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Changes in the Ripening Characteristics and Shelf Life of Mango Fruits as Related to the Application of Coating Based on Cocoa Leaf Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author KJO performed laboratory experiment and the statistical analysis. Authors KJO and PTE managed the literature searches, wrote the protocol and the first draft of the manuscript. Author ANJ designed the study and managed the final manuscript. All three authors read and approved the final manuscript.

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ABSTRACT

The influence of edible coatings based on cocoa leaf extracts on the ripening of "Belle-Dame" mangoes has been evaluated, to contribute to the improvement of the shelf life of these fruits after harvest. There were overall treatment-independent decreases in the firmness and chlorophylls levels, and increases in total soluble solids content, water content, physiological weight loss and β-carotene concentration during the ripening of mango fruits. These variations were very rapid in control fruits, reflecting an accelerated ripening process that led to the senescence of these fruits from day 9 after harvest onwards. In treated fruits, variations of different parameters were slow with significant differences between the treated fruits and controls. These inhibitory effects of coatings resulted in a delay of the ripening process and consequently to a shift of the onset of senescence to 12 days after harvest. In addition to the extension of shelf life by three days, treatments of mango

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fruits by dipping them in solutions containing cocoa leaf extracts induced a higher accumulation of β-carotene in coated fruits as compared to controls, thereby enhancing the nutritional value of the former.

Keywords: Conservation; edible coating; mangoes; cocoa leaves; ripening parameters.

1. INTRODUCTION

Food is now perceived as one of the factors of public health. Fruits and vegetables are particularly recommended because of their protective effects against the major chronic diseases such as cardiovascular, neurodegenerative and metabolic diseases (diabetes) and cancers [1]. Also, their low energy content renders them appropriate for the prevention of overweight and obesity which are increasing in modern societies. Among the tropical fruits, mangoes have very great nutritional and medicinal virtues due to their richness in minerals (calcium, phosphorus and iron), antioxidants (phenylpropanoids, carotenoids and vitamin C) and provitamin A (β-carotene) [2]. Despite this importance, mangoes that are mainly eaten fresh deteriorate rapidly because they are living products whose metabolic activities, disrupted by harvesting operations, continue during conservation [3].

Most varieties of mangoes produced are picked ripe. However, the capacity of local artisanal fruit processing industries is not enough to value the entire production. Large quantities of these fruits are lost for various reasons among which [4] the lack of professionals who, taking into account the climacteric character of the mangoes, master the particular precautions to be taken for their picking, handling and storage; the lack of commercial opportunities (99% of these fruits are consumed or processed locally); the presence of fruit flies and poor postharvest practices that favour the installation of microbial agents responsible for the rotting of these fruits; lack of modern processing industries and adequate conservation methods.

Several fruit conservation techniques have been developed, among which: refrigeration, drying at the sun or in an oven [5], chemical treatments such as the addition of firming agents to the example of calcium [6,7], heat treatments [8,9], controlled and modified atmospheres [1,9], the addition of antioxidants such as ascorbic acid and citric acid [10]. However, many studies have revealed the limitations of some of these different methods, including the high costs of cold room installations and devices for some heat treatments [11]. In most cases (refrigeration, controlled atmospheres) there is also the risk of modifying the organoleptic qualities of the fruit. FAO [12] estimates that despite the existence of these conservation technologies, post-harvest losses of 15-40% of mangoes in the tropics and subtropics remain high. These losses estimated at 17.9% for the Alphonso variety [13] are distributed as follows: orchard (3.5%), transport (4.9%), storage (4.1%) and retail sales (5.4%).

Therefore, it is necessary to develop natural conservation techniques that are accessible to even smallholders farmers, will help to slow down the ripening and ageing processes of fruit tissues and therefore contribute to the reduction of losses, which favour not only the marketing of these fruits but also their increased consumption. Among these methods are edible coatings. Coatings are thin layers prepared from various film-forming materials that, when used in liquid form, are applied directly to the surface of the food to act as a barrier to external elements to protect the product and prolong its shelf life [14]. The treatment of bananas with palm kernel oil has been used to delay ripening by acting on parameters such as respiration, water content, pigment degradation and thereby improving the conservation period of bananas [15]. Researches on cocoa leaf extracts had shown their strong antioxidant potential [16], which would probably explain the anti-ripening effect of cocoa leaf powder on plantain with the consequent extension of the shelf life of these fruits at the mature-green stage [17].

The present study aims not only to contribute to the improvement of the shelf life of fresh mangoes by the dipping technique based on coca leaf extracts, without negatively influencing their nutritional or organoleptic qualities but also to valorise the agricultural by-products generally considered as wastes.

2. MATERIALS AND METHODS

2.1 Plant Material

The cocoa (*Theobroma cacao*) leaves used in the preparation of the coating solutions were collected in a cocoa plantation located in the

South West Region (at Ekona) in Cameroon. They were transported in clean polythene bags and oven-dried at 40ºC for 72 hours for complete dehydration. Subsequently, they were finely crushed in a food-mill (Master Chef MC-BL1544).

