Effects of Chitosan Coating on the Storability and on the Ultrastructural Changes of ‘Jonagold’ Apple Fruit in Storage

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Chitosan is a high molecular compound, containing nitrogen derived from the shell of crab and shrimp. The effects of chitosan coating on respiration, ethylene production, and storage of ‘Jonagold’ apples (Malus pumila Mill. var. domestica Schneid.) were investigated. Coating the fruit with chitosan significantly reduced the respiration rate and ethylene production in storage. Postharvest coating increased the internal CO₂ and decreased the internal O₂ levels of the fruits markedly. Firmness of the treated fruits were considerably retained during storage. Observation by SEM revealed that the chitosan films covered overall surface of the treated fruits. A plenty of deep cracks were observed on the pericarp of uncoated fruits, but much less on the surface of coated fruits. Growing hyphae, which was resulted from an inoculation of conidia of apple gray mold caused by Botrytis cinerea, were recognized on the pericarp of uncoated fruits, whereas many deformed spores were observed on the surface of the coated fruits. These observations support the view that chitosan coating could not only suppress the ethylene production and respiration, but also inhibit conidial germination and fungal development resulting in preserving the quality of ‘Jonagold’ apples.

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Chitosan, a natural, non-toxic, biodegradable, high molecular weight polymer, is now produced commercially in North America and Japan on a large scale, and is being used in the wide fields of medicine, skin care products, biotechnology, agriculture, clarification and waste management, and also as functional additives for foods and feeds). Approval of chitosan as a food additive is still pending in the United States, but its acceptance is considered only a matter of time). This paper reports the effects of chitosan coating on postharvest physiology and on changes in surface structure of ‘Jonagold’ apples, to understand the mechanisms of chitosan coating on the storage of fruits.

MATERIALS and METHODS

1. Chitosan treatment

Chitosan, used in this experiment, was supplied by Nippon Kayaku Co., Ltd. having 87.0% deacetylation and molecular weight ≥ 1,000,000. The viscosity differs depending on the type and concentration of acids used to dissolve chitosan). It was 197.0 mPa·s when 1.0% chitosan dissolved in 0.5% acetic acid solution in this experiment.

‘Jonagold’ apples were harvested at the Aomori
Apple Experimental Station on November 1, 1996, and transported to Tsukuba under cooling atmosphere one week after.

'Jonagold' apples were individually dipped either in 1.0%, or 1.2% chitosan solution in 0.5% acetic acid (w/v) or in 0.5% acetic acid solution alone. Control fruits were not dipped. Each treatment consisted of three replications of 16 fruits in a randomized block design. Air-dried fruits were stored at 5°C, RH 85~90% for 4 months.

After 3 months of storage, 9 fruits each of control and 1.2% chitosan treatment, were inoculated with Botrytis cinerea conidial suspensions (1.5×10⁵ conidia per milliliter), and stored at 5°C, RH 85~90% for 2 weeks, then transferred and kept at 25°C, RH 65~70% for one more week. These materials were used for SEM studies to observe the germination of the conidia, the growth of this fungus and the changes in surface structure of the apples.

2. Gas analyses

The ethylene and carbon dioxide evolutions were determined by sealing one 'Jonagold' apple in a 2.8 liter container for 3 hours at 25°C. One ml gas sample was withdrawn with a gas-tight hypodermic syringe and analyzed by gas chromatography (Hitachi 163 equipped FID and Shimadzu GC-8A equipped TCD) for ethylene and carbon dioxide or oxygen, respectively. For each treatment, 3 gas samples were analyzed.

To assess the effect of chitosan coating on the internal gases of apples, the internal gases were attracted by using a vacuum pump from a fruit put in a bottle which submerged in water. One ml samples of the gases were withdrawn from the bottle covered with a rubber cap and analyzed for CO₂ and O₂ as mentioned above.

3. Fruit firmness

After peeling the equatorial site, the firmness (g) of flesh of apples was determined with a pressure tester (Fudoh Rheo Meter NRM-2002J), equipped with a 3 mm flat plunger.

4. SEM studies

Scanning Electron Microscope (SEM) studies were made as follows. Each portion of the peel was fixed in FAA solution (formalin : acetic acid : 60% ethanol, 5 : 5 : 90 v/v) until further use. The FAA-fixed-samples were immersed in distilled water for 5 hours, then slowly dehydrated in a graded ethanol series and postdehydrated in 100% isooamy acetate. Fruit peel segments were subsequently subjected to critical-point drying. The dried samples were attached to aluminum stubs and sputter-coated with gold. The specimens were viewed on a Hitachi Scanning Electron Microscopy (Model S-430s) at the accelerating voltage of 20 kV and photographed.

RESULTS

1. Effects of chitosan coating on storability of apples

Carbon dioxide evolution of chitosan coated fruit decreased gradually during storage (Fig. 1), and was significantly lower in levels than that of non-coated fruit after the first month of the storage.

