

**Effect of coating based on orange epicarp extract on the ripening of avocado (*Persea americana* L.)**Emmanuel Mbatchamen<sup>1,\*</sup>, Mariette Anoumaa<sup>1</sup>, Eugène Tafre-Phounzong<sup>1</sup>, Alain Tchinda Ngotio<sup>1</sup>, Jean Nguemezi Aghofack<sup>1</sup>, Roland Nnomo Douanla<sup>1</sup>, Claude Simo<sup>2</sup><sup>1</sup> Department of Plant Biology, Faculty of Science, University of Dschang, P.O Box 67 Dschang, Cameroon;<sup>2</sup> Department of Plant Biology, Faculty of Science, University of Douala, P.O Box 24157 Dschang, Cameroon

Keywords	Abstract
Avocado; Coating; Orange epicarp extracts; Ripening; Storage.	The shelf life of avocados during the post-harvest period is very limited due to rapid senescence. In order to extend the shelf life of the avocados, the effects of coating based on orange epicarp extracts were tested on the physical and physiological parameters of the ripening process. Avocados were coated with different concentrations of orange epicarp extracts, including 0.066 Kg.l <sup>-1</sup> , 0.133 Kg.l <sup>-1</sup> , 0.2 Kg.l <sup>-1</sup> and 0.26 Kg.l <sup>-1</sup> . Uncoated fruits were considered as control. Twelve parameters including loss of firmness, physiological loss of mass, water content in the pulp, soluble solids content, pH, protein content, pigment content in skin and pulp as well as time to senescence were measured during the ripening process. Results showed that, 0.133 Kg.l <sup>-1</sup> was found to be the most effective extending the time to senescence of avocado by slowing down the loss of firmness, physiological mass loss, chlorophyll degradation and protein synthesis as compared to the other concentrations of orange epicarp extracts and the control. Orange epicarp at a concentration of 0.133 Kg.l <sup>-1</sup> helped to extend the shelf life of avocados.
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**1. Introduction**

The avocado (*Persea americana*; Lauraceae) is a fruit originating from tropical regions, more precisely from Mexico and Central America [1, 2]. It is produced in about fifty countries and is the sixth most exported tropical fruit in the world. In 2010, Cameroon exported 834.4 tons of avocado to neighboring countries (Gabon, Chad and Equatorial Guinea) and Europe for an income of several million CFA francs. In terms of food, the avocado has high energy intake, with an average content of 155 Kcal per 100g. This property is attributed to high lipid content (20.6%) [3, 4]. Because of its high mineral (74.4%) and protein (7%) content, the avocado belongs to the category of building foods. Moreover, it is rich in fat-soluble vitamins (A, D, E and K) which are particularly interesting for health [5, 6].

Like most climacteric fruits, avocados begin their ripening process after harvest with intense physiological and biochemical changes and high production of carbon dioxide and ethylene. Ethylene triggers the ripening process and subsequently the senescence of the fruit, thus reducing its shelf life and marketability [6, 5]. Avocados are also considered to have a high mass loss in the post-harvest period; this mass loss is due to the loss of moisture through transpiration which has an effect on the fruit quality, hence leading to huge economic losses [7, 8]. Due to these characteristics, the control of ripening is essential to increase the fruit shelf life after harvest [2].

Edible films and coatings have been defined as thin protective layers of food that are integral to the food and can be consumed as such [10]. They are traditionally used to improve the appearance and shelf life of foods. Edible coatings are mainly used to reduce gas exchange and mass loss during transport and storage. These edible films and

coatings are generally produced from polysaccharides, proteins, lipids and other compounds with antimicrobial and antioxidant properties [9]. These antimicrobial and antioxidant substances have the ability to inhibit or slow down the growth of pathogenic microorganisms [13] and to improve the organoleptic quality of the fruit and/or extend shelf life [14]. Orange epicarp contains polyphenols and large amounts of ascorbic acid which have antioxidant properties [15]. The general objective of this study was to improve the shelf life of avocados, using the coating technique based on orange epicarp extracts.

**2. Materials and methods****2.1. Biological material**

Mature avocados of the Pollock variety were harvested in Bamendjinda, a village in the district of Mbouda in West region of Cameroon. Bamendjinda is located at 1595 m above the sea level at 5°36'46" North and 10°17'46" East.