The mangoes (*Mangifera indica* L.) used in this work were of the "Belle-Dame" variety still called the improved variety of Cameroon. They were harvested from an orchard in Penja, Littoral Region, Cameroon. Mangoes of the ‘‘Belle-Dame’’ variety are dark-green fruits in the mature, unripe state. They become yellowish-green and yellow when they are respectively ripe and too ripe [18]. This variety was chosen because of its commercial importance and for its availability at the time of the study (March 2018).

Fruits at the advanced stage of maturity showing a beginning of turning to the yellow colour of the pulp were harvested on five different mango trees. The collection of mango fruits took place on March 18, 2018. To minimize the differences between fruits, their harvest was carried out by a single climber who selected fruits of similar sizes after hand-tapping (to reassure himself of the firmness of each harvested fruit). To prevent fruit from being injured during the fall, they were thrown at people standing at ground level and who took care of catching them and gently putting them in bamboo marrow crates. The harvested fruits were immediately transported in these boxes to the laboratory.

2.2 Preparation of Coating Solutions and Treatment of Fruits

The solvent composed of a mixture of water and ethanol (1/1: v/v) was used as a dispersion medium. 200 g or 400 g of cocoa leaf powder were soaked in this dispersion medium. The mixture that was covered and homogenized with a spatula from time to time was standing for two hours at room temperature (26.6ºC). After filtration of this mixture, the residue was rinsed and filtered twice using 0.5 l of distilled water/ethanol (1/1, v/v) each time. To the total extract, glycerol was added as a plasticizer (and thus as a matrix for potential active compounds some of which might have ripening and senescence delaying properties) at a concentration of 4% (v/v); sodium hypochlorite (NaOCl) was also introduced to the mixture as a disinfectant at a concentration of 0.023% (i.e. 230 µl per litre). Each coating solution thus prepared with the various concentrations of cocoa leaf powder was completed to a total volume of three litres, using the mixture distilled water/ethanol (1/1: v/v). Following the same protocol, another solution of the same volume (three litres) containing all the ingredients used above excepted the cocoa leaf powder was prepared, to evaluate the effect of cocoa leaves only. All prepared solutions were left at room temperature for 15 hours before use.

To use a homogeneous fruit sample, mangoes were sorted based on size, colour and absence of wounds. They were then washed and rinsed with distilled water to remove any impurities and were dried using blotting papers. Four fruit set types were used, namely: T00: not treated fruits; T01: fruits that were treated with a solution containing no extract from cocoa leaves; T1: fruits that were treated with a solution containing 0.06 kg of cocoa leaf powder per litre; and T2: fruits that were treated with a solution containing 0.12 kg of cocoa leaf powder per litre. For each treatment, three repetitions of 18 fruits were used.T01, T1 and T2 fruits were soaked for thirty minutes in previously prepared solutions as described above. The fruits of four batches were kept at an ambient temperature of 25-27ºC and relative humidity of 55-72%.

2.3 Determination of Ripening Parameters

To evaluate the effect of coatings on the ripening and quality of mangoes, two randomly selected fruits in each replicate (i.e. 24 fruits in total) were used to determinate the physical and physiological parameters below. These measurements were carried out before the fruit coating (day 0), then every 3 days after the treatment for a total duration of 12 days.

2.3.1 Firmness of the mango pulp

The internal firmness of mango fruits was determined using a portable GY-2 brand penetrometer having measurement capacity of $4x10^5$ Pascal. The measurements were taken on three different sides of the same fruit and the firmness was the average of the three measurements.

2.3.2 Physiological weight loss

It was determined by weighing the fruits at various time intervals using an SF-400 brand balance, capacity 10000 g x 1 g. It is the decrease in the weight of the fruits during the ripening process and was expressed as a percentage of the initial weight according to the formula below:

PWL (%) = [(IW-WT) / (IW)] x100

Where PWL represents the physiological fruit weight loss, IW corresponds to the initial weight of the mango fruits and WT to the weight of the mango fruits at a definite time.

2.3.3 Total soluble solids content

The total soluble solids content (TSSC) is the major indicator of the sucrose content of the fruit. Its value was determined every three days by refractometry of the mango fruit juice. A small volume of the juice was collected with a pipette and deposited as 2 to 3 drops on the prism of a hand ATC-1C brand refractometer having a value range of 0 to 32ºBrix (1ºBrix is equivalent to 1 g sucrose in 100 g of fruit). The measurements were carried out at room temperature.

