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REGULAR ARTICLE

Effects of hydrophilic plasticizers added to chitosan coating for extending the storage life of *Citrus sinensis*

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ABSTRACT

The use of conventional food packaging materials is usually effective in terms of barrier. On the other hand, their non-biodegradability creates serious environmental problems, motivating researches on edible biopolymer films and coatings to at least partially replace synthetic polymers as food packaging materials. Chitosan is a biopolymer obtained by N-deacetylation of chitin, which is the second most abundant polysaccharide on nature after cellulose. Chitosan forms clean, tough and flexible films with good oxygen barrier, which may be employed as packaging, particularly as edible films or coatings, enhancing shelf life of a diversity of food products. Chitosan films (CH) were plasticized with two hydrophilic compounds, namely, olive oil (CHOO) and vegetable oil (CHVO). Our objective was to investigate the effect of plasticizers on the quality and storability of oranges with chitosan films. The following parameters were measured: weight loss, ascorbic acid content, pH, and firmness. The stability of the plasticized films was observed by storage for 7weeks at ambient temperature of 25±2°C and relative humidity of 55-67%. Prior to storage, the orange samples were surface sterilized using 100mg/L sodium hypochlorite. Results showed that chitosan films plasticized with two hydrophilic compounds was effective in extending the shelf-life of orange when compared to untreated control in the following order: CHOO>CHVO>CH>Control.

1. Introduction

The environmental impact of non-biodegradable plastic material wastes is of increasing global concern. There is an urgent need to develop renewable and environmentally friendly bio-based polymeric materials (Khwaldia et al., 2010). Edible films can help solve the waste disposal problem by partially replacing non-biodegradable plastics. They can also act as a barrier to external influences such as water vapor, oil and oxygen, as a vehicle for functional compounds, and at the same time reducing e.g. bruising and breakage and thus preserving/improving food integrity.

Edible coatings are thin layers of edible component applied to the fruit's surface in addition to or as a replacement for natural protective waxy coatings and act as physical barrier towards carbon dioxide, oxygen and moisture movement for the fruits

(Baldwin et al., 1999). Coatings can be formulated from different components such as hydrocolloids (polysaccharides and proteins), lipids (waxes and resins) and synthetic polymers. These edible materials have different barrier properties against gases and physico-chemical and mechanical characteristics.

Therefore, most coatings are made of more than one material with the addition of low molecular weight molecules including sorbitol, polyols or glycerol that serves as plasticizers (Risse & Miller, 1983; Olivas & Barbosa-Canovas, 2005). Edible coatings are applied on fruits and vegetables to improve appearance, delay ripening, reduce water loss and decay, and extend shelf life, but may also change flavour (Baldwin et al., 1995; Saucedo-Pompa et al., 2007). In fact, semi-permeable coatings can modify internal atmosphere of fruits by changing the composition or concentration of standard atmosphere gases like Control Atmosphere storage, with less expense incurred (Nisperos-Carriedo et al., 1992). The atmosphere created by coatings is strongly related to permeability of coating and fruit respiration rate, and can alter as function of environmental conditions including temperature and humidity (Baldwin et al., 1995).

Chitosan is a natural polymer obtained by deacetylation of chitin, and when compared with other polysaccharides, chitosan has several advantages such as biocompatibility, biodegradability and no toxicity, while also presenting functional properties as bacteriostatic and fungistatic (Dutta et al., 2009; Kumar, 2000). The cationic character of chitosan offers an opportunity to establish electrostatic interactions with other compounds. Due to these characteristics, chitosan has been widely used for the production of edible films (Ziani et al., 2008; Aider, 2010; Rivero, García, & Pinnoti, 2010). Chitosan films present good barrier properties when compared with other polymers such as methylcellulose and corn starch (Debeaufort & Voilley, 2009; García et al., 2009). Also, mechanical properties of chitosan films can be improved e.g. by the addition of plasticizers (Yoshida et al., 2009); however, the presence of such compounds can affect the structure of chitosan films.

