International Food Research Journal 23(5): 1902-1910 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Efficacy of nata de coco-carboxymethyl cellulose-based composite coating on the storage quality of calamundin [*xCitrofortunella microcarpa* (Bunge) Wijnands] fruits

[1*]San Pascual, J.C.K., [1]Sabularse, V.C., [2]Castillo-Israel, K.A.T. and [3]Serrano, E.P.

[1]Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna, Philippines 4031
[2]Food Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines 4031
[3]Postharvest and Seed Sciences Division, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines 4031

Article history

Received: 1 September 2015
Received in revised form: 28 December 2015
Accepted: 14 January 2016

Keywords

Calamundin
Carboxymethylcellulose
Coatings, Nata de coco
Nata cellulose

Abstract

Nata de coco-based carboxymethylcellulose or carboxymethyl-nata (CMN) was prepared from raw nata cellulose by two cycles of mercerization and etherification. The product had a degree of substitution (DS) of 0.61, a degree of polymerization (DP) of 992, molecular weight of 1.61 × 10^5 g mol^{-1}, and water solubility of 48.0 g 100 mL^{-1}. Composite coatings were prepared by blending the CMN product with either coconut or palm oil together with some additives. The coating formulations were applied on calamundin fruits to assess the effect of the coating on the storage life of the fruits. CMN and palm oil composites exhibited the most desirable effects in terms of the storage quality of calamundin fruits. These coatings caused delayed peel colour development, lowered percent weight loss, and minimal shriveling. Chemical analyses of the juice extracts showed that the pH, total soluble solids, titratable acidity and ascorbic acid content of the CMN and palm oil composite coated fruits indicate better storage quality.

Introduction

Carboxymethyl cellulose (CMC) has been widely used in edible coatings for fresh fruits and vegetables (Niazmand *et al*., 2009; Bahri and Rashidi, 2009). A cellulose derivative using the bacterial cellulose *nata de coco* has also been synthesized (Manguiat and Sabularse, 2001; Sabularse *et al*., 2009). The derivative is herein referred to as carboxymethyl-nata (CMN). *Nata de coco* is a coconut gel product of the bacterial fermentation of a mixture of coconut water or milk, refined sugar, glacial acetic acid and the mother liquor of the bacterium, *Acetobacter aceti* subspecies *xylinum* (Sanchez, 1990). The product is pure cellulose with a high degree of crystallinity and mechanical strength in the hydrated state (Ross *et al*., 1991).

Sabularse *et al*. (2009) reported the potential use of CMN as an edible coating for the shelf-life extension of bell pepper fruits. The CMN coatings reduced the rate of ripening of bell pepper fruits. Arnon *et al.* (2014) and Arnon *et al.* (2015) investigated the use of edible natural biodegradable coatings to replace commercial synthetic waxes as coatings for citrus fruits. Of the polysaccharides examined CMC gave mandarin fruits that were the most firm, had the least weight loss and adequate gloss and a CMC/chitosan bilayer coating improved fruit quality (Arnon *et al*., 2015). Adetunji *et al*. (2013) reported that a CMC coating containing a crude extract of *Moringa oleifera* applied on oranges and stored at ambient temperature was effective in extending the shelf life of the fruits.

Calamundin [x*Citrofortunella microcarpa* (Bunge) Wijnands] is a native fruit of the Philippines. It is a major fruit crop of the country. Its primary demand is for the fresh market but currently demand is high for its processed forms like juices (Pabuayon, 2000). In calamundin fruits, the most evident manifestation of postharvest physiological change is peel yellowing as well as shriveling accompanied by substantial loss of juice content. At ambient conditions, the shelf life of citrus fruits like the calamundin is only about five days to two weeks as shriveling and decay occur and account for the short shelf life (PCARRD, 1980). This study aimed to develop *nata de coco*-based carboxymethyl cellulose coatings, apply it onto calamundin fruits and determine its efficacy as a postharvest technology in extending the shelf life of calamundin.

*Corresponding author.
Email: *jksanpascual@up.edu.ph*

Materials and Methods

Nata de coco cubes were purchased from Alaminos, Laguna, Philippines and directly transported to the Biochemistry Research Laboratory of the Institute of Chemistry. Batangas-grown calamundin fruits at mature and unripe green stage were obtained from the Divisoria Public Market, Manila, Philippines and brought directly to the Postharvest Horticulture Training and Research Center (PHTRC). The calamundin has the scientific name [*xCitrofortunella microcarpa* (Bunge) Wijnands] as identified by the Museum of Natural History. All three institutions are based at the University of the Philippines Los Baños, College, Laguna, Philippines.

Preparation and physico-chemical properties of carboxymethyl-nata

The preparation of CMN was based on the method described by Sabularse *et al.* (2009) with some modifications. The *nata de coco* cubes were thoroughly washed, manually cut into smaller pieces and homogenized using a blender for three minutes. Manual pressing was done to separate the pulp. The nata pulp was washed at least three times by mixing with distilled water in a blender for one minute at liquefy speed and then pressing out the water. The nata pulp was pre-treated by soaking in analytical grade isopropyl alcohol for about seven hours at room temperature (RT) with a nata pulp to isopropyl alcohol ratio of 1:3 (w/v).

