homogenisation at room temperature, 28 mL sodium TPP aqueous solution was added into 70 mL of each chitosan solution, as a polymerising agent. The solutions were mixed with a homogenizer (Fisatom) at, with continuous addition of TPP solution at a rate of 1 mL min^{-1}. The nanoparticles solution of 110 and 300 nm presented pH of 4.23 and 4.04, respectively, corresponding to chitosan isoelectric point, ensuring stability in suspension allowing spraying.

Particle size distribution and polydispersion index

The particle size distribution was evaluated at room temperature using a Zetasizer Nano ZS (Malvern Instruments Inc., Westborough, MA, USA), with laser diffraction. All analyses were performed in triplicate. The resulting nanoparticles were observed under electronic microscopy (FEG-SEM JEOL JSM-6701F) by dropping a diluted solution (1/1000) onto a silicon wafer, allowed to dry for 24 h and carbon coated.

Processing and fruits coating

Cold room was previously washed and sanitised with 1 mL L^{-1} quaternary ammonium and utensils washed and sanitised with 200 mg L^{-1} sodium hypochlorite solution at pH 7 before processing. 'Gala' apple fruits (*Malus x domestica* Borkh.) were purchased from a local wholesale distributor at commercial maturity from a same lot. Apples were selected by uniform size,

© 2014 Institute of Food Science and Technology

discarding those with mechanical and pathological injuries. The fruit was stored at 5 ± 1 °C before processing. Previously to the cutting operation, the fruits were washed, sanitised by immersion in a 200 mg L^{-1} sodium dichloroisocyanurate dehydrate solution for 3 min, rinsed and dried. The samples were cored and manually cut into wedges (average weight at 25 ± 2 g) with a sharp stainless steel knife. The apple wedges were then rinsed in a 20 mg L^{-1} sodium dichloroisocyanurate dehydrated solution and immersed in ascorbic acid 1% for 3 min. Afterwards, they underwent treatment as follow: (i) 110-nm chitosan nanoparticles coating by spraying on the slices, (ii) 300-nm chitosan nanoparticles coating by spraying on the slices, (iii) dipping the slices into a solution of 2 g L^{-1} chitosan dissolved in 2% citric acid for 2 min. and (iv) as control: nontreated samples. Excess gel (sprayed and dipped) was allowed to drain off and the coating was then formed. Portions of 200 g of fresh-cut apples were packed in polyethylene terephthalate trays (160 μm, 750 mL, Galvanotek®, Carlos Barbosa, Brazil). Trays were sealed and stored in cold room at 5 ± 1 °C. Analyses were carried out every 2 days for 10-day storage. Preliminary essays were carried out using citric acid alone, and the results did not differ from control without citric acid in microbiological and browning response (data not shown).

Flesh colour

Colour values of the cut apple surfaces were measured with a colorimeter HunterLab MiniScan XE Plus (Hunter Associates Laboratory, Inc, Reston, VA, USA). Colour was measured using the CIELAB system: L* (lightness), C* (chroma), h° (hue angle). Hue angle ($h^{\circ} = \tan^{-1}\ [b^*/a^*]$) and chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$) were calculated from a* and b* values (Mcguire, 1992). Illuminant D65 and 10° observer angle were used. The instrument was calibrated using a standard white reflector plate. Samples were randomly taken from three trays per treatment, and five readings (five sliced surfaces) were made in each tray.

Determination of PPO and POD activities

The enzyme extraction was prepared by homogenising 10 g of apple wedges with 20 mL of 0.2 M sodium phosphate buffer (pH 7.0) and 0.2 g polyvinylpolypyrrolidone (Sigma-Aldrich). The homogenate was centrifuged at 16000 ***g*** at 4 °C for 30 min (Centrifuge Continent R, Hanil Science Industrial Co., Ltd., Incheon, South korea). The enzyme extract was obtained by filtration of the supernatant through Whatman 0.45-μm paper. Polyphenol oxidase activity was determined by measuring the rate of increase in absorbance at 420 nm in a spectrophotometer (Micronal B542, São Paulo, SP, Brazil) of a mixture containing 2.9 mL of 0.11 M catechol in 0.05 M phosphate buffer (pH 7.0) and 100 μL enzyme extract. Peroxidase activity was determined by measuring the rate of increase in absorbance at 485 nm of a mixture containing 2.7 mL of 0.05 M phosphate buffer (pH 7.0), 200 μL of ρ-phenylenediamine solution (1%), 100 μL of hydrogen peroxide solution (1.5%) and 25 μL of enzyme extract. The enzymes activities were calculated on the basis of the slope of the linear portion of the curve of changes in absorbance plotted against time (up to 3 min) and were expressed as changes in absorbance per min and gram (Cano *et al*., 1997).

