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Quality and Safety of *Citrus Sinensis* Coated with Hydroxypropylmethylcellulose Edible Coatings Containing *Moringa Oleifera* Extract Stored at Ambient Temperature

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Abstract - Edible coatings based on hydroxypropylmethyl-cellulose(HPMC) with and without an aqueous extract of Moringa leaves, were developed and applied to orange, in order to improve quality and shelf life during storage, while taking advantage of the beneficial health properties of Moringa. Weight loss, firmness and ascorbic acid content of uncoated and coated samples were determined throughout ambient storage. The two experimental coatings were: HPMC without crude extract of Moringa oleifera and HPMCME mixed with 75mg/ml of crude extract of Moringa oleifera . Four hundred and eighty (480) orange fruits were stored for seven weeks at ambient temperature of 27±3oC and relative humidity of 50- 65%. The overall result showed that polysaccharides coating from (HPMC) and (HPMCME) is effective in extending the shelf-life of orange fruits when compared to untreated in the following order:(HPMCME) > (HPMC) >Control.

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QUALITY AND SAFETY OF CITRUS SINENSIS COATED WITH HYDROXYPROPYLMETHYLCELLULOSE EDIBLE COATINGS CONTAINING MORINGA OLEIFERA EXTRACT STORED AT AMBIENT TEMPERATURE

Strictly as per the compliance and regulations of:

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I. Introduction

The sweet orange (*Citrus sinensis* (L.) Osbeck), is the most commonly grown tree fruit 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).

Moringa oleifera is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficiary property. Moringa is a special food for the tropics, because the tree is in full leaf at the end of scarce (Iwu, 1993). It is available all year round. Almost all parts are used as food and forage for livestock (Ram, 1994). The part (leaves, fruits, flowers and immature pods) are edible and form part of traditional diet in many countries of the tropics and subtropics (Odee, 1998).

Edible coatings have long been known to protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavor compounds and reducing microbial growth (Debeaufort, Quezada-Gallo, & Voilley, 1998). Specially formulated edible coatings may provide additional protection against contamination of microorganism while serving the similar effect as modified atmosphere storage in modifying internal gas composition (Park, 1999). According to their components, edible films and coatings can be divided into three categories: hydrocolloids (proteins and polysaccharides), lipids, and composites. Antioxidants, flavors and pigments, vitamins, and antimicrobial agents can be successfully incorporated into edible coatings to improve their functional properties. In the literature, several reviews reported on the efficacy of films and coatings containing antimicrobials to control microbial growth on fruits and vegetables (Ayala-Zavala et al., 2008; Cagri et al., 2004).

The polysaccharides as coating materials for fruits have been used extensively in the past few years. These natural polymers, in addition to the above mentioned benefits, present advantages due to their availability, low cost, and biodegradability. The latter in particular is of great interest, as it leads to a reduction in the large quantities of non-biodegradable synthetic packaging materials (Zhou *et al.*, 2008). In addition, their physico-chemical properties can also be improved by modifying them. Cellulose is a naturally occurring polymer which is found abundantly and is usually present as a linear polymer of anhydroglucose (Kester and

Fennema, 1986). At a molecular level, cellulose is a simple linear polymer comprising of β-[1,4] linked D-glucose molecules. Because of its regular structure and array of hydroxyl groups, it tends to form strong hydrogen-bonded crystalline microfibers, which are insoluble in several solvents (Zugenmaier, 2006).

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Several cellulose derivatives such as methyl cellulose (MC), carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC), and hydroxypropylmethyl cellulose (HPMC) are widely produced commercially (Olivas and Barbosa- Canovas, 2005). These edible coatings have been applied to a variety of fruits to provide moisture, oxygen and carbon dioxide barriers, and to improve adhesion of coating formulations (Kester and

Fennema, 1986). Coatings and films that are based on these cellulose ethers are generally transparent, flexible, odourless, tasteless, water-soluble, and resistant to O_2 and CO_2 (Nisperos-Carriedo *et al.*, 1992).

The aim of this work was to analyze the effect of hydroxypropylmethylcellulose incorporated with Moringa aqueous extract on the development of the physicochemical properties stored at ambient temperature.