**2.2. Preparation of the orange epicarp coating solution and fruits treatment**

The orange epicarp was ground in a blender, then 200g, 400g, 600g or 800g were introduced into a 5l container. A mixture of 2.5l of ethanol/distilled water was (v/v) added to each quantity of epicarp. The mixture was covered and left to stand for 3 hours and then filtered twice by adding 0.5l of ethanol/distilled water (v/v). In order to thicken the filtrate, glycerol was added at 4% rate. Then sodium hypochlorite was added at 0.023% as a disinfectant. Finally, the mixture was left to stand for 15 hours. Thus, coating solutions with different concentrations of orange epicarp extracts, namely 0.066 Kg.l<sup>-1</sup>, 0.133 Kg.l<sup>-1</sup>, 0.2 Kg.l<sup>-1</sup> or 0.26 Kg.l<sup>-1</sup> were prepared by taking 200 g, 400 g, 600 g or 800 g of epicarp paste respectively.

The treatment consisted of soaking each experimental unit of twelve (12) avocados for 30min in a coating solution. Untreated (uncoated) fruits

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were used as control treatment. Each treatment was replicated three times in a randomized design. After soaking, the fruits were stored at room temperature (24±2°C) and at a relative humidity of 80±2%.

### 2.3. Assessment of ripening parameters

Ripening parameters including physicochemical and biochemical parameters were measured every 4 days after soaking in the coating solution until senescence of control fruits. Avocado was considered senescent when black spots appeared on the epicarp.

#### 2.3.1. Physicochemical parameters

- Firmness: this parameter was determined by perforating the pulp of the fruit using a GY-2 brand penetrometer [16]. Measurements were made at three different locations on the same fruit and the firmness was obtained by averaging the three values.

- Water content: it was measured as follows: Five grams of avocado pulp was oven dried at 80 °C to a constant dry mass and the water content (WC) was calculated according to the following formula:

$$WC = \frac{\text{dry matter} - \text{fresh matter}}{\text{fresh matter}} \times 100$$

- Physiological mass loss: the fruits were weighed at the beginning of the experiment and every four days. The masses obtained were used to calculate the physiological mass loss (PML) as follow:

$$PML = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100$$

- pH: fifty grams of avocado pulp were ground in a mortar and homogenized in 50 mL of distilled water. The pH was measured using a pH meter (ATC brand) after filtration.

- Shelf life: this parameter was evaluated by recording the number of days from the date of soaking in the coating solution to the date of senescence.

#### 2.3.2. Biochemical parameters

- Soluble solids content: this parameter was quantified using a refractometer [17].

- Protein content: this parameter was evaluated in the skin using the biuret method (Cooper, 1977) as follows: five grams of skin were ground in a mortar using sand. After adding 50 mL of distilled water, the mixture was homogenized and then filtered. Four milliliters of chloroform were added to the filtrate. After decantation, the aqueous phase was collected and centrifuged at 4000 rpm for 10 minutes. Three milliliters of biuret reagent were added to 2 mL of supernatant. After homogenization, the mixture was incubated for 20 minutes at 37°C in the dark. The optical density (OD) of the extract was read at 540 nm using a spectrophotometer. The protein content was calculated from an albumin standard using the OD obtained.

- Pigment contents: 5g of avocado skin or pulp were ground in a mortar using sand. Pigments extraction was done in the dark by adding 10 mL of acetone to the ground skin or 20 mL of acetone/hexane mix (4/6 : V/V) to the ground pulp [19]. After filtration using Whatman N°1 filter paper, the optical density of the supernatant containing the pigment extracts was read at different wavelengths using a spectrophotometer. The content of each pigment was calculated using a formula developed by Lichtenthaler [18] or Nagata [19] depending on whether it was in the skin or in the pulp respectively.

The concentrations of different pigments in avocado skin were calculated using the following equations:

$$\text{Chlorophyll } a (\mu\text{g/mL}) = 11.75 \text{ A662} - 2.350 \text{ A645}$$

$$\text{Chlorophyll } b (\mu\text{g/mL}) = 18.61 \text{ A645} - 3.960 \text{ A662}$$

The concentrations of the different pigments in the pulp of avocados were calculated using the following equations:

$$\text{Chlorophyll } a (\text{mg}/100 \text{ mL}) = 0.999 \text{ A663} - 0.0989 \text{ A645}$$

$$\text{Chlorophyll } b (\text{mg}/100 \text{ mL}) = -0.328 \text{ A663} + 1.77 \text{ A645}$$