The pretreated nata pulp was subjected to mercerisation. Nata pulp and 40% NaOH solution at a 1:1 (w/v) ratio were intermittently mixed at RT for eight hours at puree speed for 2.5 min at 15-min intervals, allowed to stand overnight and then filtered. The alkali cellulose was etherified by treatment with aqueous monochloroacetic acid, $ClCH_2COOH$ (1.35 g mL^{-1} H_2O) at 1:1 (w/v) ratio at RT for eight hours with mixing for 2.5 min at 15-min intervals. The pH of the crude CMN was adjusted to 6.5-7.5 using 20% NaOH and 20% HCl. The crude CMN was treated with 95% ethanol, pressed in a cheesecloth and washed with 80% ethanol five to six times to remove sodium chloride and sodium glycolate that may have formed during carboxymethylation. The purified CMN was dried in a forced-draft oven (Boekel Scientific, Feasterville, PA, USA) at 70-75°C for four to five hours, pulverized in a Rotor Speed-mill Pulverisette (Fritsch GmbH, Idar-Oberstein, Germany) at 0.2 mesh and stored in a screw-cap polyethylene container. Derivatization was repeated to increase degree of substitution.

The synthesized CMN was analysed for its physico-chemical properties. Moisture content was determined using the oven-drying method (AOAC, 1995), degree of substitution (DS) by the ASTM (1983) D1439-83A 'Testing NaCMC' method (ASTM, 1983), and intrinsic viscosity of CMN solutions by the capillary flow method using a Cannon-Fenske viscometer (Cannon Instrument Co., State College, PA, USA). Molecular weight was calculated from intrinsic viscosity data using the Mark-Houwink equation. Degree of polymerization (DP) was determined according to the method of Swenson (1963). Solubility in water was determined by the method of Schoch (1964). A known amount of CMN was dissolved in a volume of water and centrifuged at 3,000 rpm in a tabletop centrifuge (EBA III Type 2009; Hettich, Tuttlingen, Germany) at RT to separate the undissolved portion. The residue was washed with 80% ethanol two to three times, dried to constant weight in an oven and the percentage dissolved CMN calculated.

Carboxymethyl-nata¬ as coating for calamundin

Medium-sized, green calamundin fruits were randomly grouped into two with each group divided into different sets. The sets for the first group, each assigned with different treatments, consisted of three replicates with eight fruits each. These sets were used for physical analyses that included monitoring weight loss, change in peel colour, and shriveling. The second group of 10 fruits per set was used for chemical analyses.

Preliminary experiments indicated that a 1% CMN concentration was appropriate for the coating formulations to retard peel colour change. The coatings used were the blank (additives only), 1% CMN (CMN), coconut oil (CO), palm oil (PO), 1% CMN + 1% coconut oil + additives (CMN + 1% CO), 1% CMN + 2% coconut oil + additives (CMN + 2% CO), 1% CMN + 1% palm oil + additives (CMN + 1% PO), and 1% CMN + 2% palm oil + additives (CMN + 2% PO). Uncoated calamundin fruits served as control. The additives included a protein, an acidulant/antioxidant, an emulsifier and plasticizer.

The coatings were prepared by dissolving CMN in a minimal amount of 10% NaOH to attain 80-90% dissolution while stirring with a magnetic stirrer and heating the mixture. Previously heated additives and oil were mixed with the alkali CMN solution and stirring was done until the resulting mixture was homogeneous. The coating formulations were brushed on the fruits. The coated fruits were placed in open Styrofoam trays lined with cling wrap and allowed to dry at RT prior to storage at 25 ± 2°C. Physical changes were monitored at 2-day intervals

until dramatic changes were observed. Chemical analyses were conducted on the initial and final days of the storage period.

Physico-chemical properties of coated calamundin fruits

Physical analyses of the calamundin fruits included monitoring of peel color change and shriveling. Peel color change was rated using a visual chart of numerical colour indices developed by the Postharvest Horticulture Training and Research Center (PHTRC), University of the Philippines Los Baños. The colour ratings are: 1 = full green; 2 = green with traces of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with traces of green; 6 = full yellow. Shriveling was evaluated using the shriveling indices of PHTRC where 1 = turgid, no shriveling; 2 = slightly shriveled; 3 = moderately shriveled; 4 = severely shriveled. Weight loss was expressed as percent weight loss relative to the initial weight.

The juice from ten fruit samples per treatment was extracted at day 0 and at the end of the storage and subjected to chemical analyses that included determination of pH, titratable acidity, total soluble solids and ascorbic acid content. The pH of the juice was measured using a Cyberscan 1000 pH meter (Eutech Instruments, Singapore). A given volume of juice was titrated with standard 0.1 N NaOH to determine titratable acidity (%TA), which was expressed in meq acid per 100 g (%meq) citric acid. Total soluble solids (TSS) was determined by the refractometry method (AOAC, 1995) where 2-3 drops undiluted juice extracts were placed in a hand-held Atago °Brix refractometer. Ascorbic acid (vitamin C) content of the juice extracts was determined using the method of Del Rosario *et al.* (2003). Ascorbic acid (25 mg) was dissolved in 100 mL 0.4% oxalic acid and a one mL aliquot was titrated with 0.04% aqueous indophenol solution to the rose pink end point and the dye factor was calculated. Two mL of the juice extracts was diluted to 10 mL with 0.4% oxalic acid solution and was also titrated with 0.04% aqueous indophenol solution to the rose pink endpoint. The ascorbic acid content, expressed as mg ascorbic acid per 100 g extract, was calculated from the volume of the dye used for titration and the dye factor.

