Effect of Heating and of Short Exposure to Sunlight on Carotenoids Content of Crude Palm Oil

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Abstract

This study was done to evaluate the effect of short exposure to sunlight and of heating on crude palm oil (CPO) quality particularly on its carotenoids content. Firstly, CPO was exposed to sunlight for 14 hrs. Then, samples were collected and kept at 4°C for analysis of carotenoids content, free fatty acid (FFA) and peroxide value (PV). Secondly, CPO was heated at 50°C, 120°C, 200°C or 400°C for 30 min, 60 min or 120 min and samples were cooled down and kept at 4°C until analysis of carotenoids content, FFA and PV. Finally, we studied the effect of heating of CPO in the food matrix (maize cake). Thus, maize cake was steamed on gas stove (100 ± 5°C) during exactly 1, 2, 3 or 4h and kept at 4°C until analysis of moisture and carotenoids contents. The results showed that short exposure to sunlight did not significantly affect carotenoids content, FFA and PV of CPO. However, heating accelerated the formation of peroxides and degradation of carotenoids. Destruction of carotenoids increased with both temperature and duration of exposure to heat. FFA did not significantly change during heating. Likewise, during heating of CPO in the food matrix, carotenoids content decreased significantly with cooking time. These results suggest that short exposure to sunlight does not have a significant effect on carotenoids content of CPO. But, its heating (directly or in the food matrix) results in significant degradation of carotenoids.

Keywords: Crude palm oil; Carotenoids; Heating; Sunlight; Photo-oxidation; Thermo-oxidation

Introduction

Vitamin A Deficiency (VAD) remains a major nutritional problem worldwide, despite substantial progress in recent decades [1-5]. It is estimated that more than 500,000 preschool children become blind each year because of the VAD among which 250,000 die because of resulting immune dysfunction [6]. The distribution of vitamin A (VA) capsules permitted good coverage of child populations at relatively low cost. However, it is widely recognized that food approaches should be part of means to fight against VAD in sustainable way. They must cover all approaches improving the nutrition focused on food systems or circuits taking into account nutrition education, production, marketing, processing and consumption [7]. In developing countries, these dietary approaches are very encouraging in the case of VA. Indeed, although provitamin A rich fruits and vegetables are not always available and accessible in these countries, crude palm oil (CPO) is generally available and cheap for everybody [6,8]. This oil extracted from the pulp of palm fruits (Elaeis guineensis) is the main source of provitamin A, principally β-carotene [8,9]. CPO has a special place among vegetable sources of VA, because of the absence of plant matrix and presence of lipid environment favorable to the absorption of carotenoids. Moreover, it provides to the organism energy, essential fatty acids, phytosterols, phosphatides and vitamin E [9].

The presence of unsaturated fatty acids exposes palm oil (PO) to lipid oxidation reactions. In the oxidation process, oxygen reacts with the double bonds of fatty acids to form peroxides and/or free radicals. These reactions result in the qualitative and nutritional alterations (rancidity, loss of essential fatty acids and vitamins) and even the toxicity caused by oxidative products (peroxide, aldehydes). They are influenced by extrinsic factors (temperature, light) and intrinsic factors (fatty acid composition, presence of pro- or antioxidants). Natural antioxidants found in many foods which can act as IO2 quenchers help to reduce the oxidation reaction. So, a food will be more resistant to oxidation as its natural antioxidants content is high [10]. It is the case of CPO which is very rich in carotenoids and vitamin E. However, the development of these reactions may be enhanced by cooking, refining or during storage [11]. In fact, some authors showed the negative effects of refining [12], heating [13-15] and light [14,16] on nutritional, sensory and/or physicochemical qualities of vegetable oils including carotenoids degradation.

In 2010, Cameroon was the 11th CPO producer in the world with 190 MT per year [17]. This oil is therefore the most accessible and available for consumption in this country. The survey on the CPO selling conditions and use in households in Douala town and its surroundings showed that sellers exposed oil to open air during the distribution. The maximum average exposure time to liquidate a sample of around 20l was 6 hrs. Moreover, in order to liquefy CPO (which coagulates at ambient temperature), sellers most often exposed it to sunlight and/or heated it slightly.