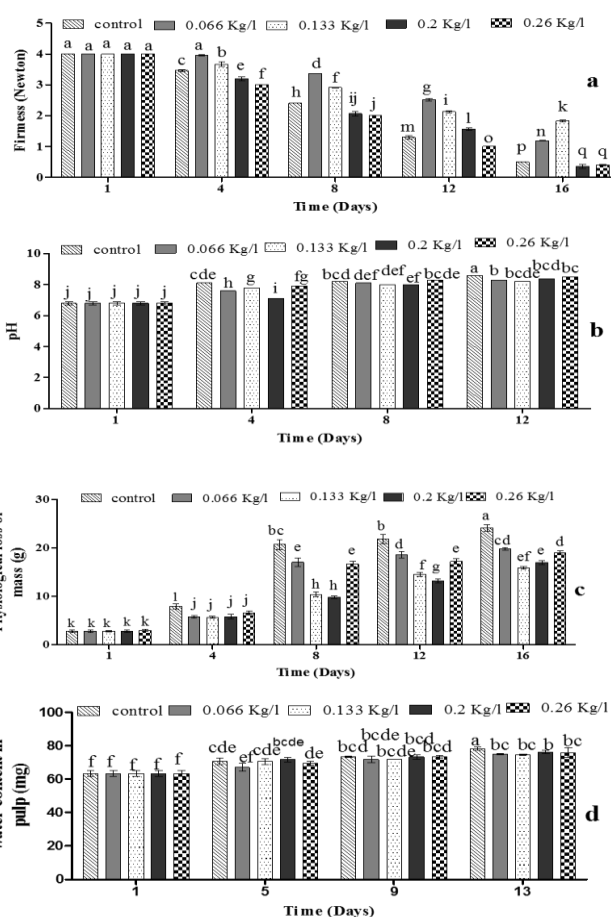
$$\beta\text{-carotene (mg}/100 \text{ mL}) = 0.216 \text{ A663} - 1.22 \text{ A645} - 0.304 \text{ A505} + 0.452 \text{ A453}$$

### 2.4. Data analysis

The data obtained were subjected to an analysis of variance (ANOVA) to check whether there were significant differences between the means. Duncan's multiple comparison test was used separate the means when difference was significant. All the statistical tests were performed on GenStat version 12.1.

## 3. Results

### 3.1. Effect of coating based on orange epicarp extracts on the physicochemical parameters of avocado



**Figure 1:** Influence of the concentration of the orange epicarp extracts on four physicochemical parameters of avocados during the conservation period. Bars with different letters are significantly different at the 5% probability level. a: firmness ; b: pH; c: Physiological mass loss; d: Water content in the pulp

Figure 1 shows firmness, physiological mass loss, water content and pH of avocados as affected by the concentration of orange epicarp extracts throughout the conservation period.

The firmness of avocados decreased from the 4th day during the conservation period. Fruits coated with 0.066 and 0.133 Kg/l of orange epicarp extract presented significantly high values of firmness compared to that the other treatments throughout the period of the experiment. However, fruits coated higher concentrations (0.2 and 0.26 Kg/l) of extract presented significantly low firmness as compared to uncoated fruits (Figure 1a).

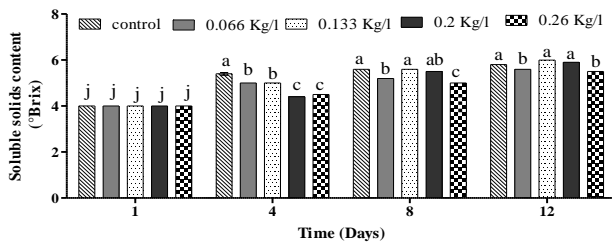
pH value increased from the 4th day with all the treatments. All coated fruits showed lower pH value as compared to uncoated fruits (Figure 1b). Physiological mass loss increased in all fruits from day 4. This parameter was significantly lower in fruits coated with 0.133 kg/l and 0.2 kg/l of orange epicarp extracts than that of the other treatments (Figure 1c).

Water content in the pulp of avocados increased from day 4 in all fruits. Coating at all concentrations of orange epicarp extract did not significantly affect the water content in the pulp of avocados as compared to the control (Figure 1d).

### 3.2. Effect of coating based on orange epicarp extracts on the biochemical parameters of avocado

#### 3.2.1. Soluble solids content

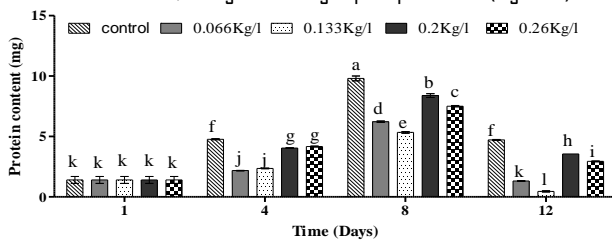
Soluble solids increased during ripening in coated and uncoated fruits. Fruits coated with the highest concentration of orange epicarp extract (0.26 kg/l) had significantly lower soluble solids content than the other treatments (Figure 2).