The sweet orange (*Citrus sinensis* (L.) Osbeck), is one of the most commonly grown tree fruits in the world (Morton, 1987).Citrus fruits are produced all around the world and world citrus production in selected major producing countries in 2005/2006 is 72.8 million metric tons. Citrus fruits are said to be the first crops in the international trade in terms of values (CIAC, 2002).

The present study was aimed at investigating the suitability of chitosan (CH) as an edible coating with additions of hydrophilic plasticizers, namely vegetable oil (CHVO) and olive oil(CHOO), to extend the shelf-life of orange.

2. Materials and Methods

2.1. Preparation of chitosan

Mature edible dark yellow crabs (*Cancer pagurus*) were collected from the brackish waters of Warri/ Sapele Delta state. They were killed after which the visera and muscles was carefully removed. The exoskeleton particularly the carapace was washed with warm tap water in order to remove foreign materials and remaining muscle particles. The shells were dried at 60^{o}C overnight, ground with a centrifugal grinding mill (Retsch/Brinkmann ZM-1, Westbury, NY), and shell particles between a mesh size of 20 (0.841 mm) and 40 (0.420 mm) were used as starting material. The methods established to extract chitin from crab fish shell waste by No and Meyers (1995) and further processing of chitin into chitosan through autoclaving (No *et al*., 2000) was used to prepare different chitosan samples for this study.

Dried crab shell particles were treated with 1 N NaOH at 65^{o}C for 1 hour at a solid: solvent ratio of 1:10, w/v for deproteinization (DP). Following a washing step, deproteinized shell particles was treated with 1 N HCl at room temperature for 30 minutes at a solid: solvent ratio of 1:1.5, w/v for demineralization (DM). Particles was treated with acetone at 1: 10 w/v concentration, washed, and bleached with 0.315% NaOCl at a 1:10 w/v ratio for 5 minutes for decoloration (DC). Resultant chitin was treated with 50% NaOH at a 1:10 w/v ratio at 121^{o}C/15 psi for 30 minutes for deacetylation (DA). Subsequent washing and drying steps yielded chitosan.

2.2. Source of oranges

Freshly harvested oranges were procured from National Horticultural Research Institute (NIHORT) in Ibadan, Oyo State. They were selected on the basis of size, color and absence of external injuries.

2.3. Surface preparation of the oranges

Surface preparation was primarily to remove all contaminants that would hinder proper coating adhesion and to render a sound clean substrate, suitable for firm bonding. Surface sterilization of the oranges was carried out by soaking them in 25% hypochlorite solution for two minutes.

2.4. Treatments

T_o (control) - Untreated oranges.

T_1 - Oranges coated with 5ml of olive oil mixed with chitosan.

T_2 - Oranges coated with 5ml of vegetable oil mixed with chitosan.

T_3 - Oranges coated with chitosan only.

The treated and untreated oranges were packed in small plastic baskets and each basket contained 20 oranges. The baskets were stored at ambient temperature (27+2oC) and at 55-67% relative humidity. physicochemical and organoleptic assessments were carried out initially, and from 1-7 weeks after coating.

2.5. Firmness

Firmness was measured as the maximum penetration force (N) reached during tissue breakage, and determined with a 5 mm diameter flat probe. The penetration depth should be 5 mm and the cross-head speed of 5 mm s_1 using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), MA. Oranges were sliced into halves and each half was measured in the central zone.

2.6. Percentage water loss

The water content of the orange fruit was determined using the following equation.

Water content (%) $[100 \times M_1\text{-}M_2] / M_1$

Where,

M_1 = Mass of sample before oven drying in g.

M_2 = Mass of sample after drying, in g.

2.7. Ascorbic acid

Ascorbic acid content was measured using 2, 5-6 dicholorophenol indophenols' method described by AOAC (1994).