2.3.4 Pigment content

The determination of levels of chlorophyll a, chlorophyll b and β-carotene in mango fruits were carried out according to the method of Nagata and Yamashita [19]. 4 g of fresh peel or pulp previously crushed in the presence of sand were introduced into a test tube containing 10 ml of acetone/hexane mixture (4/6, v/v). The tube was wrapped with aluminium paper impervious to light rays and left inside the ice for 10 minutes to obtain two separate phases. The optical densities of the extracts (supernatant) were determined at wavelengths of 453 nm, 505 nm, 645 nm and 663 nm, using a Biochrom Libra S22 brand spectrophotometer.

2.4 Statistical Analyses

The obtained data were submitted to the analysis of variance (ANOVA) to check the differences between means and the Student-Newman-Keuls test was used in case of need at the 5% probability threshold for the separation of these means. These statistical analyses were performed using the XLSTAT 2016 software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Variations of the firmness of mangoes during conservation

Table 1 shows that the internal firmness of all mango fruits generally decreased during ripening, ranging from 4.83 x10^5Pa at day 0 to 1.02x10^5Pa on day 12. Specifically, firmness of fruits treated by dipping with solutions containing 0.06 kg (T1) or 0.12 kg (T2) cocoa leaf powder per litre was significantly higher than those of all control fruits (T00 or T01) from day 3 after treatment onwards. T2 was significantly more effective than T1 in delaying the decrease in fruit firmness between day 3 and day 6 after treatment.

3.1.2 Changes in a physiological weight loss of mangoes during storage

Results presented in Table 2 show that variations of the physiological weight loss (PWL) were from 4.41% on day 3 to 10.91% on day 12 for T00 fruits; from 4.23% on day 3 to 10.44% on day 12 for T01 fruits; from 5.05% on day 3 to 8.94% on day 12 for T1 fruits; and from 6.01% on day 3 to 7.36% on day 12 for T2 fruits. The general trend was thus an increase of PWL during the experimental period. This increase was statistically significant for T00, T01 and T1 fruits on day 12 as compared to day 3 after treatment. There no significant difference in the PWL of T2 fruits during all the experimental period. However, on 12 after treatment, T2 fruits had a PWL (7.36%) significantly lower than PWls of T00, T01 and T1 fruits (10.91%, 10.44% and 8.94%, respectively).

Table 1. Variation of mango fruit firmness during storage

Time after treatment in days	Firmness (X10^5 Pa)			
	T00	T01	T1	T2
0	4.83 ±0.22^{a}	4.83±0.22^{a}	4.83±0.22^{a}	4.83±0.22^{a}
3	2.97±0.25^{de}	2.59±0.13^{e}	3.45±0.33^{cd}	3.98±0.02^{d}
6	1.16±0.32^{hi}	1.14±1.125^{hi}	2.54±0.06^{e}	3.09±0.09^{cd}
9	1.08±0.05^{hi}	1.02±0.07^{hi}	2.00±0.11^{f}	2.07±0.10^{f}
12	1.08±0.03^{i}	1.02±0.06^{i}	1.66±0.08^{fg}	1.50±0.09^{gh}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 2. Variation in a physiological weight loss of mango fruits during storage

Time after treatment in days	Physiological weight loss (%)			
	T00	T01	T1	T2
3	4.41±0.7^{a}	4.23±0.59^{a}	5.05±0.94^{a}	6.01±1.99^{ab}
6	4.42±1.14^{a}	6.64±0.92^{ab}	6.04±2.31^{ab}	6.49±0.93^{ab}
9	7.82±0.76^{bc}	3.64±0.54^{a}	7.04±0.79^{b}	6.30±1.46^{ab}
12	10.91± 6.2^{cd}	10.44±6.07^{cd}	8.94±3.15^{c}	7.36±4.99^{b}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

3.1.3 Variations of the water content in the peel and the pulp

3.1.3.1 Water content in the peel

Globally, an increase of the water content in the peel of all fruits during the first three days of storage was observed; there was a decrease of the water content in the peel of these fruits from the 6th day after treatment onwards. The water content in the peel ranged from 68.33% on day 0 to 70%, 71.67%, 73.33% and 70% on day 3 after treatment in T00, T01, T1 and T2 fruits, respectively. It then declined in T00 and T01 fruits to reach 63.33% and in T1 fruits to reach and 66.67% on day 12 after treatment. In T2 fruits, peel water content remained at 70% until day 6 before starting to decrease to 66.67% on day 12 as in T1 fruits. However, no significant temporal or treatment-dependent difference in the water content of the peel of mango fruits could be observed during the experimental period (Table 3).

3.1.3.2 Water content of the pulp

The water content of the pulp increased during the first 6 days of storage in fruits of control batches T00 and T01 and the first 9 days in treated fruits T1 and T2. It increased from 73.33% on day 0 to 83.33% and 85% on day 6 for control fruits T00 and T01 respectively, and to 85 and 81.67% on day 9 for treated fruits T1 and T2 respectively. The water content of the pulp then decreased in the fruits of control batches T00 and T01 to 70 and 71.33% respectively on the 12th day. From day 9 to day 12 after treatment, the water content in the pulp of T1 and T2 fruits decreased from 85 % to 81.67% and from 81.67% to 78.33%, respectively (Table 4).