Firmness measurement

Apple firmness evaluation was performed using a TA.XTPlus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) by measuring the maximum penetration force required for a 4-mm-diameter probe to penetrate into an apple wedge to a depth of 5 mm at a rate of 5 mm s^{-1}. Apple wedges were placed perpendicular to the probe to allow penetration in their centre, and the measurements expressed in Newton (N). Five wedges from each tray were measured.

Microbiological analysis

The microflora was measured every 2 days for 10 days of samples storage at 5 °C. One sample from each treatment was dipped in equivalent volume of sterile peptone water 1% (e.g. a slice of 10 g was dipped in 1 mL of peptone water) for 1 min. A 1 mL aliquot of the solution was then serially diluted in sterile peptone water 1% in (1:10) to a final dilution of 10^3. Separate 1 mL aliquots of each of the dilutions from 10° to 10^3 were withdrawn and poured on 3M™ Petrifilm™ YM (AOAC Official Method 997.02) to detect mould and yeast (Three plates per dilution), 3M™ Petrifilm™ EC (AOAC Official Method 991.14 and 998.08) to detect *E. coli*/coliform (three plates per dilution), 3M™ Petrifilm™ AC (AOAC Official Method 990.12) to detect psychrotrophic and mesophilic aerobic bacteria, and 3M™ Tecra™ Salmonella Visual Immunoassay (AOAC Official Method 998.09) for *Salmonella spp*. (three kit wells per sample). The plates for yeast and mould were incubated at 25 °C for 72 h and 120 h; plates for E. *coli* and coliform were incubated at 35 °C for 24 h and 48 h, respectively, and plates for psychrotrophic and mesophilic bacteria were incubated at 21 °C for 25 h and 35 °C for 48 h, respectively. The microorganisms were counted with an automated colony counter Phoenix CP 600 Plus (Araraquara, SP, Brazil). The results were expressed as CFU g^{-1} of apples. For *Salmonella*, the samples were primary enriched in Buffered Peptone Water for 24 h at 35 °C

© 2014 Institute of Food Science and Technology

followed by the secondary enrichment in Rappaport Vassiliads Broth for 20 h at 42 °C. For postenrichment, samples were enriched in M-Broth for 8 h at 36 °C. The results were interpreted comparing wells of the samples to the well of positive control.

Statistical analysis

A completely randomised design was used with evaluation of eight slices from four replicate trays per treatment and sampling time. The effects of treatments, storage days and interaction for each original isolated variable were analysed using general linear model (GLM) procedure of SAS® 9.2, using a complete factor analysis in the analysis of variance (ANOVA). Significant differences among the levels of main effects (treatment and storage day) were compared by least significant differences (LSD) test at the 5% level. The results of nanoparticles size distribution (Zetasizer Nano ZS) were mathematically fitted by Gaussian function and the values obtained by the mean ± standard deviation (sigma error).

Results and discussion

Particle size and polydispersion index

Chitosan–tripolyphosphate (CS-TPP) nanoparticles with different particle sizes were successfully produced by varying the solution citric acid content. It can be observed that the CS-TPP nanoparticles size increases upon the increase in acid content in the feeding solution (NPCS1 minor concentration; NPCS2 higher concentration) (Table 1). It should be pointed out that the production of nanoparticles with controlled particle size is usually highly desirable. In other studies found in the literature (Saloko *et al*., 2013; Venkatesan *et al*., 2013), chitosan nanoparticles are produced using acetic acid as solvent. In the present work, the nanoparticles were prepared with citric acid solution, which may be extremely important for several applications in food area. This fact is relevant as the pH of citric acid also makes it useful as a preservative.

Table 1 Particle size and polydispersion index (PDI) for chitosan nanoparticles suspension

Particles	*D* (nm)*	PDI†
NPCS1	111.72 ± 55.86	0.351 ± 0.030
NPCS2	295.30 ± 64.97	0.410 ± 0.021

*D (nm): average particle size, reported as mean ± SD (sigma value).
†PDI: polydispersion index, reported as mean ± SD.

Polydispersion index indicates how the particle size distribution is spread. Small PDI values are related to narrow size distributions (Lemarchand *et al*., 2003). Overall, the synthesised chitosan nanoparticles showed a narrow pattern of size distribution, as presented in Table 1. Both size distributions fit a Gaussian function (Fig. 1) with average particle size measured as 111.72 ± 55.86 nm and 295.30 ± 64.97 nm (Table 1). Such dimensions are similar to those obtained by de Moura *et al*. (2008) and Martelli *et al*. (2013), using the same process. The low polydispersity found in this work shows that the particles are uniformly sized, which can also be evidenced by the images of FEG electronic microscopy (Fig. 2).