The survey also showed that housewives frequently used CPO to cook many foods. They usually used it heated either before cooking (bleaching) or during cooking. To bleach oil, they heated it either for long time at low temperature or for short time at high temperature. Moreover, those using the frying at commercial scale (doughnuts, chickens, fish etc.) declared that they always heated oil at high temperature for a long time and often recycled it. Depending on the food, CPO is added either at the beginning, during or at the end of the cooking. So, it results in dishes with oil distributed on the surface of...
food and those with oil uniformly (mixed with all) integrated to the matrix of food.

The objective of this study was to evaluate the effect of heating and of short exposure to sunlight on carotenoids from CPO. More specifically, we firstly assessed the effect of exposure to sunlight and of bleaching on carotenoids content and chemical quality (free fatty acids, peroxide value) of CPO. Secondly, we evaluated the retention of carotenoids from CPO during cooking of maize (*Zea mais*) cake, food commonly cooked in many households and eaten by many people in Cameroon.

**Materials and Methods**

**Sampling**

CPO used in this study was obtained in the area of *Moungo*, Littoral Region Cameroon. It was collected in the plastic cans of 20l and brought to Biochemistry Laboratory (University of Douala). Maize, wraps (banana leaves) and others ingredients were obtained in *Bonamoussadi* market, Douala Cameroon. We used white variety of maize because it was not only the most used by the housewives, but also the most available as compared to the yellow variety. Moreover, contrary to white variety, yellow variety contains carotenoids (around 0.4 mg/100 g flesh mater for dry seed). Thereby, these carotenoids could influence the study by interfering with carotenoids from CPO. Maize flour was obtained from maize seeds by crushing with grinder, then packed in plastic bags and brought in Laboratory. We firstly determined the composition of this flour and of Banana leaves used as wraps. We found that the flour contained around 12.4% of water; 70.7% of carbohydrates; 9.4% of proteins; 1.4% of fibers; 1.5% of ashes, 4.2% of lipids and 11.4µg of carotenoids per 100g of fresh mater. As for banana leaves, they contained around 87% of water; 12.6% of organic mater and 1.4% of ashes.

**Methods**

Three independent samples were done for each study of this work.

**Study of the effect of exposure to sunlight:** Three samples of 10l each of CPO contained in green plastic basins were exposed to sunlight during 14 hrs (7 hrs of exposure per day during 2 days) at 79359 ± 41628 Lux (1200-144000 Lux). Basins used had an interior diameter of 38 cm and a wall with 0.3 mm of thickness. Surface of oil directly exposed to sunlight was therefore 1134 cm² and expose volume to surface ratio was 8.82 ml/cm². Between the two exposition days, the containers were sealed and kept hideaway of light. In Parallel, a control of oil without light (<2 Lux). Samples of 50ml were collected after 1, 2, 3 and 4 hrs. After cooking, the samples were cooled down for the analysis of moisture and carotenoids contents. 500 ml of CPO and maize cake without oil were done under the same conditions as controls.

**Analysis methods:** Moisture content was analyzed by gravimetric method according to AOAC protocol [18]. Briefly, samples were dried until constant weight in an electric oven (Binder FDL 115) at 105°C. Moisture content was then calculated as percent of water loss.

Carotenoids content was evaluated by photometry (ichekTM Carotene; BioAnalyt GmbH, Teltow, Germany) at 446 nm. The procedure of analysis in CPO and in maize cake was different. Concerning CPO, a sample of 20 µl of oil was diluted with 2 ml of hexane. After vigorous shakeup, the mixture was read with photometer which gives the carotenoids content of solution in mg/l. The carotenoids content of CPO was calculated from this value by taking into account the dilution factor. As for maize cake, previous extraction of carotenoids was necessary. Thus, approximately 2 g of crushed food was added to a tube containing the mixture of ethanol/hexane (1:1). After centrifugation at 3000 revs/min for 5 min, the hexane phase was transferred to another tube. The procedure was repeated until the total discoloration of the residue. The volume of the hexane phases obtained was noted and 2 ml was used to read carotenoids content in photometer. The carotenoids content of maize cake in mg/100 g fresh mater was calculated from this value according to the mass of food used and the total volume of hexane phase obtained. As for carotenoids contents in mg/100 g dry mater, it was evaluated taking into account the moisture content of cake. Carotenoids losses were deducted from carotenoids contents in mg/100 g dry mater of food before and after cooking.