3.1.4 Variations of total soluble solids content

The total soluble solids content, which reflects the overall sugar content, gradually increased in the pulp of control fruits T00 and T01 and reached its maximum on the 9th day after treatment with values of 20.5 and 20ºBrix respectively. It then decreased in the pulp of these fruits to attain respectively 16.5 and 16.33ºBrix on day 12 after treatment. In treated fruits, T1 and T2, the increase in the total soluble solids content were progressive until day 12 after treatment where it reached 19.58 and 20.25ºBrix, respectively. There were significant differences in the total soluble solids content in the pulp of controls (T00 and T01) and treated fruits (T1 and T2) on day 12 after treatment, this content being higher in latter than in the former (Table 5).

3.1.5 Variations of pigment contents in the peel and the pulp

3.1.5.1 Variations of the concentration of pigments in the peel

Chlorophylls a and b contents: As it can be noticed from Tables 6 and 7, levels of chlorophylls *a* and *b* decreased overall in fruit peel during ripening regardless of treatments. Decreases in chlorophyll levels in T00, T01, T1 and T2 fruits ranging from 15.66 µg / g on day 0 to 3.36, 4.61, 7.18 and 6.83µg/g on the 12th day after treatment were respectively observed (Table 6). Concerning the chlorophyll *b* content in the peel of T00, T01, T1 and T2 fruits, it decreased from 8.59µg/g on day 0 to 3.28, 3.44, 6.18 and 6.36 µg/g on day 12 after treatment, respectively.

β-Carotene content: The β-carotene content in the peel gradually increased in all fruits regardless of treatment (Table 8). Being 0 µg/g on day 0, it attained 1.05, 1.23, 1.98 and 1.93 µg/g on day 12 after treatment, respectively, in the fruits of batches T00, T01, T1 and T2. On day 9 after treatment, maximum values of β-carotene content of 1.56 and 1.64 µg/g were recorded in the peel of respectively T00 and T01

fruits. These values decreased thereafter and reached respectively 1.05 and 1.23 µg/g on day 12 after treatment. In treated fruits (T1 and T2), the concentration of β-carotene increased gradually from day 0 to day 12 after harvest. The maximum levels of β-carotene reached on day 12 in treated fruits were higher than those found in controls (T00 and T01) on day 9 after treatment. Levels of β-carotene in the peel of control fruits were significantly higher than those determined in the peel of treated fruits on the 9th day after treatment. On the contrary, on day 12 after treatment β-carotene contents in the peel of treated fruits were significantly higher than those found in the peel of controls (Table 8).

3.1.5.2 Variations of the concentration of pigments in the pulp

Chlorophylls a and b contents: The concentrations of chlorophyll *a* and *b* in the pulp presented in Tables 9 and 10 decreased overall in all fruits independently of the treatments. The chlorophyll content decreased from 2.98 µg/g on day 0 to 0.22, 0.45, 1.02 and 1.05 µg/g respectively in the pulp of T00, T01, T1 and T2 fruits 12 days after treatment. There were significant differences between chlorophyll content in the pulp of controls (T00 and T01) and those in the pulp of treated fruits (T1 and T2) on day 12 after treatment, chlorophyll-*a* levels in treated fruits being significantly higher than those found in controls.

The Chlorophyll *b* level decreased from 2.29 µg / g on day 0 to 0.25, 0.36, 0.86 and 0.66 µg/g in the pulp of respectively T00, T01, T1 and T2 fruits on day 12 after treatment. Similarly to chlorophyll a, on day 12 after treatment, chlorophyll *b* contents in the pulp of treated fruits were significantly higher than those found in the pulp of controls.

β-Carotene content: The concentration of β-carotene significantly increased in the pulp of all fruits during ripening regardless of treatments (Table 11). However, in contrast to treated fruits (T1 and T2), this increase was not linear in the pulp of controls (T00 and T01). It thus ranged from 0.18 µg/g on day 0 to 3.44 and 3.62 µg/g respectively in the pulp of T00 and T01 fruits on day 9 after treatment, then dropped to 1.28 and 1.02 µg/g respectively on day 12 after treatment. In the pulp of T1 and T2 fruits β-carotene increased from 0.18 µg/g on day 0 to 4.27 and 4.38 µg/g respectively on day 12 after treatment. Moreover, similarly to chlorophyll levels, β-carotene contents of controls were significantly

Table 3. Variation of water content in the peel of mango fruits during storage

Time after treatment in days	Water content (%)			
	T00	T01	T1	T2
0	68.33±2,22^{a}	68.33±2.22^{a}	68.33±2.22^{a}	68.33±2.22^{a}
3	70±0^{a}	71.67±2.22^{a}	73.33±4.44^{a}	70±0^{a}
6	66.66±4.44^{a}	70±0^{a}	70±0^{a}	70±0^{a}
9	63.33±2,22^{a}	66.67±4.44^{a}	66.67±4.44^{a}	68.33±2.22^{a}
12	63.33±4,44^{a}	63.33±4.44^{a}	66.67±4.44^{a}	66.67±4.44^{a}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 4. Variation of the water content in the pulp of mango fruits during storage