Statistical analyses

Analysis of Variance (ANOVA), using the SAS System employing the Least Significant Difference (LSD) Test, was used to evaluate physical analyses data and Duncan's Multiple Range Test (DMRT) in the chemical analyses data.

Table 1. Physico-chemical properties of synthesized CMN

Physico-Chemical Properties	*Synthesized CMN*
Appearance	White powder
DS	0.61
Intrinsic Viscosity	0.0648
DP	991.65
MW (g/mol)	160,650

Results and Discussions

The CMN product was a white powder with a DS of 0.61, moisture content of 29.15%, DP of 992, molecular weight of 1.61×10^5 g mol^{-1}, and water solubility of 48.0 g 100 mL^{-1} (Table 1). The DS indicates the efficiency of the derivatization process. This represents the number of carboxymethyl groups attached to the anhydroglucose unit of cellulose. The DS of the CMN product was comparable to reported values (Fundador *et al*., 2003; Sabularse *et al*., 2009). This satisfactory DS can be attributed to the greater accessibility of the mercerizing and etherifying agents to the cellulose. Moreover, the second cycle derivatization allowed further swelling of the amorphous regions not accessed in the first cycle, thereby facilitating reaction with the etherifying agent (Fundador *et al*., 2003).

CMN coating formulations for calamundin

From preliminary experiments (data not shown) a coating consisting of 1% CMN + additives generally exhibited the desired characteristics of coated fruits. These included minimal moisture loss, less shriveling and minimal physiological defects. Using this CMN concentration and the additives, coating formulations with a lipid component added, were prepared. The hydrophobicity of the lipid enhances the moisture barrier properties of the coating (Bourtoom, 2009).

In general, edible coatings contain a lipid, carbohydrate, and protein component to improve their properties. Acidulants are added for their antimicrobial properties (Baldwin, 1994). Plasticizers and emulsifiers are used to improve mechanical and permeability characteristics (Turhan *et al*., 2007; Seixas *et al.*, 2013) while proteins improve the mechanical strength of a coating (Bourtoom, 2009).

Physico-chemical changes of coated calamundin fruits during storage

Newly harvested mature calamundin fruits are generally full green in colour and change to yellow with time. The change is caused by photodegradation that occurs upon bleaching of chlorophyll pigments by light and oxygen. Photochlorophyllases and pH

Table 2. Peel colour changes of calamundin fruits as affected by different coating during storage at 25°C

	Peel Colour Index*					
	Storage Time (Days)					
	0	2	4	6	8	10
Control	1.96^{ab}	2.13^{ab}	2.58^{ab}	3.13^{a}	3.58^{a}	4.46^{a}
Blank	1.79^{c}	1.83^{dc}	2.13^{cd}	2.75^{ab}	3.04^{b}	3.50^{bc}
CMN	1.88^{bc}	1.88^{bc}	2.25^{bcd}	2.67^{ab}	2.67^{ab}	2.96^{cd}
Coconut oil (CO)	2.00^{ab}	2.00^{ab}	2.25^{bcd}	2.42^{bc}	2.42^{bc}	2.58^{de}
Palm oil (PO)	1.75^{c}	1.75^{c}	2.00^{d}	2.13^{c}	2.08^{d}	2.29^{d}
CMN + 1% CO	2.00^{ab}	2.13^{ab}	2.33^{abcd}	2.75^{ab}	2.88^{bc}	3.13^{bcd}
CMN + 2% CO	2.00^{ab}	2.08^{abc}	2.42^{abc}	2.96^{a}	3.08^{ab}	3.67^{b}
CMN + 1% PO	2.08^{a}	2.29^{a}	2.63^{a}	2.96^{a}	3.08^{ab}	3.58^{bc}
CMN + 2% PO	2.04^{a}	2.25^{a}	2.46^{abc}	2.46^{bc}	2.79^{bc}	3.67^{b}

* Each value is a mean of 3 replicates with each replicate consisting of 8 fruits. Means within the same column with the same superscript are not significantly different using LSD test at $\alpha = 5\%$.

changes contribute to photodegradation (Wills *et al*., 1981). Temperature and humidity also influence chlorophyll loss. Changes in chlorophyll content cause dramatic alterations in postharvest colour. However, lipid membranes and other pigments like the carotenoids protect chlorophyll (Whitaker, 1996).

Citrus fruits, being non-climacteric, synthesize very small quantities of ethylene (Baldwin and Biggs, 1983) but generally respond to the plant hormone on exposure (Brecht *et al.*, 2009). Degreening of citrus fruits is associated with ethylene (Grierson *et al*., 1986) as it induces chlorophyll degradation by stimulating gene expression for chlorophyllase (Brecht *et al*., 2009). The ultimate breakdown of chlorophyll requires chlorophyllase (Purvis and Barmore, 1981).