II. Materials and Methods

a) *Source of Materials*

Freshly harvested oranges were procured from the local market of Ilorin, kwara state, Nigeria. They were selected on the basis of sized, color and absence of external injuries. Fresh leaves of *Moringa oleifera* was obtained from Nigeria stored products research institute(NSPRI) garden.

b) *Prepration of aqueous extract Moringa oleifera (AEMO)*

Moringa oleifera leaves was dried in an open laboratory and ground into very fine powder using an electric blender (supermaster ®, Model SMB 2977, Japan). The powder was further sieved to pass through 1 mm^2 perforations. The powder was then packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use. Fifty grams of the dried *Moringa oleifera* leaves was weighed and introduced into conical flask containing 250ml of distilled water. The conical flask was then covered with aluminum foil and placed on mechanical shaker. The suspensions was then shaked for 48hours at 190rev.per.min. The extract was decanted and passed through different clean muslin cloth and later filtered with whatman fitter paper. The filtrate obtained was evaporated to dryness at 50^{0}c and the residues obtained are kept in an aluminum foil.

c) *Reconstitution and sterilization of extract*

The dried residue was weighed into McCartney bottles and appropriate volume of distilled water was added to make a stock solution of 75mg/ml, for example 750mg in 10mls of distilled water. The stock solution was then sterilized using 0.65 membrane filter by suction pump. The sterilized extract were stored inside McCartney bottle and kept in a refrigerator.

d) *Surface preparation of the oranges*

The primary purpose of surface preparation was to remove all contaminants that would hinder proper coating adhesion and to render a sound clean substrate suitable for firm bonding. The surface should be in paint ready condition. Mold, mildew and/or algae should be removed and sterilized with a 25% hypochlorite solution (1 gallon household bleach to 3 gallons water). The oranges will be soaked in the 25% hypochlorite solution for two minutes.

e) *Film-forming dispersions methodology*

1. Preparation of edible coatings HPMC

Hydroxypropylmethylcellulose **(HPMC)** coatings (3%) was prepared by dissolving 6.0 g of hydroxypropylmethylcellulose powder (Hangzhou Hongbo Chemical Co. Ltd, China) in 200 ml of water ethyl alcohol mixture (3:1L/L) at 80°C and stirred for 10 min by using magnetic stirrer. Ethyl alcohol was used to reduce drying time and obtain a transparent and shinny coating. 2% propylene glycol was also added in the formulation as plasticizer.

2. Preparation of edible coatings HPMCME:

75mg/ml of **(AEMO)** was added to the resulting solution of HPMC coatings. The mixtures was emulsified at room temperature using a rotor stator homogenizer ultraturrax (DI25 Yellow Line, IKA®, Germany) at 13,500rpm for 4min and then degassed at room temperature by means of a vacuum pump.

f) *Application of the coatings*

Oranges fruits were dipped in the film forming dispersions for 1min. Afterwards, they were hung up and dried at room temperature with natural convection for 2–3 h and then stored in perforated basket at 27±3°C and 50–60% R.H.

g) *Treatments*

T_o (control):- T_0 was selected as the control (untreated oranges)

T_1 Oranges was coated with hydroxypropyl-methylcellulose in addition to 75% *Moringa oleifera* (HPMCME)

T_2 Oranges was coated with hydroxypropyl-methylcellulose without 75% *Moringa oleifera* (HPMC)

The treated and untreated will be packed in small plastic basket and each basket contain 20 orange fruits. The basket will be stored at ambient temperature (27+2°C) and at 50-60% relative humidity .Physiochemical analysis will be carried out from 1-7weeks of coating.

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 was 5 mm and the cross-head speed was 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.

Ascorbic acid: - Ascorbic acid content was measured using 2, 5-6 dicholorophenol indophenols' method described by A.O.A.C 1994.

Percentage weight loss:- The water content of the orange fruit was determined using the Equation (1).Water content(%) $100 \times \frac{M_1 - M_2}{M_1}$

Where: M_1 = Mass of sample before drying in g. M_2 = Mass of sample after drying, in g.

h) Statistics

The results of this investigation are means of seven measurements. To verify the statistical significance of all parameters the values of means ± S.E. were calculated. SPSS software (version 12.0, SPSS Inc., US) was used for all statistical analysis for Analysis of variance. The significance level used was 0.05.