Time after treatment in days	Water content (%)			
	T00	T01	T1	T2
0	73.33±4.44^{cd}	73.33±4.44^{cd}	73.33±4.44^{cd}	73.33±4.44^{cd}
3	76.67±4.44^{c}	76.67±4.44^{c}	75±3.33^{c}	80±0^{b}
6	83.33±2.22^{ab}	85±3.33^{a}	80±0^{b}	81.67±4.44^{ab}
9	73.33±4.44^{cd}	76.67±4.44^{c}	85±3.33^{a}	81.67±2.22^{b}
12	70±0^{cd}	71.67±2.22^{cd}	81.67±2.22^{b}	78.33±4.44^{bc}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 5. Variation of total soluble solids content in mango fruits during storage

Time after treatment in days	Total soluble solids content (°Brix)			
	T00	T01	T1	T2
0	12±0.33^{e}	12±0.33^{e}	12±0.33^{e}	12±0.33^{e}
3	16.83±0.78^{bc}	17.5±0.33^{b}	15.17±0.44^{cd}	14.75±0.5^{d}
6	18±0.67^{ab}	19.33±0.78^{a}	16.5±0.22^{b}	16.67±0.44^{bc}
9	20.5±0.33^{a}	20±0.33^{a}	17.87±0.33^{b}	19.83±0.28^{ab}
12	16.5±1^{bc}	16.33±0.22^{bc}	19.58±0.61^{a}	20.25±0.83^{a}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 6. Evolution of chlorophyll content in the peel of mango fruits during conservation

Time after treatment in days	Chlorophyll *a* content (µg/g)			
	T00	T01	T1	T2
0	15.66±0.29^{a}	15.66±0.29^{a}	15.66±0.29^{a}	15.66±0.29^{a}
3	9.53±0.55^{c}	7.17±0.23^{de}	11.53±0.99^{b}	12.14±1.18^{b}
6	9.37±0.52^{c}	6.34±.3^{ef}	9.48±0.56^{c}	13.11±1.09^{b}
9	5.68±0.88^{ef}	5.61±0.28^{ef}	7.48±0.34^{de}	8.85±0.78^{cd}
12	3.36±0.07^{g}	4.61±0.93^{fg}	7.18±0.29^{de}	6.83±0.50^{e}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 7. Evolution of chlorophyll *b* content in the peel of mango fruits during storage

Time after treatment in days	Chlorophyll *b* content (µg/g)			
	T00	T01	T1	T2
0	8.59±0.27^{a}	8.59±0.27^{a}	8.59±0.27^{a}	8.59±0.27^{a}
3	6.17±0.27^{bcd}	6.64±0.40^{bcd}	8.49±0.54^{a}	7.44±0.49^{abc}
6	5.89±0.51^{cd}	6.36±0.58^{bcd}	7.29±0.27^{abcd}	7.93±0.71^{ab}
9	5.53±0.30^{d}	3.88±0.12^{e}	6.63±1.05^{bcd}	6.64±0.15^{bcd}
12	3.28±0.38^{e}	3.44±0.58^{e}	6.18±0.79^{bcd}	6.36±0.54^{bcd}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

lower than those of treated fruit 12 days after treatment.

3.2 Discussion

3.2.1 Firmness

The gradual decline in the firmness of control mango fruits as observed in this study is supported by the results of Jha, et al. [20] who reported a gradual decline in the firmness of seven varieties of Indian mangoes ranging from 5 to 0.3 Newton over a 10 day storage period; similar results were obtained by Kouame, et al. [21] during the ripening of banana fruits. The decline in mango firmness was significantly mitigated in this study by coatings based on cocoa leaf extracts. The coating containing 0.06 kg of cocoa leaf powder per litre of the solution was found to be the most significantly effective in maintaining the firmness of "Belle-Dame" mango fruits. Thai-Thi [22] also demonstrated that the 4% hydroxypropyl methyl cellulose-based coating and composite-based coating were very effective in maintaining the external and internal firmness of Tommy Atkins mango variety.