**Figure 2 :** Soluble solids content of avocados of avocados as affected by the concentration of orange epicarp extracts during ripening. Bars with different letters are significantly different at the 5% probability level.

#### 3.2.2. Protein content

Protein content in the skin of avocado increased during ripening till day 8 where it started decreasing. This parameter was lower in coated fruits than in uncoated fruits. The lowest value of protein content was recorded in fruits coated with 0.133 Kg<sup>-1</sup> of orange epicarp extracts (Figure 3).

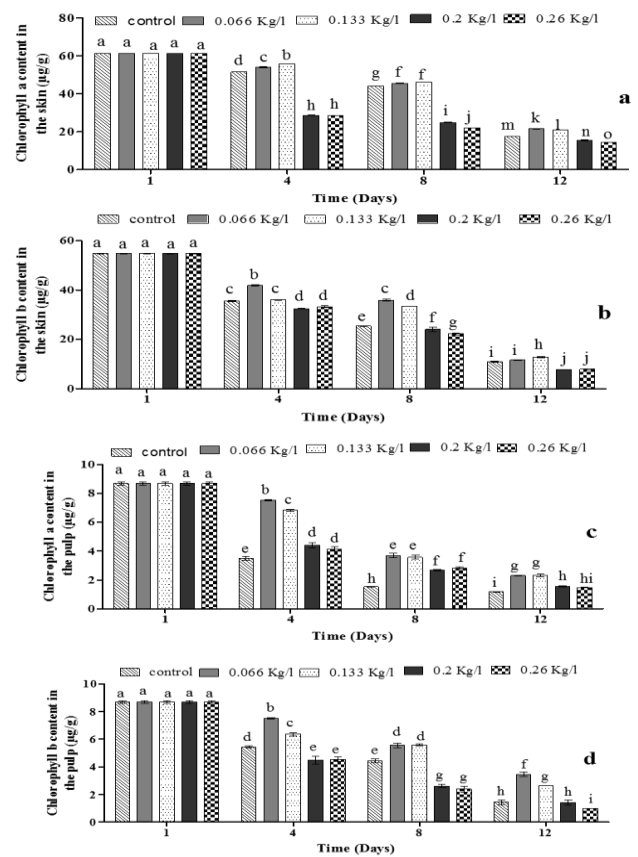


**Figure 3 :** Protein content in the skin of avocados as affected by the concentration of orange epicarp extracts during ripening. Bars with different letters are significantly different at the 5% probability level.

#### 3.2.3. Pigment contents

##### 3.2.3.1. Chlorophylls

Figure 4 shows chlorophyll *a* and *b* contents in the skin and pulp of



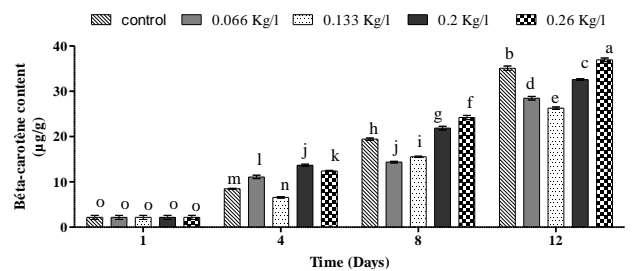
**Figure 4:** Chlorophyll *a* and *b* content in the skin and pulp of avocados as affected by the concentration of orange epicarp extracts during ripening. Bars with different letters are significantly different at the 5% probability level. a: Chlorophyll *a* in skin; b: Chlorophyll *a* in pulp; c: Chlorophyll *b* in skin; d: Chlorophyll *b* in pulp

avocados as affected by the concentration of orange epicarp extracts throughout the conservation period.

Chlorophyll *a* and *b* content in the skin and pulp decreased during ripening in all fruits whether coated or uncoated. Lower concentrations of orange epicarp extract (0.066 and 0.133 kg/l) enabled to obtain higher values of chlorophyll *a* and *b* contents in the skin and pulp as compared to the other treatments. In the skin, higher concentration, reduced chlorophyll *a* and *b* contents as compared to the control; meanwhile, in the pulp, no significant difference was observed between the values obtained with uncoated fruits and fruits coated with higher concentrations of extracts.