Control fruits exhibited the fastest rate of peel colour change (Table 2). Almost all fruits were predominantly yellow, PCI 5, on the 10th day of storage. The fruits coated with CO and PO only significantly showed the slowest rate of peel colour change. On day 10, these fruits had peel colour indices between PCI 2 (green with traces of yellow) and PCI 3 (more green than yellow). Lower peel colour indices throughout storage were observed in fruits with coatings containing palm oil. The lipid may have protected chlorophyll from direct oxidation. The susceptibility of the double bond of the oleic acid residues in palm oil may have protected the underlying peel layer pigments from direct oxidation. Palm oil acted as the sacrificial lipid. The fruits were still coloured green with a tinge of yellow (PCI 2) on day 10.

The use of CMN only and CMN in combination with coconut oil and palm oil delayed peel colour development compared to the uncoated fruits. However, no significant differences were noted among the sets of fruits with coatings containing combinations of CMN and lipid probably because CMN being a polysaccharide has good gas barrier properties. Polysaccharide coatings have the ability to reduce O_2 and increase CO_2 levels in internal atmospheres, reduce respiration rates and prolong shelf life of fresh produce (Nisperos-Carriedo, 1994). In the CMN coated fruits, CO_2 levels may have been elevated such that through competitive inhibition of ethylene action and inhibition of ethylene biosynthesis by the reduced levels of O_2 (Brecht *et al*., 2008), the green colour of calamundin was retained. Ethylene is necessary for chlorophyll degradation in calamundin and Robinson tangerine fruits, and thus, the build up of CO_2 inhibited chlorophyll degradation (Purvis and Barmore, 1981).

The marketability of shriveled fruits is greatly reduced; they become less appealing to consumers. Moisture loss causes fruit shriveling. Shriveling is accompanied by peel contraction and sometimes loss of firmness. The fruits coated with CMN only significantly showed the greatest shriveling, even greater than the control, due to enhanced hydophilicity of CMN (Table 3). This was observed as early as the 2nd day of storage. However, shriveling indices for fruits coated with CMN and PO were generally lower than that of the CMN only coated fruits. It was surmised that CMN was primarily responsible for these effects. The formulations may have restricted volatile flavours and moisture from escaping the fruit. Shrivelling indices of fruits coated with CMN and 2% PO were significantly less than those coated with CMN and coconut oil starting on the 6th day of storage. No significant differences were observed in fruits coated with formulations containing 1% and 2% of the lipid. Results imply that the type of oil may be an important contributing factor in determining the shriveling retardation. However, lipid concentration did not exhibit any significant effect. After harvest, fruits exhibit metabolic activity and continue to respire. In nonclimacteric fruits like

Table 3. Shriveling of calamundin fruits coated with formulations and stored for 10 days at 25°C

	Shrivelling Index*					
	Storage Time (Days)					
	0	2	4	6	8	10
Control	1.00	1.04^{c}	1.58^{d}	2.13^{cd}	2.17^{cd}	2.33^{d}
Blank	1.00	1.00^{c}	1.21^{e}	2.08^{cd}	2.21^{cd}	2.67^{bcd}
CMN	1.00	1.54^{a}	2.58^{a}	2.94^{a}	2.92^{a}	3.13^{a}
Coconut oil (CO)	1.00	1.00^{c}	1.58^{d}	1.83^{d}	1.96^{d}	2.58^{cd}
Palm oil (PO)	1.00	1.00^{c}	1.71^{cd}	2.08^{cd}	2.08^{d}	2.50^{cd}
CMN + 1% CO	1.00	1.08^{bc}	2.08^{b}	2.71^{ab}	2.79^{ab}	3.08^{ab}
CMN + 2% CO	1.00	1.21^{b}	2.04^{b}	2.42^{bc}	2.50^{bc}	2.92^{abc}
CMN + 1% PO	1.00	1.04^{c}	1.83^{bcd}	2.00^{d}	2.17^{cd}	2.50^{cd}
CMN + 2% PO	1.00	1.04^{c}	1.92^{bc}	1.92^{d}	2.00^{d}	2.42^{d}

* Each value is a mean of 3 replicates with each replicate consisting of 8 fruits. Means within the same column with the same superscript are not significantly different using LSD test at α = 5%.

Table 4. Percent (%) weight loss of calamundin fruits coated with formulations and stored for 10 days at 25°C

	Weight loss (%)	
	Day 0	Day 10
Control	0.00	29.77^{a}
Blank	0.00	29.99^{a}
CMN	0.00	27.40^{ab}
Coconut oil (CO)	0.00	26.21^{bc}
Palm oil (PO)	0.00	25.77^{bc}
CMN + 1% CO	0.00	24.04^{cd}
CMN + 2% CO	0.00	21.77^{de}
CMN + 1% PO	0.00	20.83^{e}
CMN + 2% PO	0.00	20.23^{e}

* Each value is a mean of 3 replicates with each replicate consisting of 8 fruits. Means within the same column with the same superscript are not significantly different using LSD test at α = 5%.

citrus fruits, respiration gradually declines during ripening (Tucker, 1993). Loss of moisture and volatile compounds accompany these biochemical processes resulting in weight loss.