Flesh colour

There was a decrease in L* value indicating flesh browning of fresh-cut apples over the 10 days of storage, with L* values from 87.7 to 81.7 (Fig. 3). Browning in the slices coated with conventional chitosan solution and the control was slightly intense than those coated with colloidal solution of chitosan nanoparticle 110 nm and chitosan nanoparticle 300 nm. The browning on apples cut surface may be attributed to the enzymatic oxidation of endogenous phenols into quinones, which polymerise into brown products. Despite significant effect of treatments on L* (Table 2), the L* changes were so small that it would be practically impossible to detect such differences among fruit by visual evaluations. Hue angle (h°) decreased for all of the samples, but although there was statistical differences (Table 2), the slices were maintained with yellowish-orange hues (h° from 83.8

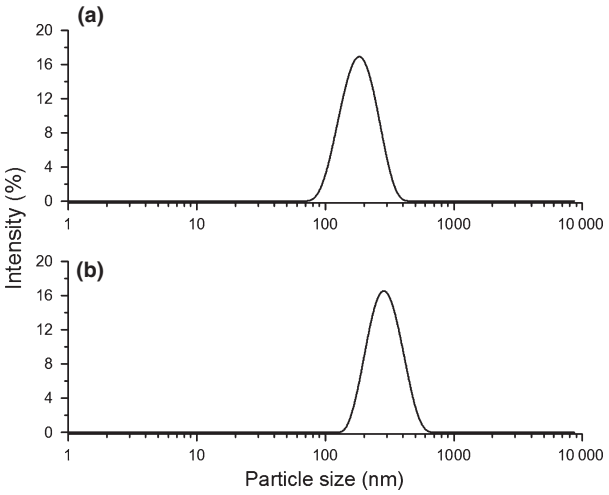

Figure 1 Particle size distribution of chitosan nanoparticles solution: average size (a) 110 nm and (b) 300 nm.

© 2014 Institute of Food Science and Technology

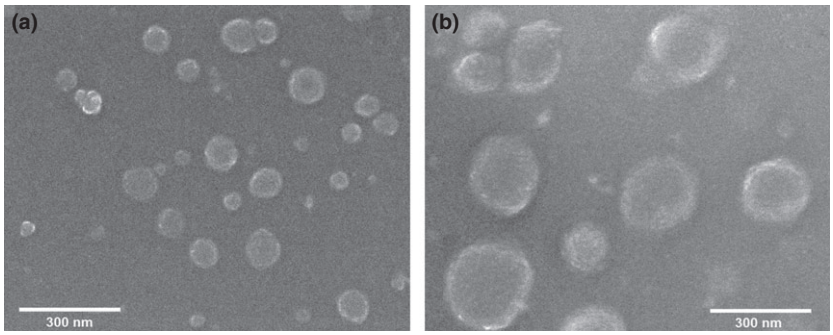

Figure 2 Nanoparticles observation under FEG-SEM: (a) 110 nm (× 30 000); (b) 300 nm (× 50 000).

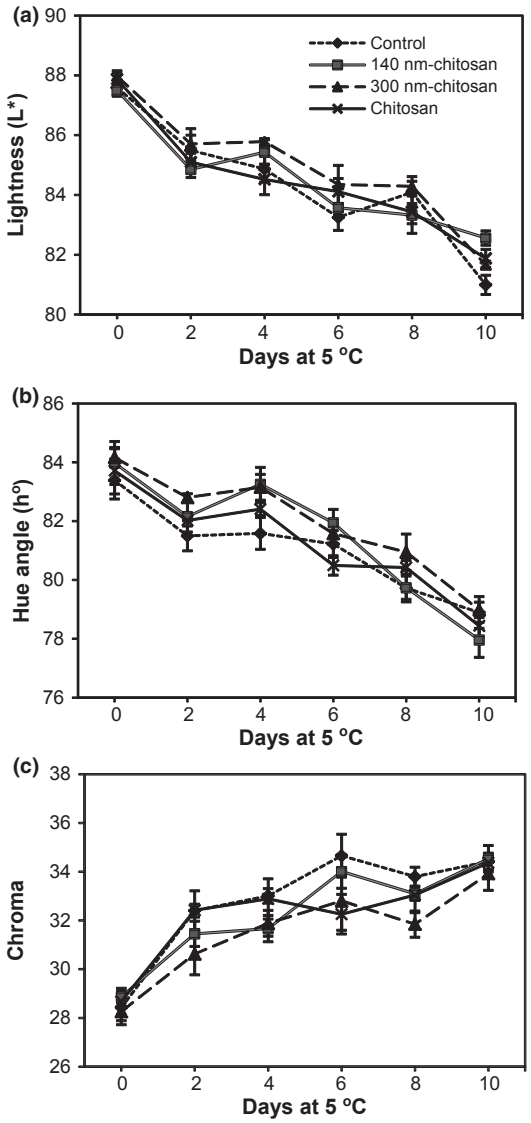

Figure 3 Changes in lightness, chroma and hue angle during storage of fresh-cut 'Gala' apple slice surfaces treated with 2 g L^{-1} chitosan, 110-nm chitosan, 300-nm chitosan and control (noncoated). Data represent the means (± SE) of four replicates by the LSD test.