Indeed, the loss of firmness observed during fruit ripening is due to the action of enzymes such as pectinases (pectin esterases, pectate lyase, pectin lyase), polygalacturonases and amylases whose activities lead to a reduction of pectic compounds, cellulose, hemicellulose, starch and an increase in soluble solids and total soluble sugars [22]. The reduction of pectic compounds, following their hydrolysis by pectinases and polygalacturonases is accompanied by the degradation of intercellular bonds [23]. This fleshy climacteric fruit softening mechanism is normally triggered by the fixation of the ethylene molecules to their specific site located on the cell membrane. This attachment to a protein receptor requires the presence of oxygen and is inhibited by high levels of carbon dioxide [22]. The slowing effect of treatments on the decrease in firmness observed in this study indicated that the coatings based on cocoa leaf extracts would have served as a barrier to gases, resulting to low absorption of O_2 and a high CO_2 concentration around these fruits. This would have induced inhibition of the binding of ethylene to its specific receptor and therefore slowed down the ripening related to pectinase activity. Besides, the polyphenols compounds found in cocoa leaves, through their antioxidant properties, also have a barrier effect on gases and moisture [24]. They are also vascular protectors that improve the resistance and permeability of vessels by protecting perivascular connective tissue from enzymatic degradations [25]. In plants, they would have also protected the cell walls from enzymatic degradations. On the other hand, polyphenols by their free radical scavenging effect would prevent the oxidative degradation of membrane lipids.

3.2.2 Physiological weight loss

The mass losses that result from physiological water losses are because the fruit peel bears stomata and transpiration continues after harvest. During ripening, physiological water losses that are normally low become very important when the stage of senescence begins [26] due to degenerative changes in the peel and resulting from both transpiration and respiration.

The results obtained showed the efficacy of coating based on cocoa leaf extracts to limit physiological weight loss. Similar results have been reported by Das, et al. [24] when assessing the "antimicrobial and antioxidant effects of

Table 8. Variation of the β-carotene content in the peel of mango fruits during storage

Time after treatment in days	β-Carotene content (µg/g)			
	T00	T01	T1	T2
0	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}
3	0.88 ± 0.08^{c}	0.17 ± 0.21^{cd}	0.18 ± 0.04^{e}	0.31 ± 0.07^{de}
6	1.04 ± 0.08^{bc}	1.12 ± 0.10^{bc}	1.16 ± 0.32^{bc}	0.95 ± 0.18^{c}
9	1.56 ± 0.35^{ab}	1.63 ± 0.18^{ab}	1.22 ± 0.11^{bc}	1.07 ± 0.06^{bc}
12	1.05 ± 0.23^{bc}	1.23 ± 0.24^{bc}	1.98 ± 0.05^{a}	1.93 ± 0.16^{a}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 9. Evolution of the content of chlorophyll *a* in the pulp of mango fruits during storage

Time after treatment in days	Chlorophyll *a* content (µg/g)			
	T00	T01	T1	T2
0	2.98 ± 0.28^{a}	2.98 ± 0.28^{a}	2.98 ± 0.28^{a}	2.98 ± 0.28^{a}
3	1.58 ± 0.22^{cd}	1.73 ± 0.14^{c}	2.36 ± 0.07^{b}	2.82 ± 0.21^{a}
6	1.35 ± 0.18^{cd}	1.24 ± 0.13^{cde}	1.71 ± 0.07^{c}	1.71 ± 0.08^{c}
9	0.47 ± 0.05^{f}	0.71 ± 0.09^{ef}	1.25 ± 0.09^{cde}	1.13 ± 0.08^{cde}
12	0.22 ± 0.03^{f}	0.45 ± 0.03^{f}	1.02 ± 0.04^{de}	1.05 ± 0.04^{de}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 10. Evolution of the chlorophyll *b* content in the pulp of mango fruits during conservation

Time after treatment in days	Chlorophyll *b* content (µg/g)			
	T00	T01	T1	T2
0	2.29±0.12^{a}	2.29±0.12^{a}	2.29±0.12^{a}	2.29±0.12^{a}
3	2.25±0.24^{a}	1.58±0.13^{cd}	2.06±0.09^{ab}	1.85±0.32^{bc}
6	1.28±0.16^{de}	1.13±0.11^{ef}	1.62±0.02^{cd}	1.56±0.03^{cd}
9	0.38±0.07^{ij}	0.52±0.08^{hij}	1.02±0.12^{efg}	0.81±0.05^{fgh}
12	0.25±0.01^{i}	0.36±0.05^{ij}	0.86±0.05^{fgh}	0.66±0.04^{ghi}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

Table 11. Variation of β-carotene content in the pulp of mango fruits during storage

Time after treatment in days	β-Carotene content (µg/g)			
	T00	T01	T1	T2
0	0.18±0.04^{i}	0.18±0.04^{i}	0.18±0.04^{i}	0.18±0.04^{i}
3	1.27±0.04^{gh}	1.13±0.04^{h}	1.24±0.08^{gh}	1.57±0.26^{g}
6	2.25±0.16^{e}	2.48±0.08^{e}	1.87±0.06^{f}	2.16±0.08^{e}
9	3.44±0.10^{d}	3.62±0.06^{d}	4.00±0.28^{bc}	3.73±0.30^{cd}
12	1.28±0.13^{gh}	1.02±0.03^{h}	4.27±0.04^{ab}	4.38±0.15^{a}