In citrus fruits, most of the water vapour and other gases moving through detached fruits flow freely throughout the spongy parenchyma of the mesocarp (albedo) and the central axis, diffuses through the exocarp and coloured portion of the rind (flavedo), and evaporates on the surface (Kaufmann *et al*., 1956). Diffusion of these compounds is restricted by the presence of waxes that are predominantly made up of long chain alcohols, fatty acids and esters. Coating formulations with lipid components have been shown to significantly reduce moisture loss and subsequent shriveling by acting as moisture barriers (Tanaka *et al*., 2001; Prodpan *et al.*, 2005).

The fruits coated with pure lipid exhibited significant reductions in weight loss compared to the control but more so when CMN was incorporated (Table 4). The percent weight loss of fruits coated with formulations containing CMN in combination with palm oil was significantly lower overall. The conjugation of CMN with the lipid component may enhanced moisture barrier properties due to the combined hydrophobicity of the lipid preventing the escape of water and gas exchange regulation by CMN by retarding the escape of volatile components of the fruit. The type of oil incorporated influenced weight loss of calamundin. The fruits coated with formulations containing CMN and palm oil had significantly lower weight loss than those coated with CMN and coconut oil formulations. These agree with results obtained by Prodpran *et al.* (2005) who reported significantly lower water vapour permeability (WVP) of films containing palm oil or butter than films with shortening. Fatty acid chain lengths affect WVP and hence weight loss. Longer chain fatty acids are expected to decrease WVP because of their greater hydrophobicity regulating water vapour transmission through the coating (Tanaka *et al*., 2001; Prodpran *et al*., 2005). The predominant fatty acid in palm oil is oleic acid, a C18 fatty acid, while that in coconut oil is lauric acid, a C12 fatty acid. The crystallinity of the lipid also plays a role in water vapour permeability (Prodpran *et al*., 2005). When the coating dried up on the surface of the calamundin fruit, the triacylglycerol

Table 5. pH, total soluble solids (TSS), titratable acidity (TA) and ascorbic acid content of juice extracts from calamundin fruits coated with formulations and stored for 10 days at 25°C

	pH (Day 0 = 2.63)	TA (%) (Day 0 = 8.48)	TSS (°Brix) (Day 0 = 7.60)	Ascorbic acid (mg/100 g) (Day 0 = 7.70)
Control	2.53^{g}	7.47^{e}	7.80^{d}	7.64^{c}
Blank	2.60^{de}	7.47^{e}	7.80^{d}	7.28^{c}
CMN	2.63^{de}	9.38^{a}	8.60^{ab}	9.18^{c}
Coconut oil (CO)	2.73^{ab}	7.75^{cde}	8.27^{c}	50.00^{a}
Palm oil (PO)	2.77^{a}	7.67^{de}	8.60^{ab}	43.90^{a}
CMN + 1% CO	2.70^{a}	8.82^{b}	8.73^{a}	30.49^{b}
CMN + 2% CO	2.67^{cd}	9.05^{ab}	8.67^{a}	31.06^{b}
CMN + 1% PO	2.60^{ef}	8.13^{c}	8.40^{bc}	17.23^{c}
CMN + 2% CO	2.57^{fg}	8.86^{b}	8.27^{c}	11.31^{c}

* Each value is a mean of 3 replicates with each replicate consisting of 8 fruits. Means within the same column with the same superscript are not significantly different using LSD test at α = 5%.

molecules in coconut oil must have been arranged in an orderly fashion, giving rise to a more crystalline structure, creating micro-cracks (Prodpran *et al*., 2005) in the surface coating. These cracks could increase water vapour transfer. The long chains of oleic acid in the triacylglycerol molecules of palm oil possibly interspersed with the CMN polymer chains to create a more compact network that regulated moisture transfer from the fruit to the surrounding environment more effectively.

The dates and duration of citrus maturation are related to the time of onset of rapid physiological changes, mainly increases in sugar content (measured as TSS), decreases in acid (measured as %TA), and changes in the amount of extractable juice (Soule and Grierson, 1986). Concomitant with the decrease in acid content is the rise in alkalinity of the juice. However, since citrus fruits are nonclimacteric, postharvest ripening per se has already occurred upon harvest. Thus, postharvest measures are applied in order to preserve the physiological maturity or "ripe" stage of the fruit and to delay senescence.

Table 5 shows the data on pH, total soluble solids, percent titratable acidity and ascorbic acid content. The pH of the juice extracts ranged from 2.5-2.8 after the 10-day storage period. A decrease in pH relative to the initial pH was observed in juices extracted from control fruits, the blank and CMN + 1% PO coated fruits. The decrease in pH can be attributed to the high percentage of moisture loss in these treatments as inferred from their weight loss. When water is lost, the concentration of the solvated hydrogen ions become more concentrated, thus making the juice more acidic. Also, the possible existence of active synthetic pathways producing soluble acids contributing hydrogen ions in fruits during storage would cause a decrease in pH. The CMN only coated fruits retained its initial pH. An increase in pH was observed in all the other treatments. In this case, organic acids may have been utilized as respiratory substrates (Tucker, 1993).