2.8. pH

After firmness analysis, oranges were cut into small pieces and homogenized in a grinder, and 10 g of ground orange was suspended in 100 ml of distilled water and then filtered. The pH of the samples were assessed using a pH meter (pH-526; WTW Measurement Systems, Wissenschaftlich, Technische Werksta¨tten GmbH, Wellhelm, Germany).

3.0 Result and discussion

3.1. Firmness

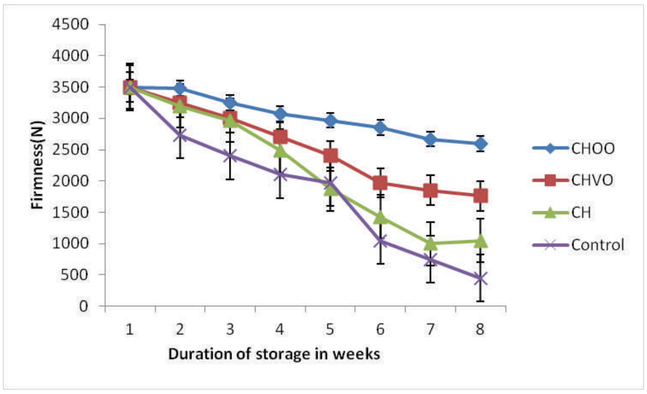

Figure 1: Effect of coatings from chitosan on firmness of orange fruits during storage at ambient temperature. The mean ± SE value for the Firmness on chitosan coatings from CHOO, CHVO, CH on orange fruits were these respectively: 3046.88 ± 108.70 lb/kg/N, 2555 ± 210.57 lb/kg/N, 2187.5±312.45 lb/kg/N, while the mean ± SE value for the uncoated was 1869.5 ± 331.41 lb/kg/N.

Chitosan coatings exerted a beneficial effect on fruit firmness such that, by the end of the storage period, all the treatments gave rise to fruit with higher flesh firmness values than untreated fruit ($P < 0.05$). The beneficial effect of the elevated chitosan concentration on firmness has also been reported for tomato (El Ghaouth et al., 1992b), peach, Japanese pear, kiwifruit (Du et al., 1997) and 'Murcott' tangor (Chien et al., 2007).

Overall better retention of firmness in coated fruits as compared to untreated can be explained by retarded degradation of insoluble protopectins to the more soluble pectic acid and pectin. During fruit ripening, depolymerization or shortening of chain length of pectin substances occurs with an increase in pectin-esterase and polygalacturonase activities (Kashappa & Hyun, 2006). Less availability of oxygen to the coated fruit may be responsible for reduction in the activities of these enzymes and hence retention of the firmness of fruits during storage (Salunkhe et al., 1991). According to the studies by Intalook et al. (2006) chitosan coating materials affected postharvest quality changes of mango fruit cv. Chok Anan. One other factor involved in maintaining the structure of fruits is chitosan coating contained calcium which demonstrated the best results, probably because calcium may interact with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining structure of the fruit (Rolle & Chism, 1987).

3.2. Ascorbic acid

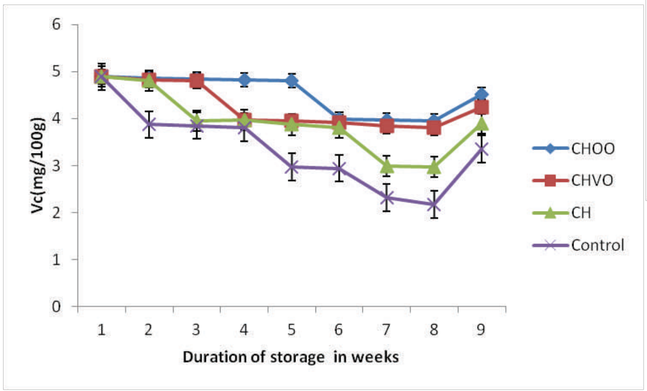

Figure 2: Effect of coatings from chitosan on Vitamin C of orange fruits during storage at ambient temperature. The mean ± SE value for the vitamin C on chitosan coatings from CHOO, CHVO, CH on orange fruits were these respectively: 4.52 ± 0.14 mg/100g, 4.25 ± 0.16 mg/100g, 3.91 ± 0.29 mg/100g, while the mean ± SE value for the uncoated was 3.35 ± 0.29 mg/100g.