Values followed by the same letters in the same row or column are not significantly different at p≤0.05 threshold level according to the Student-Newman-Keuls. T00: fruits that have not received any treatment; T01: fruits that were treated with the coating solution without cocoa leaf powder; T1: fruits that were treated with the solution containing 0.06 kg of cocoa leaf powder per litre; T2: fruits that were treated with the solution containing 0.12 kg of cocoa leaf powder per litre

edible rice starch and tea leaf extracts based coatings on tomato fruit preservation". Similarly, Baldwin [27] and Thai-Thi [22] found a considerable limitation in weight loss in Tommy Atkins and Early Gold mangoes varieties treated with the zein-based coating and a so-called "Nature Seal 2020" coating available in commerce. The reduction in the mass loss in coated fruit can be attributed to the barrier properties to the diffusion of gases through the stomata, organs that regulate the process of transpiration and gas exchange between the fruit and the environment [22]. The effect of coatings on the limitation of mass loss in fruits suggests that the rate of transpiration was reduced by the blocking of lenticels of the fruits. This could be due to the thin layer formed by the polymeric compounds that are constituents of the coating (cocoa leaf powder in the case of this study) and which partially closed the pores (or lenticels) at the level of the epidermis of fruits and therefore limited gaseous exchange, the flow of water vapour and the movement of solutes [28].

The physiological weight loss is mass loss due to water loss through evapotranspiration, consumption through respiration of the reserves necessary for the survival of the fruit and whose products are discharged in the form of CO_2 and H_2O. Any reduction in water loss can help the fruit to extend its shelf life by a few days but the latter does not exceed the limit value which is estimated at around 6% of the initial weight of the fruit [29]. It has been shown that even if the presence of water favours local necrosis and microbial development, not to mention the acceleration of the metabolism which leads to the depletion of reserves, the excess water loss accelerates the senescence which represents a loss of material and product quality [30]. This could have been the case of the control fruits batchesT00 and T01 that not only showed high physiological weight loss values but also presented visible necrosis on their peel at the 9th day of conservation. The coating, in this case, would have acted as a barrier thus limiting the exchange of water vapour between the fruit and its environment (thus reducing transpiration) and would have allowed the maintenance of the water losses around the limit value. Best results were obtained with the coating containing 0.12 kg cocoa leaf powder per litre solution and which maintained the physiological weight at 7.36% on the 12th day of storage.

3.2.3 Water content in the peel and pulp

The increase in water content in the peel observed during the first three days of storage, although not significant, is related to the fruit ripening process and could be explained by the hydrolysis of carbohydrates and pecto-cellulosic compounds [23]. The decrease in the water content of the peel recorded from the 6th day of storage onwards could be explained not only by the complete ripening of the fruits (as in the case of T00 and T01 batches), but also by the phenomenon of physiological losses of water that accompanies mass loss. Indeed, when the fruits are already ripe, the degradation reactions that accompany the ripening process are slowed down and the mass losses related to the flow of water between the fruit and its environment become more and more important. Also, the high concentration of reductive sugars in the pulp of ripe fruits leads to an increase in its osmotic potential and consequently to an increase of the movement of water from the peel to the pulp.

The increase in water content of the pulp observed up to the 6th day of storage in the control fruits and up to the 9th day in the treated fruits indicated a faster ripening of control fruits compared to the coated fruits. On the other hand, the drop in the water content in the pulp observed from the 9th day in the controls and the 12th day in the treated fruits would be a sign of the beginning of senescence. Indeed, Létang [29] studying the variation of water content in mango pulp during refrigerated storage, found an increase in water content followed by a decrease during a 15 days storage period. The increase in water content in the fruit pulp is due to the ripening process which is accompanied not only by the hydrolysis of carbohydrates (mainly starch), but also by the osmotic migration of the water from the peel to the pulp because of the high concentration of sugars in the pulp; for, the hydrolysis of the starch to soluble sugars is maximal at the peak of ethylene production [2]. On the other hand, the following decrease of the same water content in the pulp could be explained by the fact that water losses cause a slowing down of the metabolic reactions in the purpose of extending the shelf life. However, if this decline is rapid and important, it leads directly to senescence. The drop in water content, that started early in the control fruit batches (day 9) and later in the treated batches (day 12), could be related to an acceleration of water losses leading to a beginning of senescence. Senescence was thus retarded in treated fruits as compared to controls. This is what would justify the deterioration found in the control fruits from the 9th day of storage onwards as compared to the treated fruits which remained visibly healthy at that period, the best results having been obtained with the fruits of the T1 batch (fruits treated by coating with a solution containing 0.06 kg cocoa leaf powder per litre). Also, a time frame comparison of the water content of the peel to that of the pulp showed significant differences between the peel and the pulp of all the fruits; the water content in the pulp was always higher than that in the peel. This explains why the pulp is juicier than the peel [31].