Titratable acidity was observed to increase in fruits coated with CMN only and formulations containing CMN. This increase may be due to the elevated concentration of the acids because of moisture loss or due to the possible production of organic acids during storage. Deshpande and Ramkrishnan (1961) demonstrated that the requisite enzyme systems for the transformation of sugar to citric acid in fruit tissue are active. A decrease in TA was observed in the control, CO, PO and CMN + 1% PO coated fruits. Mbogo *et al.* (2010) reported a decrease in TA of oranges during storage. The decrease in TA may be attributed to metabolic pathways that utilize organic acids (Tariq *et a*l., 2001) as a primary energy and carbon source (Huberman *et al*., 2005). In ripe Garcinia fruit, the decrease in citric acid was ascribed to the presence of an enzyme system responsible for the partial breakdown of the acid (Deshpande and Ramkrishan, 1961).

On storage, pH values decreased and TA values increased for fruits coated with CO, PO and CMN + PO formulations. This relationship is to be expected. Solutions with high TA values would have more hydrogen ions and hence have lower pH. However, in the other treatments this inverse relationship between pH and TA was not observed. Weak acids with very low dissociation constants such as citric acid and ascorbic acid and buffer systems may have been present in the fruit juices (PCARRD, 1980).

The TSS range of the juice extracts was 7.8-8.8 °Brix. All fruit samples had TSS values greater than that prior to storage. Similarly, Mbogo *et al*. (2010) and Hassan *et al.* (2014) also observed an increase in soluble solids content on storage in Valencia and Navel oranges and tangerine fruits, respectively. The

CMN coated fruits had generally significantly higher TSS values than the control and blank fruits. These treatments had low weight loss values and their pH values increased relative to the initial, particularly those coated with CMN + 1% CO and CMN + 2% CO. The increase in TSS suggests that citric acid may have been utilized in the synthesis of sugars (Wills *et al*., 1981). The possible enzymatic degradation of polysaccharides may be another reason for the TSS increase. Echeveria *et al.* (1988) reported that Brix readings increased upon action of cell wall digesting enzymes on citrus pulp due to the solubilization of reducing sugars.

Normally, ascorbic acid contents of citrus fruits decrease after or during storage (Ladaniya, 2008). The ascorbic acid content of the control and blank calamundin fruits decreased on storage. A decrease in ascorbic acid content and an increase in weight loss were observed on storage in coated and uncoated tangerine fruits (Hassan *et al*., 2014) and in Kinnow mandarin fruits (Mahajan and Singh, 2014). In contrast, fruits coated with CMN, lipids and formulations of CMN + lipids had higher ascorbic acid contents than the initial. Some of the glucose produced from the enzymatic degradation of the cell wall polysaccharides may have undergone secondary oxidation pathways resulting in its conversion to glucuronic acid and ascorbic acid (Eskin *et al*., 1971) in the fruit. Barata-Soares *et al.* (2004) reported that L-galactose and L-galactono-1,4-lactone are effective precursors for the biosynthesis of ascorbic acid. These precursors may be derived from glucose through its conversion from one sugar to another to eventually form galactose. The glucose utilized may have been the product of cell wall polysaccharide degradation. This supports the conjecture that biosynthetic pathways are still active in postharvest fruit.

The increase in ascorbic acid content on storage may also be attributed to the pronounced intrafruit ascorbic acid (Soule and Grierson, 1978). The ascorbic acid in the peel is usually five times higher than in the juice. Lincoln (1949) observed in lime that shriveling, TSS and juice amounts increased with storage. He suggested that when citrus fruits undergo shriveling due to water loss, its peel components tend to migrate to the juice. This may imply that with less oxidation, the active form of ascorbic acid can be transferred from the peel to the flesh thereby increasing its concentration. The incorporation of CMN in the coating resulted in a significantly lower final ascorbic acid content compared to those coated with pure lipid. Thus, the coatings may have retarded postharvest changes in the calamundin fruits.

Conclusion

Postharvest changes in the physicochemical characteristics of coated calamundin fruits indicated that the incorporation of CMN in composite coatings with palm oil as the lipid component had desirable effects in extending the postharvest life of the fruits. Results suggested that CMN in the composite coatings functioned mainly as a gas exchange regulator. Along with this, the moisture regulating property of the lipid component, particularly palm oil, contributed to the coatings' effectiveness in maintaining quality and integrity of the calamundin fruits for a relatively longer storage period compared to the uncoated fruits. The CMN + PO composite coatings caused a significant delay in peel colour development compared to the uncoated fruits. Weight loss of these fruits was significantly lower than that of the other coated fruits and the control. Shriveling was minimal and was significantly less than the CO and CMN + CO coated fruits but was comparable to that of the control, blank, PO and CO coated samples. The pH, TSS, TA and ascorbic acid content showed that fruits coated with CMN + PO formulations had better storage quality.

Acknowledgements

The authors wish to thank the Institute of Chemistry, CAS, UPLB for the financial support for this study and the Postharvest and Seed Sciences Division, Crop Science Cluster, CA, UPLB, for accommodating the storage studies.