to 78.6) during storage (Fig. 3). The colour of the slices became slightly saturated (C* from 28.5 to 34.6) (Fig. 3), deeper in colour with a significant increase in chroma over the storage period (Table 2). However, no major impact of particle size of chitosan on overall visual quality was noticed, showing that colour of fresh-cut apples was not influenced by the nanoparticles size. Indeed, chitosan conventional coatings on processed apple have already demonstrated that they have positive effect on firmness conservation, however bringing no benefits in controlling apple mesocarp browning during storage (Assis *et al.*, 2012). In contrast, Mustafa *et al.* (2013) reported five-day delay in skin colour change, from green to red, and also in other indicators of ripening in tomatoes coated with chitosan-surfactant with nanoparticles of 400, 600 and 800 nm when compared to noncoated samples, demonstrating that chitosan nanoparticles do have potential for maintaining postharvest quality of fresh tomato.

PPO and POD activities

The concentration and composition of phenolic compounds and the PPOs activity are frequently attributed as the main causes of browning development in cut fruit and vegetables (Joslyn & Ponting, 1951; Lamikanra, 2002). The PPO activity significantly increased during storage (Table 2), with the highest values after the sixth day in all of the treatments (Fig. 4). Despite statistical variance, small differences in the PPO activity occurred among treatments. The control remained with the PPO activity a little higher than the other treatments after the second day of storage. The enzymatic activity was slightly lower in samples coated with nanoparticulated chitosan. The presence of citric acid and sodium tripolyphosphate in the coating composition and the ability of chitosan to act as barriers to oxygen, necessary for the reactions of this enzyme to occur, may explain this slight preservative effect. Similarly occurred for the POD activity, which increased with time in all samples during the storage period (Fig. 4) and despite the statistical significance among treatments (Table 2), the difference was

© 2014 Institute of Food Science and Technology

Table 2 Analysis of variance of the colour parameters, polyphenoloxidase, peroxidase and firmness of fresh-cut 'Gala' apple slices stored at 5 °C for 10 days

		F-value					
Source of variation	**d.f.**	**Lightness (L*)**	**Hue angle (h°)**	**Chroma**	**PPO[†](A min^{-1} g^{-1})**	**POD[‡] A min^{-1} g^{-1})**	**Firmness (N)**
Control/110 nm/300 nm/Chitosan	3	2.69*	3.89**	3.93**	5.34**	29.55***	0.64 ns
Storage period	5	110.48***	61.05***	41.24***	63.70***	166.36***	0.74 ns
Period x Trat		1.42 ns	0.96 ns	0.86 ns	1.69 ns	2.63**	0.26 ns

[†]Polyphenoloxidase;
[‡]Peroxidase.
ns = nonsignificant; *Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

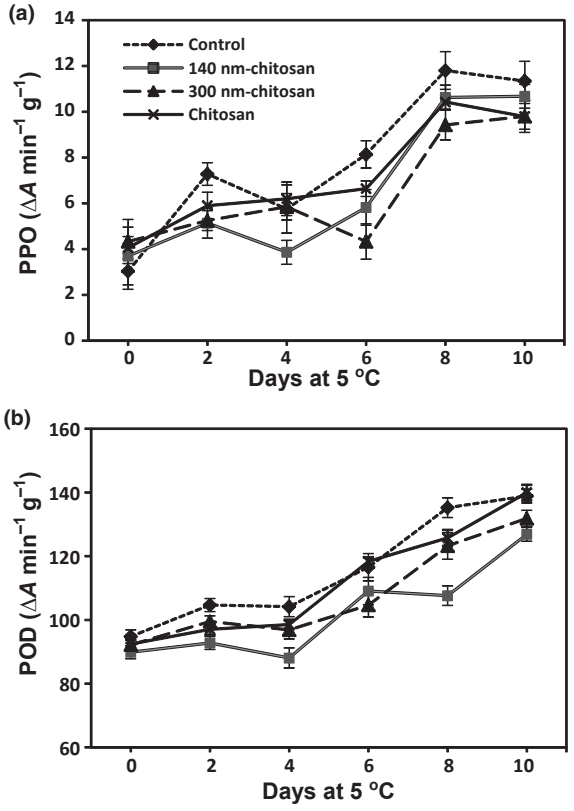

Figure 4 Changes in polyphenol oxidase (PPO) and peroxidase (POD) during storage of fresh-cut 'Gala' apple slices treated with 2 g L^{-1} chitosan, 110-nm chitosan, 300-nm chitosan and control (noncoated). Data represent the means (± SE) of four replicates by the LSD test.

irrelevant, not visually noticeable on the apple slices surfaces. Kim *et al*. (2013) used chitosan nanoparticles 228 nm as a carrier of ascorbyl palmitate (AP) to inhibit PPO in bananas and reported that the PPO inhibitory activity of AP was effectively improved when AP was nano-encapsulated by chitosan compared to no encapsulation.