##### 3.2.3.2. β-carotene content

β-Carotene content increased in avocado pulp during ripening (Figure 5). Lower concentrations of extract contributed to lower β-carotene content in the pulp as compared to the control.



**Figure 5 :** β-carotene content in the pulp of avocados as affected by the concentration of orange epicarp extracts during ripening. Bars with different letters are significantly different at the 5% probability level

The lowest value of  $\beta$ -carotene content was obtained with 0.133 Kg.l<sup>-1</sup> of orange epicarp extracts. The highest concentration of orange peel extract used in this study (0.26 Kg.l<sup>-1</sup>) increased  $\beta$ -carotene content in the pulp as compared to the control.

#### 4. Discussion

Avocados treated with lower concentrations of orange epicarp extract (0.066 Kg.l<sup>-1</sup> and 0.133 Kg.l<sup>-1</sup>) showed the highest firmness at the end of the experiment. These concentrations in solution enabled to form a thin layer suitable to create a medium probably composed of appropriate balance of CO<sub>2</sub> and O<sub>2</sub> which lead to moderate respiration while reducing fermentation as previously reported [24, 25]. Appropriate volume of carbon dioxide inhibits ethylene binding to membrane receptors, which may result in a decrease in the enzymatic activity of  $\alpha$ -galactosidase, polygalacturonase, and pectinmethyl-esterases in fruits hence slowing down the process of firmness loss.

Since water loss is a marker of ripening, low water loss lead to delayed ripening. Orange epicarp extract at all concentrations reduced water loss in avocados in this study. Orange epicarp is rich in antioxidants [27] which have the property of slowing down the aging process of tissues, hence slowing down water loss.

Results also showed that, avocados coated with 0.133 Kg.l<sup>-1</sup> and 0.2 Kg.l<sup>-1</sup> of orange epicarp extracts had the lowest values of the loss of physiological mass. This could be due to a decrease in the transpiration of the coated fruits [23]. In fact, polysaccharides have gelling properties that form, slightly thick layer which reduces transpiration when used as coating [24]; such molecules are abundantly found in orange epicarp [25]. Late ripening is associated to low respiration and transpiration as it has been reported that loss of physiological mass is fasten by the increase in physiological processes such as respiration and transpiration [26, 27].

During ripening, avocados use organic acids for their metabolic activities, which leads to a decrease in total acidity and, consequently, an increase in pH [28]. In this study, coated avocados showed less pH variations as compared to control, suggesting that the orange epicarp extract delayed the biochemical reactions due to ripening. In fact, enzymes involved in the degradation of organic acids may have been inhibited due to antioxidants contained orange epicarp extract, hence reducing pH in coated avocados. İlhami [29] reported that, antioxidants inhibit enzyme activity.

Soluble solids content increased during ripening; this is due to the hydrolysis of complex sugars into simple sugars which is an important characteristic of fruit ripening [30,31]. The highest amount of total soluble sugars was obtained with fruits coated with 0.26 kg.l<sup>-1</sup> of orange epicarp extract. At higher concentration, orange epicarp extract contain enough phenolic compounds to inhibit the activity of enzymes involved in the breakdown of complex sugars into simple sugars as previously reported [29, 32].

Proteins biosynthesis is a physiological hallmark of the process of fruit ripening. During this experiment, coating contributed to lower the protein content. This may be due to the inhibition of the activity of enzymes involved in the protein synthesis as well ribosome activity by the antioxidants contained in the coating solution. Ribosome is the location of protein synthesis and its activity can be inhibited by antioxidants [33, 34]. The chlorophyll content of the peel and pulp decreased during ripening. The highest values were found in avocados coated with 0.066 and 0.133 kg.l<sup>-1</sup> of orange peel extract. This may be explained by the fact that ascorbic acids contained in orange epicarp extracts [35] would have either inhibited chlorophyllase activity [36].

#### 5. Conclusion

This study aimed at determining the effect of coating based on orange epicarp extracts on the ripening parameters and storage of the avocado. Results showed that lower concentrations (0.066 and 0.133 Kg.l<sup>-1</sup>) of orange epicarp extracts delayed ripening and improved the shelf life of avocados while high concentrations (0.2 and 0.26 Kg.l<sup>-1</sup>) accelerated ripening. Indeed, avocados treated with 0.066 and 0.133 Kg.l<sup>-1</sup> slowed down the loss of firmness, the physiological loss of mass, the increase in water content, protein biosynthesis, chlorophylls degradation. The coating with orange epicarp extracts significantly influenced each of the ripening parameters either by slowing down or improving the ripening process.

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