The results illustrated in (Figure 2) revealed that there was a significant decrease in ascorbic acid values of chitosan coated fruits along with the storage period. However, the rate of decrease in vitamin C was significantly higher in untreated control fruits as compared with coated fruits. Present studies showed that vitamin C was mostly high in mature but unripe mango fruits and it decreased as the ripening progressed. The reason for high vitamin C content in coated fruit can be attributed to slow ripening rate of chitosan treated fruit. Oxidation of ascorbic acid may be caused by several factors including exposure to oxygen, metals, light, heat and alkaline pH (Sritananan et al., 2005). Coatings served as a protective layer and control the permeability of O_2 and CO_2 (Srinivasa et al., 2002). The ascorbic acid contents in chitosan coated fruits were higher than uncoated fruits at the end of storage. The effect of chitosan was reported with the break of glycosides link to produce different lower molecular weight fragments, which help in protecting the outer and inner surface of fruits (Park et al., 1993). The results congregates with the findings of Jiang et al. (2004) who narrated that ascorbic acid content decreased when longan fruit was coated with chitosan at low temperature 2°C.

3.3. Percentage water loss

Water can increase the rate of several reactions in fruits such as browning, vitamin degradation and enzyme activity, enhance the rate of microorganisms growth and cause texture changes (Smith et al., 1989). Figure 3 shows the weight loss of coated orange as function of chitosan with olive oil and vegetable oil concentrations. As clearly observed, concentration of olive oil and vegetable oil added to chitosan, significantly ($P < 0.05$) decreased the weight loss. This result can be explained by the fact that attractive forces between chitosan molecules (cohesion) increase by increasing the chitosan concentration in edible coating formulation.

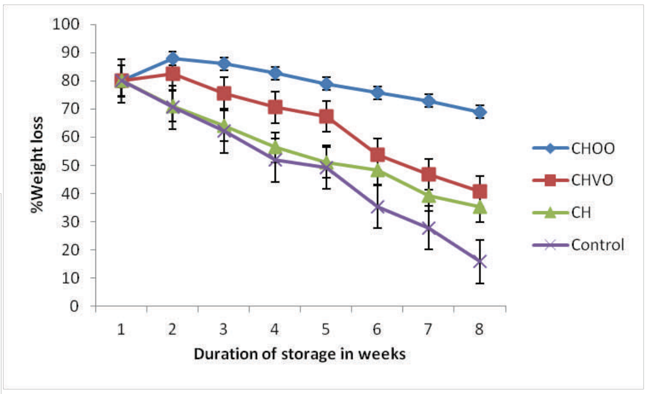

Figure 3: Effect of coatings from chitosan on percentage weight loss of orange fruits during storage at ambient temperature. The mean ± SE value for the percentage weight loss from chitosan coatings CHOO, CHVO, CH on orange fruits were these respectively: 49.09±6.91%, 64.66±4.96 %, 55.64±4.86 %, while the mean ± SE value for the uncoated was 79.15 ±2.04 %.

Result of present study was in agreement with findings of Cien et al. (2007). These authors found that by increasing the concentrations of chitosan in edible coating formulations with low concentration of plasticizer, weight loss of coated fresh cut mango decreased.