Firmness measurement

No treatment and storage effect was found for the flesh firmness (Table 2). The measured firmness has not changed nor followed any trend in function of the used coating, as measured within sample replicates. The apples slices coated with nanoparticulated chitosan as well as the slices coated with conventional chitosan solution and the control remained with similar firmness range from 8.2 to 7.7 N during the storage period (data not shown).

Microbiological analysis

Microbial growth was found for all samples along the storage time. As expected, the use of chitosan in nanoparticles format resulted in a relevant reduction on the microbial count when compared to noncoated samples (Fig. 5). It is worth noticing that *Salmonella* and total and faecal coliforms were also evaluated in this study; however, none of these microorganisms were detected in any samples.

Mesophilic microorganisms are among those most important in food, being the most of the microorganism related to public health concern. The temperature range for mesophilic microorganisms' growth is between 10 and 47 °C, being 30–40 °C the optimum growth condition, at the human body temperature (ICMSF, 1980). In raw fruit and vegetable, an increased number of total mesophilic counts may indicate an excessive contamination probably caused by inadequate manipulation during harvest, storage and processing (Doyle *et al*., 1997). In our investigation, the nanoparticulated chitosan suppressed the growth of mesophilic microorganisms compared to the conventional coating (Fig. 5). The mesophiles were significantly lower (Table 3) for the fruit coated with the smaller size of chitosan particle (110 nm), reaching a mean count of 2.0 log CFU g^{-1} over the period. This count was 1 log cycle lower than those found for the treatment with 300 nm nanoparticles size and to the conventional chitosan coating, and 3 log cycles than in noncoated samples.

© 2014 Institute of Food Science and Technology

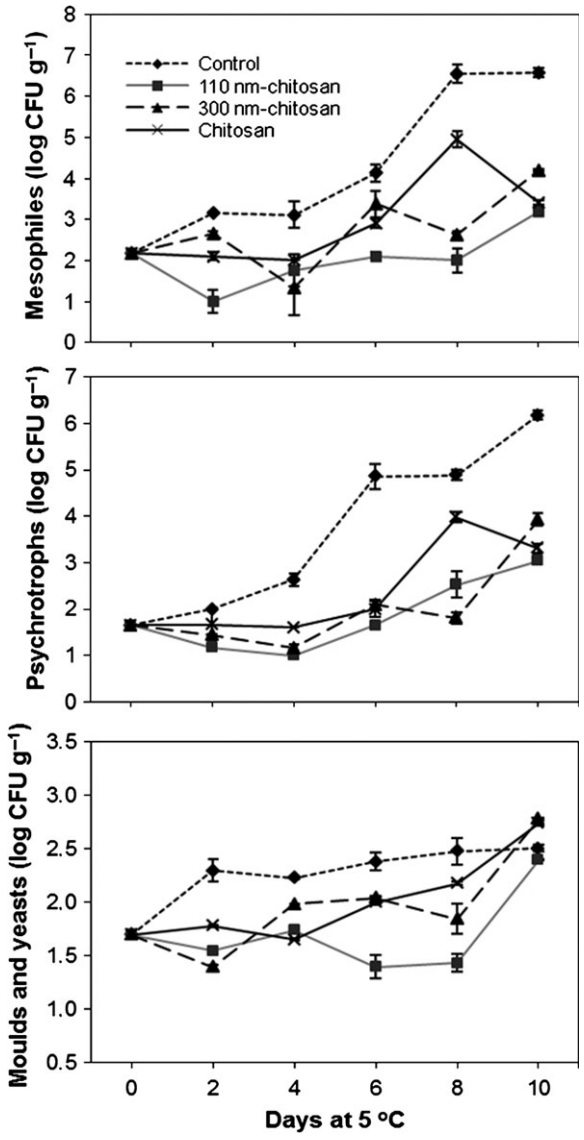

Figure 5 Microbial count (log CFU g^{-1}) during storage of fresh-cut 'Gala' apple slices treated with 2 g L^{-1} chitosan, 110-nm chitosan, 300-nm chitosan and control (noncoated). Data represent the means (± SE) of four replicates by the LSD test.