III. Results and Discussion

a) Weight loss

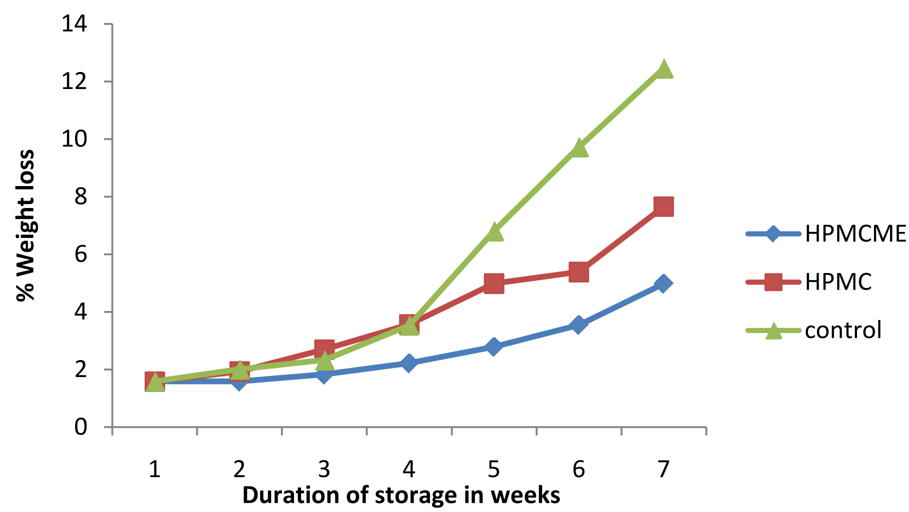

Fig1 : Effect of edible coatings from HPMCME and HPMC on % weight loss of orange stord at amblent temp

Weight loss which occurs in orange fruit during ripening is due to both two transpiration and respiration. Transpiration is associated with water vapour pressure of the surrounding atmosphere and the orange surface. Weight loss contributed by respiration is a result of carbon atoms, in the form of carbon dioxide molecules, leaving the fruits (Park, 2000).

The mean±SE value for the weight loss of HPMCME and HPMC were 40.58±0.47 and28.56± 0.98 while the mean±SE value for the weight loss of uncoated oranges was 26.71±1.04.

The result was most likely due to the fact that edible coatings based on hydrocolloids, because of their hydrophilic natures have poor barrier properties towards water vapour transition (Olivas and Barbosa-Canovas, 2005). Because of the regular structure and array of hydroxyl groups of cellulose derivatives, they tend to absorb and form strong hydrogen- bonds with water molecules of fruits and environment (Togrul and Arsalan, 2004).. Banks (1984), Maftoonazad and Ramaswamy (2005) and Navarro-Tarazaga *et al.*, (2008) found that edible coatings based on Na-CMC, HPMC and MC effectively decreased weight loss of banana, mandarin and avocado, respectively.

a) Firmness

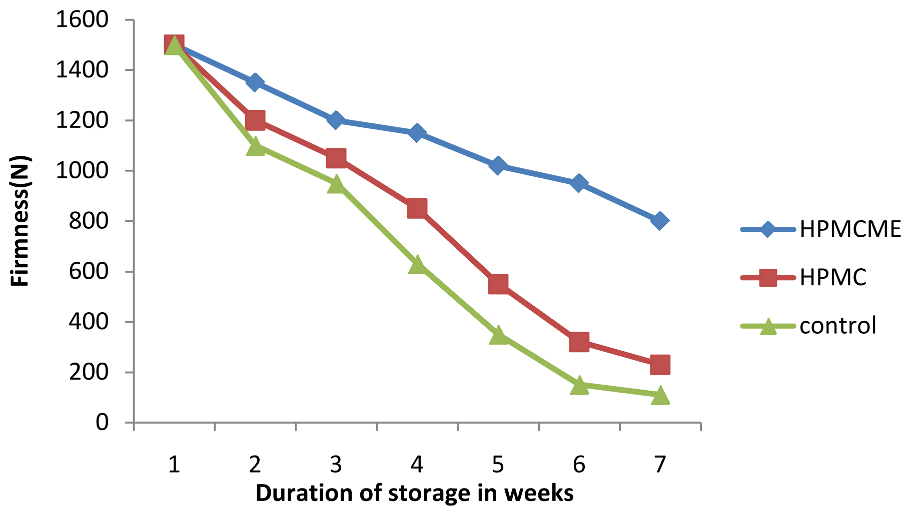

Fig 2 : Effect of edible coatings from HPMCME and HPMC on firmness of orange at ambient temp