Weight loss is caused by respiratory weight loss and evaporation of water from the fruit (Amarante et al., 2001). The main mechanism contributing to weight loss is the evaporation of water activated by a gradient of water vapor pressure at different locations in fruit (Yaman, Ö & Bayoındırlı, L., 2002). Water diffuses preferentially through a liquid aqueous phase in the cuticle, where water conductance is considerably higher, rather than through pores (Amarante et al., 2001). Water loss can cause flesh softening, fruit ripening, and senescence by ethylene production and other metabolic reactions (Bai et al., 2002).

Chitosan coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration (Ribeiro et al., 2007). Chitosan coatings have been effective in controlling water loss from other commodities, including cucumber and pepper (El Ghaouth et al., 1991a), longan fruit (Jiang & Li, 2001), banana and mango (Kittur et al., 2001) and strawberries (Ribeiro et al., 2007).

3.4. pH

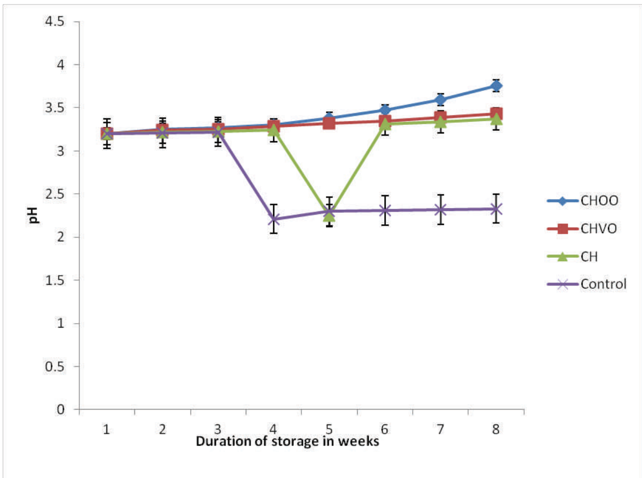

Figure 4: Effect of coatings from chitosan on pH of orange fruits during storage at ambient temperature. The mean ± SE value for the pH on chitosan coatings from CHOO, CHVO, CH on orange fruits were these respectively: 3.4 ± 0.06 , 3.31 ± 0.03 , 3.15 ± 0.12, while the mean ± SE value for the uncoated was 2.64 ± 0.29.

This was probably because the semi-permeable chitosan film formed on the surface of the fruit might have modified the internal atmosphere, i.e., the endogenous CO_2 and O_2 concentration of the fruit, thus retarding ripening (Lowings & Cutts, 1982; Bai et al., 1988).

The pH increased and the titratable acidity decreased significantly ($p<0.05$) along with increased storage time in both coated and uncoated fruits (Figure. 2a). These results agreed with those reported by El-Ghaouth et al. (1991) and Garcia et al. (1998a) that the decrease of acidity during storage demonstrated fruit senescence. It was determined as a small change in pH represents a large change in hydrogen ion concentration (Ball, 1997).

The change in pH is associated with number of reasons; it might be due to the effect of treatment on the biochemical condition of the fruit and slower rate of respiration and metabolic activity (Jitareerat et al., 2007). Coatings slowed the changes on pH and titratable acidity, effectively delaying fruit senescence. This was probably because the semi-permeable chitosan film formed on the surface of the fruit might have modified the internal atmosphere i.e., the endogenous CO2 and O2 concentration of the fruit, thus retarding ripening (Lowings & Cutts, 1982; Bai et al., 1988). The increase in pH may be due to the breakup of acids with respiration during storage (Pesis et al., 1999). Increased activity of citric acid during ripening or reduction in acidity may be due to their conversion into sugars and their further utilization in the metabolic processes of the fruit. Doreyappa & Huddar (2001) reported the similar pattern in different varieties of mango fruits stored at 18-34°C. They observed a series of physicochemical changes during ripening and the major changes were decrease in acidity. The acidity of the fruit is an important character to determine its quality and acceptability. Very high or very low values of the acidity are not recommended for good fruits. Jiang et al. (2004) also reported the effect of chitosan coatings on longan fruit and found that titratable acidity decreased during storage.

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