References

Adetunji, C.O., Fawole, O.B., Arowora K.A., Nwaubani, S.I., Oloke J.K., Adepoju, A.O., Adetunji, J. B. and Ajani, A. O. 2013. Performance of edible coatings from carboxymethylcellulose (CMC) and corn starch (CS) incorporated with Moringa oleifera extract on Citrus sinensis. Agrosearch 13: 77–85.

AOAC. 1995. Official methods of analysis of AOAC International. (16th ed.). Vol. 1. Maryland: Association of Official Analytical Chemists.

Arnon, H., Granit R., Porat R. and Poverenov, E. 2015. Development of polysaccharide-based edible coatings for citrus fruits: A layer-by-layer approach. Food Chemistry 166: 465–472.

Arnon, H., Zaitseva Y., Portat R., and Poverenova, E. 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus. Postharvest Biology and Technology 87: 21–26.

ASTM. 1983. Standard methods of testing sodium carboxymethylcellulose (NaCMC): D 1439-83A, Pennsylvania: American Society for Testing Materials.

Bahri, M.H. and Rashidi, M. 2009. Effects of coating methods and storage periods on some qualitative characteristics of carrot during ambient storage. International Journal of Agriculture and Biology 11: 443–447.

Baldwin, E.A. 1994. Edible coatings for fresh fruits and vegetables: Past, present and future. In: Krochta, J.M., Baldwin, E.A. and Nisperos-Carriedo, M.O. (Eds). Edible Coatings and Films to Improve Food Quality. p. 25–88. Lancester: Technomic Publishing Co., Inc.

Baldwin, E.A. and Biggs, R.H. 1983. Ethylene biosynthesis of citrus peel explants as influenced by cycloheximide. Proceedings of the Florida State Horticultural Society, p. 183. Florida: Florida State Horticultural Society.

Barata-Soares, A.D., Gomez, M.L.P.A, de Mesquita, C.H. and Lajolo, F.M. 2004. Ascorbic acid biosynthesis: A precursor study on plants. Brazilian Journal of Plant Physiology 16: 147–154.

Bourtoom, T. 2009. Edible protein films: Properties enhancement. International Food Research Journal 16: 1–9.

Brecht, J.K., Ritenour, M.A., Haard, N.F. and Chism, G.W. 2009. Postharvest physiology of edible plant tissues. In Damodaran, S., Parkin, K.L. and Fennema, O.R. Food Chemistry. p. 975–1049. New York: CRC Press.

Del Rosario, R.R., Del Rosario, O.M. and Dia, V.P. 2003. Food Chemistry Laboratory Manual. College, Laguna, Philippines: CA Publications Office.

Deshpande, W.M. and Ramakrishnan, C.V. 1961. Formation and breakdown of citric acid in Garcinia fruit *(Xanthochymus guttiferae)*. Journal of Biological Chemistry 236: 2377–2380.

Echeveria, E., Burns, J.K. and Wicker, L. 1988. Effect of cell wall hydrolysis on Brix in citrus fruit. Proceedings of Florida State Horticultural Society. p. 150. Florida: Florida State Horticultural Society.

Eskin, N.A.M., Henderson, H.M. and Townsend, R.J. 1971. Biochemistry of Foods. New York: Academic Press.

Fundador, E.O.V., Sabularse, V.C. and Revilleza, M.J.R. 2003. Immobilization of invertase on carboxymethylcellulose prepared from *nata de coco* for the inversion of sucrose. ASEAN Journal of Science and Technology for Development 20: 37–47.

Grierson, W. 1981. Physiological disorders of citrus fruits. Proceedings of the International Citrus Congress. p. 764. Japan: International Society of Citriculture.

Grierson, W., Cohen, E. and Kitagawa, H. 1986. Degreening. In Wardowski, W.F., Nagy, S. and Grierson, W. (Eds). Fresh Citrus Fruits. p. 254–274. New York: AVI/Van Nostrand Reinhold Company, Ltd.

Hassan, Z.H., Lesmayati, S., Qomariah, R. and Hasbianito, A. 2014. Effect of wax coating applications and storage temperatures on the quality of tangerine citrus *(Citrus reticulate)* var. Siam Banjar. International Food Research Journal 12: 641–648.

Huberman M., Zehavi U., Stein, W.D., Etxeberria, E. and Goren, R. 2005. In vitro sugar uptake by grapefruit *(Citrus paradise)* juice-sac cells. Functional Plant Biology 32: 356–366.

Kaufmann, J., Hardenburg, R.E. and Lutz, J.M. 1956. Weight losses and decay of Florida and California oranges in mesh and perforated consumer bags. Proceedings of the American Society for Horticultural Sciences 67: 244–250.

Ladaniya, M.S. 2008. Citrus fruit: biology, technology and evaluation. London: Academic Press Elsevier, Inc.

Lincoln, F. 1949. Investigation of the proper maturity of Tahiti limes for marketing. Proceedings of the Florida State Horticultural Society. p. 232. Florida: Florida State Horticultural Society.