In general, the firmness of orange fruit during ripening decreases. Softening in orange fruit may be largely due to the breakdown of starch to form sugars since starch granules could have a structural function in the cells (Cano *et al*., 1997). This could possibility be due to the breakdown of celluloses and other non pectic polysaccharides under the catalysis of cell wall hydrolysis enzymes (Wills *et al*., 1998). Figure 2 shows the firmness of coated and control oranges samples. As clearly shown in Figure 2, all edible coatings significantly ($P< 0.05$) retard the changes in orange firmness as compared to control samples. The effects of HPMCME and HPMC coatings on firmness of orange fruits stored at ambient temperature are shown in Fig.1 above .The mean ± SE values for the firmness of coated HPMCME and HPMC oranges were 7970±88.94 and 5700± 177.76 respectively while the mean±SE value for the firmness of uncoated oranges was 4790±197. Navarro-Tarazagav *et al*. (2008) also found that by increasing the concentration of fatty acids in edible coating based on HPMC, the weight loss of coated mandarins decreased but permeability of coating towards O_2 and CO_2 increased.

b) Ascorbic acid (AsA)

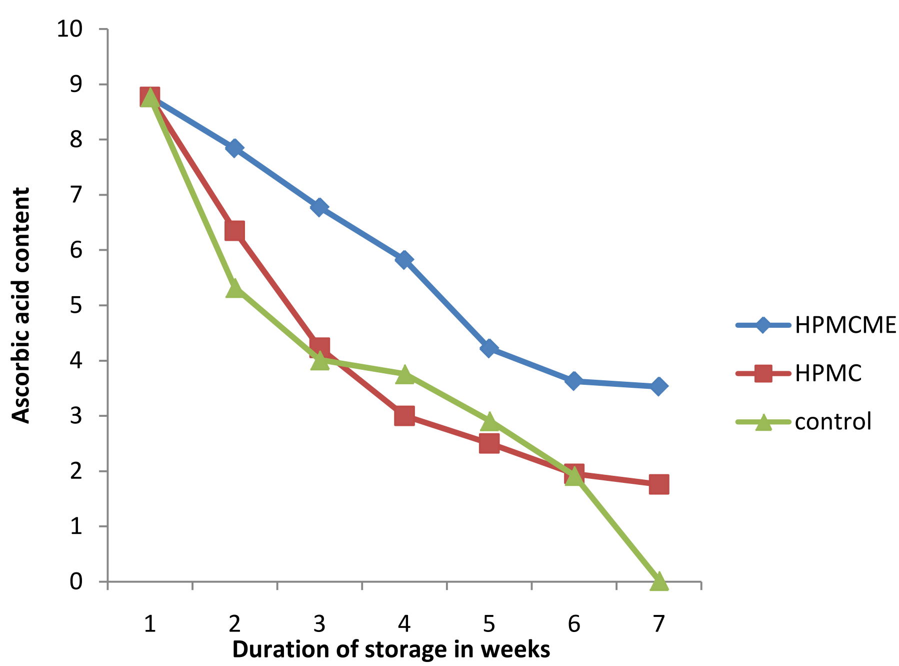

Fig 3 : Effect of edible coatings from HPMCME and HPMC on ascobic acid content of orange stored at ambient temp

The AsA content in the orange of storage at ambient temperature shown in Figure 3 above. The mean±SE value for the coated HPMCME and HPMC on vitamin C were 18.48 ± 0.47 and 27.77±0.81 for coated oranges while The mean±SE value for the vitamin C for uncoated oranges was 38.40±1.61.

The AsA content of coated HPMCME and HPMC treatment decreased gradually with prolonged storage time. Throughout the storage period, there were significant differences between control, coated HPMCME and HPMC ($P < 0.05$). The decrease in AsA level was associated with a reduced capability of preventing oxidative damage and with the incidence of physiological disorders during storage (Lin et al., 2008). AsA in a higher level in coated HPMCME and HPMC fruits than in control, similar to that reported by Dang et al. (2010), who found that the content of AsA of CA-coated fruits was higher than that of the control.

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