The 110-nm nanoparticle chitosan coating also exhibited the highest antimicrobial activity against psychrotrophic microorganisms (Table 3; Fig. 5). These microorganisms have optimal growth temperature in the mesophilic range but are capable to grow even at low refrigeration temperatures. Although both nanoparticles and conventional chitosan-coated samples presented similar counts until the sixth day, both nanoparticles coatings (110 and 300 nm) were more effective in inhibiting microbial growth. Evidently, the noncoated samples showed the highest count for psychrotrophs during the storage period (Fig. 5).

Moulds and yeasts were significantly inhibited by chitosan nanoparticles coating with 110 nm until the eighth day of storage (Table 3; Fig. 5). The effect of the antimicrobial activity of both conventional and nanoparticulated chitosan noticeably decreased in the last day of storage. Such effect can be understood by the saturation of the protonated amino groups in the chitosan backbone. Because the main antimicrobial action of the chitosan is based on the electrostatic interaction, as the population of microorganism grow, the number of charged sites remains the same. After a few days, all chitosan sites are linked, reducing its overall antimicrobial efficiency.

The activity of chitosan against different groups of microorganisms, mainly moulds, is assumed to have a mechanism similar to that described for bacteria, that is, the activity against moulds is regulated by electrostatic interactions (Rabea *et al.*, 2003; Devlieghere *et al.*, 2004; Goy *et al.*, 2009; Elsabee & Abdou, 2013). Generally, chitosan has been reported as being very effective in inhibiting spore germination, germ tube elongation and radical growth (Sashai & Manocha, 1993). As summarised by Jung *et al.* (1999), the mechanisms behind the antimicrobial activities of chitosan can be described as follows: (i) the positively charged amino groups on chitosan bind with sialic acid in phospholipids, consequently inhibiting the movement of microbial substances and (ii) oligomeric chitosan penetrates into the cells of microorganisms and preventing the growth of cells by prohibiting the transcription of DNA into RNA (Rabea *et al.*, 2003). The chitosan also activates chitinases, β-glucanases and

Table 3 Analysis of variance of mesophiles, psychrotrophs and moulds and yeasts of fresh-cut 'Gala' apple slices stored at 5 °C for 10 days

		F-value		
Source of variation	**d.f.**	**Mesophiles (log CFU g^{-1})**	**Psychrotrophs (log CFU g^{-1})**	**Moulds and Yeasts (log CFU g^{-1})**
Control/110 nm/300 nm/Chitosan	3	106.48***	347.18***	209.34***
Storage period	5	81.15***	369.46***	277.35***
Period x Trat		14.76***	41.93***	40.16***

†Polyphenoloxidase; ‡Peroxidase.
***Significant at $P < 0.001$.

© 2014 Institute of Food Science and Technology

lipoxygenases, stimulating the generation of reactive oxygen species (Vasyukova *et al*., 2001). The same antimicrobial mechanism is assumed for both chitosan as gel (continuous coating) or as nanoparticles formats.

Conventional chitosan promotes the formation of a continuous film by polymer deposition, coating and acting on the entire fruit surface. This coating may be mono- or multilayered structured, with several dipping sequences, achieving different thicknesses up to micrometres (Assis & Britto, 2010; Sui *et al*., 2010). It differs when compared to nanoparticle coatings as the nanoparticles are sprayed, forming a noncontinuous coating. Although the sprayed nanoparticles result in a much dispersed coating, the nanosize enhances the chitosan properties by providing higher surface interaction, with consequent effects on microorganisms.

Thus, the smaller the particle, the higher will be its mobility and surface interaction, resulting in enhancing on the antimicrobial activity. In fact, the treatments using colloidal solution of chitosan nanoparticles (110 and 300 nm) resulted in the highest antimicrobial activity against mesophilic and psychrophilic bacteria and moulds and yeasts, reducing total microbial contamination levels when compared to conventional coating solution and to the control samples throughout the storage period.

Conclusion

The CS-TPP nanoparticles using citric acid as solvent were successfully prepared and used as colloidal spray for processed apple coating. The tests carried out on fresh-cut 'Gala' apples revealed a better antimicrobial efficiency of nanoparticles coatings when compared to conventional chitosan coating and the control. The chitosan-based 110-nm nanoparticles were the most effective coating in inhibiting microbial growth for both bacteria and moulds. No major differences among the coatings were observed on the other quality attributes investigated in our study along 10 days under cold storage. This investigation supports the potential use of chitosan nanoparticles formulations as edible coatings in controlling microbial activity on fresh-cut apples.

References

Assis, O.B.G. & Britto, D. (2010). Evaluation of the antifungal properties of chitosan coating on cut apples using a non-invasive image analysis technique. *Polymer International*, **60**, 932–936.

