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Editors:
Caue Ribeiro
Odílio Benedito Garrido de Assis
Luiz Henrique Capparelli Mattoso
Sergio Mascarenhas

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Chitosan Edible Coating as a Fungistatic Agent on Cut Apples

O.B.G. Assis* and D. Britto

Embrapa Instrumentação Agropecuária. São Carlos, SP. Brazil, e-mail: odilio@cnpdia.embrapa.br
* Corresponding author.

Abstract – The inhibitory activity of chitosan edible coatings was evaluated against fungus on minimally processed apples. Apples, cv. Gala (*Malus domestica*) were sliced and coated with an acidic formulation of 2.0 g/L chitosan concentration. Non-classified fungus (predominantly *Penicillium* sp. and *Alternaria* sp.) were used to contaminate the cut surfaces and infection extension assessed by means of image analysis. The results show the fungistatic activity of the chitosan with significant reduction on infected area and on the number of infected samples along 10 days of storage time at 25°C.

Chitosan is a natural polymer currently obtained by deacetylation of chitin, regarded as being the second most abundant renewable organic compound on earth (second only to cellulose) and found in crustacean shells, insect exoskeletons and plankton. Chitosan is a linear polymer, formed primarily of repeating units of beta (1-4) 2-amino-2-deoxy-D-glucose which a structure similar to cellulose, present broad antimicrobial activity in conjunction with excellent film forming ability. Chitosan coatings have been evaluated, for example, on carrots, on mangos, on strawberries and on apples. Inhibitory activity has been observed for a broad range of concentrations upon several spoilage yeasts. In this study cut apples are exposed to fungus and the fungistatic effect of chitosan coating evaluated via image analysis

Commercial chitosan of shrimp origin (Polymar, Brazil) was used. A gel was prepared by dispersing the chitosan in 100 mL distilled water whilst stirring on a magnetic stirrer at room temperature. Acetic acid (0.5 M) was added initially to adjust the pH to approximately 4.0. Solution at 2.0 g/l chitosan concentration was prepared. Apples, cv. Gala (*Malus domestica*) were first sliced into two halves and then displayed separately in two groups of 20 samples each. The first group underwent chitosan coating by direct dipped into the gel. The resultant coatings were highly transparent. Groups of 20 coated and 20 non-coated apple slices were put into a controlled temperature chamber where petri dishes containing non-classified cultures of fungi (predominantly *Penicillium* sp. and *Alternaria* sp.) were allocated amongst the samples, so as to allow spontaneous inoculation by ambient contamination. Qualitative and quantitative analysis were performed using a commercial scanner for image capture. All cut surfaces were individually scanned twice a day with 512 by 512-pixels resolution and a grayness level ranging from 0 to 255. The acquired images were directly imported into the Image Tool v. 3 software, binary transformed and quantification was performed considering a two dimensional growth. The infected area was then isolated and automatically estimated by pixel counting and numerically compared to each precedent data.

As expected, the non-protected faces entail higher marked fungal growth and proliferation with time, as can be seen when comparing the evolution data as plotted in Figure 2. Infected samples were considered as those having at least 10% of the total scanned area identified as covered by fungus pattern. After 10 days of image acquisition, it was apparent that 90% non-protected samples and 40% chitosan-coated fruits slices were infected. The counting of pixels in the acquired images corresponds to the isolated area. A simple comparison of the evolution area in the same set of samples over a period reveals the kinetic tendency of the fungal growth. Figure 3 summarizes the percentage of infected area. The dissemination is, as expected faster for non-protected surfaces, with a reduced rate of proliferation on chitosan-coated surfaces, mainly in the first 1-4 days of exposure to fungi.

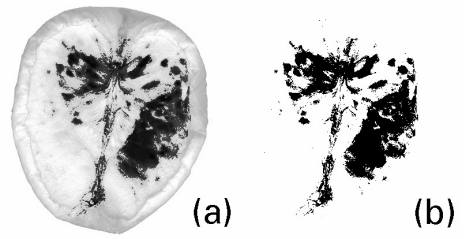

Figure 1: An example of image processing and infected area measurement. The image of a fungi-contaminated non-coated sample is digitally recorded (a), and the fungal pattern

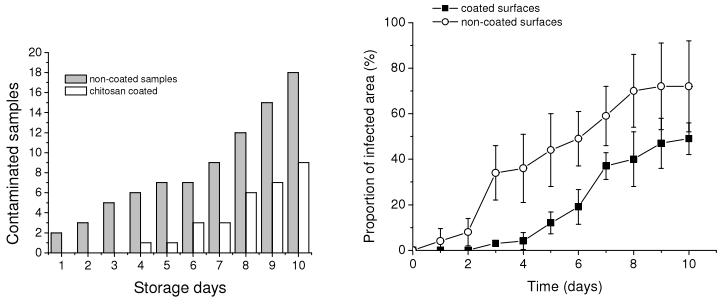

Figure 2: Evolution of the number of infected samples during 10 days of storage, over a total of 20 samples (chitosan coated and non-coated), and the proportion of Infected area for sliced surfaces versus exposure time, as measured by image analysis.