Ethylene production generally increased in the first month and then decreased toward the end of the storage (Fig. 2). Apples coated with chitosan had a significantly lower rate of ethylene production than those of non-coated fruit.

Chitosan coating also raised the internal CO₂ levels and decreased the internal O₂ levels within the fruits stored at 5°C; the greater effect was found at the higher concentration (Figs. 3, 4). One month after coating, the internal CO₂ level of 1.2% chitosan-coated apple increased up to 8.9%, and retained above 7.5% to the end of the storage (Fig. 3). The internal O₂ level of 1.2% chitosan-coated

Fig. 1 Effect of chitosan coating on carbon dioxide evolution of 'Jonagold' apple fruits during storage at 5°C, RH 85~90%
Ultrastructural changes of chitosan-treated apple fruits decreased to 4.7% two months after coating, and retained below 6.3% to the end of the storage (Fig. 4). However, neither fermentation odor nor off-flavor was noted.

Although the firmness of fruit decreased sharply during one month-storage in all treatments, fruit coated with chitosan had a significantly higher firmness than that of non-coated fruit after first month of storage (Fig. 5).

2. Effects of chitosan coating on the changes in pericarp structure

The chitosan films covered overall surface of the treated fruits (Fig. 6-A). There was no difference in coating patterns of three parts—the upper, the middle and the lower part of the fruit surface. The films could be retained well up to the end of storage and the thickness of this film is approximately 3 μm (Fig. 7-A, B).

Control apple fruit stored for about 4 months showed shrinkage of the epidermal cells on the skin, and cleavages became very apparent (Fig. 6-D), while on the surface of 1.2% chitosan coated fruits, the epidermal cells arranged well, and only a very few cracks were observed (Fig. 6-A).

When the apples were stored at 5°C, no growing hyphae were observed in 2-week storage, even if B. cinerea conidia were inoculated. However, after one week of transferring the fruits to 25°C, there were many growing hyphae on the pericarp of uncoated fruits (Fig. 6-E, F). The hypha was growing and invading into cleavage of the epidermal cells (Fig. 6-E). Whereas, only few germinated conidia and many deformed spores were seen on the pericarp of 1.2% chitosan coated fruits (Fig. 6-B, C). The deformed spores changed in morphology and became less roundish, because of losing their moisture (Fig. 6-C).
Fig. 6 Scanning electron micrographs (SEMs) of the pericarp surface of ‘Jonagold’ apples. A, B, C, show the surface of 1.2% chitosan coated fruits: note chitosan covered overall on the pericarp surface (A), germinated conidia of *Botrytis cinerea* (B), and deformed conidia of *B. cinerea* (C). D, E, F show the surface of the control fruits: note deep cracks on the skin (D), hypha of *B. cinerea* growing into crevice (E), some growing hyphae of *B. cinerea* (F)
DISCUSSION

Postharvest applications of chitosan are promising in preserving the qualities of tomatoes\(^4\), peaches, Japanese pears\(^5\) and plums\(^6\). In this study, chitosan treatments decreased the respiration rates and ethylene production (Figs. 1, 2), and retained the firmness during storage of 'Jonagold' apples (Fig. 5). The advantages for storage in chitosan coated 'Jonagold' apples may be attributed to higher internal CO\(_2\) and lower internal O\(_2\) levels (Figs. 3, 4).

SORNSRIVICHAI et al.\(^8\) reported that a sucrose ester wax coating was better than film packaging in delaying loss of fruit firmness and retarding ripening of 'Pien Pu' pear. Cut apple pieces coated with double layers of buffered polysaccharide / lipid showed a 50–70% reduction in the rate of CO\(_2\) evolution and about 90% decrease in C\(_2\)H\(_4\) as compared with uncoated controls\(^9\). LAU and YASTREMSKI\(^10\) found that NS-coated 'Golden Delicious' apples were greener and firmer and had higher titratable acidity and more shrivelled and injured fruit than the control. Contrarily, chitosan coating neither affected the appearance nor caused phytotoxicity of several fruits\(^5\).

BANKS\(^11\) demonstrated that coating fruits with Prolong wax had little effect on the internal O\(_2\) and CO\(_2\) concentration of apples stored at 4°C. But, NS applications led to an accumulation of CO\(_2\) and C\(_2\)H\(_4\) and a small reduction of O\(_2\) in the 'Golden Delicious' apple core cavities\(^10\). In this experiment, 'Jonagold' apples coated with 1.2% chitosan, the internal CO\(_2\) level increased to 8.9%, and the internal O\(_2\) level decreased to 4.7% (Figs. 3, 4). However, at the end of storage, no adverse effect on flavors was noted.

HAGENMAIER and SHAW\(^12\) suggested that permeability control may lead to a general improvement in the technology of fruit coatings. Data are needed on the performance of coatings of known permeability and thickness. Unfortunately, thickness of coating has scarcely been reported. Regards to the permeability of the film as a coating material, it should be affected by the property of material, especially the viscosity. The chitosan used in this experiment was relatively high molecular weight showing very viscous as mentioned previously. The thickness of this chitosan film was about 3 μm (Fig. 7-A, B). GEMMA and DU\(^13\) reported that high molecular weight (≈ 1,000,000) and medium MW (≈ 600,000) chitosan coating greatly prolonged the shelf life at 5°C for more than 6 weeks compared to the control. But low MW (≈ 94,000) chitosan coating, less viscous, showed no effect on extending the shelf life of 'Jonagold' apples.