Assis, O.B.G., Scramin, J.A., Correa, T.A., Britto, D. & Forato, L.A. (2012). A comparative evaluation of integrity and colour preservation of sliced apples protected by chitosan and zein edible coatings. *Revista Iberoamericana de Tecnología Postcosecha*, **13**, 76–85.

Baldwin, E.A. (1994). Edible coatings for fresh fruits and vegetables: past, present and future. In: *Edible Coatings and Films to Improve Food Quality*. (edited by J.M. Krochta, E.A. Baldwin & M.O. Nisperos-Carriedo) Pp. 25–64. Lancaster, USA: Technomic Publishing Company Inc.

Belbekhouche, S., Bras, J., Siqueira, G. *et al*. (2011). Water sorption behavior and gas barrier properties of cellulose whiskers and microfibrils films. *Carbohydrate Polymers*, **83**, 1740–1748.

Caner, C., Vergano, P.J. & Wiles, J.L. (1998). Chitosan film mechanical and permeation properties as affected by acid, plasticizer, and storage. *Journal of Food Science*, **63**, 1049–1053.

Cano, M.P., de Ancos, B., Matallana, M.C., Cámara, M., Reglero, G. & Tabera, J. (1997). Differences among Spanish and Latin-American banana cultivars: morphological, chemical and sensory characteristics. *Food Chemistry*, **59**, 411–419.

Chien, P.J., Sheu, F. & Yang, F.H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering*, **78**, 225–229.

Costa, C., Conte, A., Buonocore, G.G., Lavorgna, M. & Del Nobile, M.A. (2012). Calcium-alginate coating loaded with silver-montmorillonite nanoparticles to prolong the shelf-life of fresh-cut carrots. *Food Research International*, **48**, 164–169.

Devlieghere, F., Vermeulen, A. & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology*, **21**, 703–714.

Doyle, M.P., Beuchat, L.R. & Montville, T.J. (1997). *Food Microbiology: Fundamentals and Frontiers*. Washington, USA: ASM Press.

Du, W.L., Niu, S.S., Xu, Y.L., Xu, Z.R. & Fan, C.L. (2009). Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydrate Polymers*, **75**, 385–389.

Elsabee, M.Z. & Abdou, E.S. (2013). Chitosan based edible films and coatings: a review. *Materials Science and Engineering*, **33**, 1819–1841.

Goy, R.C., Britto, D. & Assis, O.B.G. (2009). A review of the antimicrobial activity of chitosan. *Polímeros: Ciência e Tecnologia*, **19**, 241–247.

Han, Y.S., Lee, S.H., Choi, K.H. & Park, I. (2010). Preparation and characterization of chitosan–clay nanocomposites with antimicrobial activity. *Journal of Physics and Chemistry of Solids*, **71**(464), 467.

Hirano, S. (1999). Chitin and chitosan as novel biotechnological materials. *Polymer International*, **48**, 732–734.

ICMSF - International Commission on Microbiological Specifications for Foods. (1980). *Microbial Ecology of Foods, Vol. 1: Factors Affecting Life and Death of Microorganisms*. Pp. 274. London: Academic Press.

Jiang, T., Feng, L. & Wang, Y. (2013). Effect of alginate/nano-Ag coating on microbial and physicochemical characteristics of shiitake mushroom (Lentinus edodes) during cold storage. *Food Chemistry*, **141**, 954–960.

Joslyn, M.A. & Ponting, J.D. (1951). Enzyme-catalyzed oxidative browning of fruit products. *Advances and Food Research*, **3**, 1–44.

Jung, B.-O., Kim, C.-H., Choi, K.-S., Lee, Y.M. & Kim, J.-J. (1999). Preparation of amphiphilic chitosan and their antimicrobial activities. *Journal of Applied Polymer Science*, **72**, 1713–1719.

Kester, J.J. & Fennema, O.R. (1986). Edible films and coatings: a review. *Food Technology*, **40**, 47–59.

Kim, M.K., Lee, J., Kim, K.Y. & Lee, H.G. (2013). Ascorbyl palmitate-loaded chitosan nanoparticles: characteristic and polyphenol oxidase inhibitory activity. *Colloids and Surfaces B: Biointerfaces*, **103**, 391–394.

Krochta, J.M. (2002). Proteins as raw materials for films and coatings: definitions, current status and opportunities. In: *Protein-Based Films and Coatings*. (edited by A. Gennadios) Pp. 1–42 Boca Raton, USA: CRC Press.

Lamikanra, O. (2002). Enzymatic effects on flavor and texture of fresh-cut fruits and vegetables. In: *Fresh-cut Fruits and Vegetables: Science, Technology and Market*. (edited by O. Lamikanra) Pp. 133–193 Boca Raton, USA: CRC Press LLC.