FFA content of oil was evaluated by titrimetric method according to AOAC protocol [18]. Briefly, 25 ml of ethanol was added to around 2 g of oil sample. The mixture was brought to boil in a water bath and then cooled down. After adding 2 drops of phenolphthalein as indicator, 0.1N NaOH was used to titrate the mixture with constant shaking for proper mixing until end-point (appearance of violet color). The free fatty acid content was calculated as followed, FFA=VxNxA/100gW, where V=volume of NaOH, N=normality of NaOH (0.1N), M=molecular weight of palmitic acid (256 g/mol) and W=weight of the sample.

As for PV, it was analyzed by titrimetric method according to AOAC protocol [18]. Briefly, mixture of CH3COOH/CHCl3 (3:2) was added to 2 g of oil sample. Then, added 0.5 ml of saturated KI; agitated
during 1 min and added 15 ml of distilled water and 0.5 ml of starch. Therefore, this solution was titrated with 0.1N Na₂S₂O₃ until total disappearance of blue color. Meanwhile, we performed a blank test without oil. Peroxide value was calculated from the equation, PV (meq/Kg)=1000x(V₂–V₁)xM/N, where M=mass of oil taken, V₁=volume of Na₂S₂O₃ for essay, V₂=volume of Na₂S₂O₃ for blank and N=normality of Na₂S₂O₃ (0.1N).

Data analysis: Data were processed using format designed in Microsoft Excel version 2010. Statistical analyses were performed with Graph Pad Prism package version 5.00 (San Diego California USA). T-Student and one-way ANOVA (analysis of variance) tests were used to determine the influence of one factor. Two-way ANOVA was used to determine simultaneous influence of two factors and of their interaction. P-values were used as measure of significance and P<0.05 was considered significant.

Results

Effect of sunlight on CPO

Sunlight did not have a significant effect on carotenoids content of CPO after 14 hrs of exposure (Table 1). Indeed, carotenoids content did not significantly vary during exposure and there was no significant difference between test and control (from 629.80 ± 39.00 mg/l to 629.63 ± 38.95 mg/l for test and from 629.80 ± 39.00 mg/l to 629.77 ± 38.95 mg/l for control).

Likewise, FFA did not vary significantly during exposure as well for test as for control (Table 2). In fact, FFA passed from 3.500 ± 0.200% to 3.540 ± 0.200% for test and from 3.500 ± 0.200% to 3.533 ± 0.215% for control.

Similarly, PV did not change significantly during exposure. But, contrary to carotenoids content and FFA, it varied more quickly in the test compared to the control. Indeed, the results showed a slight increase of PV in the test at the 2nd hour of exposure whereas it is only at the 5th hour that we noted this increase in the control. Moreover, after 14h of exposure, the PV increased of around 0.07 meq/kg in the test against only 0.02 meq/kg for the control. Although this result was not significant, it confirms that sunlight carries a pro-oxidizing activity on CPO (Table 3).

Effect of heating on CPO

For the study of the effect of direct heating, the results presented in Table 4 showed that it neither significantly changed with heating time nor with heating temperature.

As for the study of the effect of heating in the food matrix, the results (Table 7) showed that carotenoids content of maize cake with CPO and of controls (CPO and maize cake without oil) significantly increased as well with temperature (P<0.0001) as well as with time (P<0.0001) of heating. There was a significant interaction (P=0.0224) between the effect of the temperature and time of heating: at the higher temperature, the shorter time needed for carotenoids degradation. Nevertheless, let us note that the results also showed that at high temperatures (from 120°C), PV increased at the beginning of heating and decreased after certain heating time (from 60 min depending of temperature).

Contrary to carotenoids content and PV, FFA did not significantly vary during heating. Indeed, the result presented in Table 6 showed that it neither significantly changed with heating time nor with heating temperature.