3.2.4 Variation of total soluble solids content

The increase in total soluble solids content (TSSC) during ripening can be explained by the hydrolysis of starch and other polysaccharides to soluble sugars [22,32]. In this study, the increase in TSSC was initially rapid at the beginning of ripening but tended to slow down thereafter, certainly because of the decrease in starch content, the hydrolysis of which produces diholosides such as maltose and sucrose which are then hydrolyzed by various enzymes into glucose and fructose. The overall level of these sugars is proportional to the value of the Brix index, the most abundant sugars in the mango being sucrose, glucose and fructose [2]. The maximum TSSC average value obtained in this study was 20ºBrix, reflecting the intense sweetness of ripe ‘‘Belle Dame’’ mangoes. Similar results were obtained by Thaï-Thi [22] with the Kent mango variety on day 10 after harvest after treatments with different commercial coatings (coating based on Carnauba wax or hydroxypropyl methylcellulose). The TSSC of the treated fruits increased continuously until the 12th day after storage contrary to the control fruits in which the maximum value of the TSSC was reached on the 9th day. This could be linked to the slowing down of ripening induced by coating, T1 is the most effective treatment (TSSC = 19.58ºBrix on the 12th day after harvest). Moreover, the values of 18 and 18.33ºBrix obtained on day 6 in the controls T00 and T01, respectively, showed that these fruits were already ripe at that date, unlike treated fruits (T1 and T2) which ripened rather on the 9th day with TSSC of 17.88 and 19.83ºBrix, respectively. Several authors who have worked on the biochemical characterization and conservation improvement of mangoes [9,2] have shown that the average value of the TSSC at the stage of consumption varies between 17.5

and 20ºBrix. The decrease of the sugar level observed in the control fruits between day 9 and day 12 after harvest would reflect the overripening and the senescence that was already taking place in these fruits. In fact, during senescence, the fermentation process that takes place at the expense of respiration stimulates the conversion of glucose into ethanol, causing a decrease in the level of soluble sugars [29].

3.2.5 Evolution of pigment content

The evolution of pigment concentrations in the peel globally showed a decrease in chlorophyll *a* and *b* levels and an increase in the content of β-carotene. These results are in agreement with those of Youmbi, et al. [33] on "Morphological and biochemical changes during the development and maturation of the fruit of *Spondias cytherea* (*Anacardiaceae*)". This evolution of pigment concentrations is linked to the process of fruit ripening which is reflected externally by the gradual shift of the green colouration of the peel to yellow, orange, red, in short to a more brilliant colour that varies according to species and varieties. The external peel colour shift is a reflection of the internal transformation of chloroplasts into chromoplasts, which is accompanied by the degradation of chlorophylls by a group of enzymes including chlorophyllase [34]. The degradation of these chlorophylls leads to the appearance of the colour of carotenoids initially present in chloroplasts and those newly synthesized during the fruit ripening process [32]. The coating of the fruits would have induced inhibition of the activity of the chlorophyllase with the consequence of slowing the degradation of the chlorophylls in the treated fruits thus causing a delay of ripening. According to Mamiro, et al. [32], the decrease of oxygen in the atmosphere surrounding the fruit reduces its metabolic activity and the production of ethylene, resulting in a decrease in the rate of chlorophyll degradation. Thus, the slowing down of chlorophyll degradation in the peel of treated fruits could be linked to the barrier effect of coating on gases. This slowing down of chlorophyll degradation in treated fruits would have favoured a better accumulation of β-carotene in these treated fruits compared to that of the control fruits.

The general gradual increase of β-carotene content in the pulp of mangoes independently to treatment would justify the orange-yellow colour of the pulp of ripe fruits. However, the maximum β-carotene level was observed in control fruits on day 9 after harvest followed by a decrease related to the senescence observed on these fruits. On the other hand, in treated fruits, the β-carotene level continuously increased and was significantly higher therein than that determined in the pulp of controls 12 days after harvest. Since β-carotene is a nutritional quality trait, treatments of mango fruits by coating with solutions containing cocoa leaf extracts led to an improvement of the nutritional value of these fruits and thus to their “biofortification”. Enhancements of β-carotene and volatile compounds contents in tomato fruits after applications of coatings based respectively on coffee leaf extracts and cocoa leaf extracts have also previously been reported by Aghofack-Nguemezi, et al. [35].

4. CONCLUSION

A new method for the improvement of the conservation of fresh mango fruits using coatings based on cocoa leaf extracts has been studied in this work. The two concentrations of coatings tested 0.06 kg/l and 0.12 kg/l had significant effects. Indeed, they induced significant slowdowns in the loss of fruit firmness, physiological water loss, degradation of chlorophylls and increase in total soluble solids content. By causing these inhibitory effects, coatings retarded the ripening process and consequently favoured an extension of the shelf life of treated fruits by three days compared to controls. Also, in comparison to controls, these coatings led to a higher accumulation of β-carotene in the pulp of treated fruits, rending their nutritional quality better than that of untreated fruits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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