Mahajan, B.V.C. and Singh R. 2014. Influence of coatings on postharvest physiology and shelf life of Kinnow fruits under super market conditions. Journal of Postharvest Technology 2: 37–44.

Manguiat, L.S. and Sabularse VC. 1996. Physico-chemical and functional properties of carboxymethyl cellulose prepared from *nata de coco*. UP Home Economics Journal 23–24:14–30.

Mbogo, G.P., Mubofu, E.B. and Othman, C.C. 2010. Post harvest changes in physico-chemical properties and levels of some inorganic elements in off vine ripened orange *(Citrus sinensis)* fruits cv (Navel and Valencia) of Tanzania. African Journal of Biotechnology 9: 1809–1815.

Nisperos-Carriedo, M.O. 1994. Edible coatings and films based on polysaccharides. In Krochta, J.M., Baldwin, E.A. and Nisperos-Carriedo, M.O. (Eds). Edible Coatings and Films to Improve Food Quality. p. 305–335. Lancester: Technomic Publishing Co., Inc.

Niazmand, A., Ghodusi, H.B., Shahidi, F. and Niazmand, R. 2009. Effects of three polysaccharide coatings on physiochemical and organoleptic properties of mushroom *(Agaricus bisporus)*. American-Eurasian Journal Agricultural & Environmental Science 5: 740–744.

Pabuayon, I.M. 2000. Fruits program area research planning and prioritization: background analysis. Discussion paper series no. 2000-18. Philippines: Philippine Institute of Development Studies.

PCARRD. 1980. Philippine recommends for citrus. Los Baños, Laguna, Philippines: Philippine Council for Agriculture, Forestry and Natural Resources Research and Development.

Prodpan, T., Chinabhark, K. and Benjakul, S. 2005. Properties of composite film based on bigeye snapper surimi protein and lipids. Songklanakarin Journal of Science and Technology 27: 775–787.

Purvis, A.C. and Barmoor, C.R. 1981. Involvement of ethylene in chlorophyll degradation in peel of citrus fruits. Plant Physiology 68: 854–856.

Ross, P., Mayer, R. and Benziman, M. 1991. Cellulose biosynthesis and function in bacteria. Microbiological Reviews 55: 35–38.

Sabularse, V.C., Montalbo M.N.D., Hernandez H.P. and Serrano, E.P. 2009. Preparation of *nata de coco*-based

carboxymethylcellulose coating and its effect on the post-harvest life of bell pepper (*Capsicum annuum* L.) fruits. International Journal of Food Sciences and Nutrition. 60(7): 206–218.

Sanchez, P.C. 1990. *Nata de coco*. In Banzon, J.A., Sanchez, P.C., Gonzales, O.N. and de Leon, S.Y. (Eds). Coconut as Food. p. 185–199. Manila, Philippines: Philippine Coconut Research and Development Foundation, Inc.

Schoch, T.J. 1964. Swelling power and solubility of granular starches. In Whistler, R.L. (Ed). Methods of Carbohydrate Chemistry vol 4. p. 106–108. New York: Academic Press, Inc.

Seixas, F.L., Turbiiani, F.R.B., Salomao, P.G., Souza R.P., Gimenes M.L. 2013. Biofilms composed of alginate and pectin: effect of concentration of crosslinker and plasticizer agents. Chemical Engineering Transactions 32: 1693–1698.

Soule, J. and Grierson, W. 1986. Anatomy and physiology. In Wardowski, W.F., Nagy, S. and Grierson, W. (Eds). Fresh Citrus Fruits. p. 1–22. New York: AVI/Van Nostrand Reinhold Company, Ltd.

Swenson, H.A. 1963. Intrinsic viscosity and its conversion to molecular weight. Molecular size distribution in cellulose. In Whistler, R.L. (Ed). Methods of carbohydrate chemistry Vol 4. p. 84–95. New York: Academic Press, Inc.

Tanaka, M., Ishizaki, S., Suzuki, T. and Takai R. 2001. Water vapor permeability of edible films prepared from fish water soluble proteins as affected by lipid type. Journal of Tokyo University of Fisheries 87: 31–37.

Tariq, M.A., Tahir F.M., Asi A.A. and Iqbal, J. 2001. Effect of washing and seal packaging on scuffing damaged citrus fruit quality. International Journal of Agriculture and Biology 3: 461–463.

Tucker, G.A. 1993. Introduction. In Seymour, G., Taylor, J. and G. Tucker, G. (Eds). Biochemistry of Fruit Ripening. p. 1–51. London: Chapman & Hall

Turhan, K.N., Sancak, Z.O.E., Ayana, B. and Erdogdu, F. 2007. Optimization of glycerol effect on the mechanical properties and water vapor permeability of whey protein-methylcellulose films. Journal of Food Process Engineering 30: 485–500.

Whitaker, J.R. 1996. Enzymes. In Fennema, O.R. (Ed). Food chemistry. p. 431–530. New York: Marcel Dekker

Wills, R.B.H., Lee, T.H., Graham, D., McGlasson, W.B. and Hall, E.G. 1981. Postharvest: An introduction to the physiology and handling of fruit and vegetables. Westport, CT: AVI Publishing Co. Inc.