EL GHAOUTH\(^14\) reported that chitosan coating reduced water loss of cucumber and bell pepper. Surface morphology, as revealed by SEM, provided
visual evidence of what happened in apple fruits. At the end of storage, epidermal cells became shrivelled and cracks appeared on the wax layer (Fig. 6-A, D). This was more pronounced deeper and wider on the surface of the control fruits. It is likely that the cracking of the waxy cuticle on the surface of apple fruits further facilitated the invasion of fungi.

The chitosan films covered overall surface of the treated fruits (Fig. 6-A). When these chitosan films were removed, only a few cracks could be observed on bare surface.

CHEAH et al.\textsuperscript{15}) found that 2\% or 4\% chitosan coating reduced the incidence of sclerotinia rot, caused by \textit{Sclerotinia sclerotiorum}, from 80\% to about 20\% and also reduced the extent of the rot on a carrot. DU \textit{et al.}\textsuperscript{5}) reported that chitosan significantly inhibited the growth of \textit{B. cinerea} cultured on artificial media. After the 3-day culture, the diameter of colony of this pathogen on Potato Dextrose Agar containing 0\%, 0.05\% and 0.20\% chitosan were 77.4mm, 65.4mm and 25.6mm, respectively. EL GHAOUTH \textit{et al.}\textsuperscript{16}) found that 0.6\% chitosan inhibited radial growth of \textit{B. cinerea} and \textit{Rhizopus stolonifer} by 95.5\% and 71.5\%, respectively. As our results coincided with these findings, chitosan could be considered an antifungal material.

Besides, there were some findings concerning about the antifungal mechanism of chitosan. HIRANO \textit{et al.}\textsuperscript{17}) reported that chitinase activity is induced by coating seeds of Japanese radish, soybean and rice with chitosan. The seedlings are generally resistant against the attack of pathogens and insects, probably due to the prevention of their adhesion on plant tissues by these enzymic activities. Chitosan was inhibitory to various soilborne phytopathogenic fungi\textsuperscript{18-19}). STRUSZCZYK \textit{et al.}\textsuperscript{10}) demonstrated that chitosan also interferes with formation of local lesion induced by viral infection in plants and so chitosan treatment could reduce the plant virus infection. HADWIGER and BECKMAN \textsuperscript{20}) discovered that chitosan is an elicitor of phytoalexin pisatin, plays a prominent role in the host-parasite interaction and then could protect the pea tissue from \textit{Fusarium Solani f. sp. pisi}, a pathogen of peas.

In this experiment, 1.2\% chitosan treatment markedly inhibited the conidial germination and hypha growth of \textit{B. cinerea} on 'Jonagold' apple fruits (Fig. 6). The advantages for antifungi are attributed to: ① some phytoalexin and enzymes induced by chitosan could inhibit the conidial germination, and ② there were almost no deep cracks on the surface of chitosan coated fruit in which the harboring and invading of the hyphae are prone. From these observations chitosan can be used as an antifungal coating material for fruit.

\textbf{LITERATURE CITED}

13) GEMMA, H. and DU, J.: Conference Handbook,
Ultrastructural changes of chitosan-treated apple


収穫後キトサン処理が"ジョナゴールド”リンゴ
果実の鮮度保持と果皮構造に及ぼす影響

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“ジョナゴールド”リンゴ（Malus pumila Mill. var. domestica Schneid.）果実にキトサンを果実表面に被膜処理して、鮮度保持に及ぼす効果を検討した。果実は5℃、RH85〜90%で4ヶ月間貯蔵し、貯蔵中の果実の硬度、呼吸活性とエチレン生成量の変化などを調べた。その結果、キトサン処理により、果実の顕著な呼吸活性とエチレン生成の抑制がみられ、果実の硬度が保持された。キトサン処理では果実内部のCO2が増加し、O2が低下したが、オフ・フレーバーは認められなかった。

また、走査型電子顕微鏡（SEM）の観察により、処理果実はキトサン被膜が約3μmの厚さで全面に覆われ、細胞間に亀裂はほとんど認めてなかった。これに対して、無処理果実では細胞間に顕著に亀裂が観察された。果実にリンゴ灰色かび病菌、Botrytis cinereaの分生胞子を接種した3週間後、無処理果実の表面では、多くの生長した菌糸が観察されたが、キトサン処理果実の表面では、わずかに発芽した分生胞子がみられたのみで、ほとんどは変形していた。本研究の結果から、キトサン処理は、“ジョナゴールド”リンゴ果実の貯蔵およびB. cinereaの分生胞子の発芽と生長の抑制に有効であると認められた。

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