© 2014 Institute of Food Science and Technology

Lemarchand, C., Couvreur, P., Vauthier, C., Costantini, D. & Gref, R. (2003). Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *International Journal of Pharmaceutics*, **254**, 77–82.

Martelli, M., Barros, T.T., Moura, M.R., Mattoso, L.H.C. & Assis, O.B.G. (2013). Effect of chitosan nanoparticles and pectin content on mechanical properties and water vapor permeability of banana puree films. *Journal of Food Science*, **78**, N98–N104.

Mcguire, R.G. (1992). Reporting of objective color measurements. *HortScience*, **27**, 1254–1255.

Medeiros, B.G.S., Pinheiro, A.C., Teixeira, J.A., Vicente, A.A. & Carneiro-Da-Cunha, M.G. (2012). Polysaccharide/protein nanomultilayer coatings: construction, characterization and evaluation of their effect on 'Rocha' Pear (*Pyrus communis* L.) shelf-Life. *Food and Bioprocess Technology*, **5**, 2435–2445.

de Moura, M.R., Aouada, F.A. & Mattoso, L.H.C. (2008). Preparation of chitosan nanoparticles using methacrylic acid. *Journal of Colloid and Interface Science*, **321**, 477–483.

de Moura, M.R., Aouada, F.A., Avena-Bustillos, R.J., McHugh, T.H., Krochta, J.M. & Mattoso, L.H.C. (2009). Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. *Journal of Food Engineering*, **92**, 448–453.

Mustafa, M.A., Ali, A., Manickam, S. & Siddiqui, Y. (2013). Ultrasound-assisted chitosan-surfactant nanostructure assemblies: towards maintaining postharvest quality of tomatoes. *Food and Bioprocess Technology*, **7**, 2102–2111.

Park, H.J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science & Technology*, **10**, 254–260.

Qi, L., Xu, Z., Jiang, X., Hu, C. & Zou, X. (2004). Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydrate Research*, **339**, 2693–2700.

Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G. & Steurbaut, W. (2003). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*, **4**, 1457–1465.

Rhim, J.-W., Hong, S.-I., Park, H.-M. & Ng, P.K.W. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agricultural and Food Chemistry*, **54**, 5814–5822.

Rodríguez-Núñez, J.R., López-Cervantes, J., Sánchez-Machado, D.I., Ramírez-Wong, B., Torres-Chavez, P. & Cortez-Rocha, M.O. (2012). Antimicrobial activity of chitosan-based films against *Salmonella typhimurium* and *Staphylococcus aureus*. *International Journal of Food Science and Technology*, **47**, 2127–2133.

Romanazzi, G., Nigro, F., Ippolito, A., Divenere, D. & Salerno, M. (2002). Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. *Journal of Food Science*, **67**, 1862–1867.

Saloko, S., Darmadji, P., Setiaji, B., Pranoto, Y. & Anal, A.K. (2013). Encapsulation of coconut shell liquid smoke in chitosan-maltodextrin based nanoparticles. *International Food Research Journal*, **20**, 1269–1276.

Sashai, A.S. & Manocha, M.S. (1993). Chitinases of fungi and plants: their involvement in morpogenesis and host-parasite interaction. *FEMS Microbiological Review*, **11**, 317–338.

Shahidi, F., Arachchi, J.K.V. & Jeon, Y. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology*, **10**, 37–51.

Sui, L., Huang, L., Podsiadlo, P., Kotov, N.A. & Kieffer, J. (2010). Brillouin light scattering investigation of the mechanical properties of layer-by-layer assembled cellulose nano-crystal films. *Macromolecules*, **43**, 9541–9548.

Tsai, G.J. & Su, W.H. (1999). Antibacterial activity of shrimp chitosan against Escherichia coli. *Journal of Food Protection*, **62**, 239–243.

Vasyukova, N.I., Zinov'eva, S.V., Il'inskaya, L.I. *et al*. (2001). Modulation of Plant Resistance to Diseases by Water-Soluble Chitosan. *Applied Biochemistry and Microbiology*, **37**, 103–109.

Venkatesan, C., Vimal, S. & Hameed, A.S. (2013). Synthesis and characterization of chitosan tripolyphosphate nanoparticles and its encapsulation efficiency containing Russell's viper snake venom. *Journal of Biochemical and Molecular Toxicology*, **27**, 406–411.

Wiles, J.L., Vergano, P.J., Barron, F.H., Bunn, J.M. & Testin, R.F. (2000). Water vapor transmission rates and sorption behavior of chitosan films. *Journal of Food Science*, **65**, 1175–1179.

Zhang, D. & Quantick, P.C. (1998). Antifungal effect of chitosan coating on fresh strawberries and raspberries during storage. *Journal of Horticultural Science and Biotechnology*, **73**, 763–767.

© 2014 Institute of Food Science and Technology