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decreased (P<0.0001) during cooking. Figure 1 gives the variations of carotenoids losses in the maize cake with CPO and in the controls. It shows that carotenoids losses significantly increased (P<0.0001) with cooking time as well for maize cake with CPO as for controls. In fact, these losses passed from 15.33 ± 3.75% after 1 hrs of cooking to 51.00 ± 3.00% after 4 hrs in maize cake with CPO; from 22.33 ± 3.06% to 65.67 ± 4.73% in maize cake without CPO and from 10.33 ± 1.51% to 39.33 ± 4.93% in CPO. Moreover, it was found that whatever the cooking time, the carotenoids losses were significantly lower in CPO compared to maize cake. Likewise, they were significantly lower in maize cake

<table>
<thead>
<tr>
<th>Carotenoids content (mg/l)</th>
<th>Heating temperature (°C)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>Heating time (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>626.60 ± 2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>626.60 ± 2.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>621.40 ± 2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>613.60 ± 5.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>616.20 ± 2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>542.10 ± 3.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>613.60 ± 5.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>368.94 ± 13.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are given as mean ± standard deviation
The values of the same line superscripted with the same letter are not significantly different at P<0.05
Comparison of carotenoids content between different heating temperatures at the same heating time
Comparison of carotenoids content between different heating times at the same heating temperature
ns: No significant difference at P<0.05

Table 4: Variation of carotenoids content of heated CPO according to the time and the temperature of heating.

<table>
<thead>
<tr>
<th>PV (meq/kg)</th>
<th>Heating temperature (°C)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>Heating time (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.983 ± 0.104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.983 ± 0.104&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>2.103 ± 0.105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.260 ± 0.078&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1.050 ± 0.118&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.390 ± 0.171&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.110 ± 0.115&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.363 ± 0.151&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are given as mean ± standard deviation
The values of the same line superscripted with the same letter are not significantly different at P<0.05
The values of the same column superscripted with the sign #, ≠, † or ‡ are not significantly different at P<0.05
Comparison of PV between different heating temperatures at the same heating time
Comparison of PV between different heating time at the same heating temperature
ns: No significant difference at P<0.05

Table 5: Variation of PV of heated CPO according to the time and the temperature of heating.

<table>
<thead>
<tr>
<th>FFA (%)</th>
<th>Heating temperature (°C)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>Heating time (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.567 ± 0.379</td>
<td>3.567 ± 0.379</td>
</tr>
<tr>
<td>0.5</td>
<td>3.587 ± 0.405</td>
<td>3.583 ± 0.390</td>
</tr>
<tr>
<td>1</td>
<td>3.597 ± 0.422</td>
<td>3.610 ± 0.419</td>
</tr>
<tr>
<td>2</td>
<td>3.660 ± 0.478</td>
<td>3.620 ± 0.419</td>
</tr>
</tbody>
</table>

The values are given as mean ± standard deviation
Comparison of FFA between different heating temperatures at the same cooking time
Comparison of FFA between different heating time at the same heating temperature
ns: No significant difference at P<0.05

Table 6: Variation of FFA of heated CPO according to the time and the temperature of heating.

<table>
<thead>
<tr>
<th>Before cooking</th>
<th>After cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
</tbody>
</table>

Crude palm oil
- mg/l
- µg/100 g of fresh mater
- µg/100 g of dry mater

Maize cake without oil
- mg/l
- µg/100 g of fresh mater
- µg/100 g of dry mater

ASSAY (Maize cake with oil)
- mg/l
- µg/100 g of fresh mater
- µg/100 g of dry mater

The values are given as mean ± standard deviation
The values of the same line superscripted with the same letter are not significantly different at P<0.05

Table 7: Variation of carotenoids content of maize cake with CPO and of controls according to the cooking time.
with CPO compared to maize cake without oil. By example, after 1h of cooking, we noted 10.33 ± 1.51% of losses in CPO, 15.33 ± 3.75% in maize cake with CPO and 22.33 ± 3.06% in maize cake without oil. After 4 hrs of cooking, we noted 39.33 ± 4.93%; 51.00 ± 3.00% and 65.67 ± 4.73% of losses respectively in CPO, cake with CPO and cake without oil.

**Discussion**

The objective of this work was to evaluate the effect of sunlight and of heating on the CPO quality particularly on its carotenoids concentration. We noted that short exposure to sunlight did not significantly affect carotenoids content, FFA and PV of CPO. This result can be partially explained by the exposure time which was very short. In fact, some study showed that it is after five day of exposure to sunlight that one could observe a significant variation of physico-chemical parameters of vegetables oil [16]. Besides, another authors noted that FFA and PV significantly varied in CPO only after two weeks of storage [19]. This result could be explained by the distance of incident radiations on exposed oil and indirectly by the radiations intensity. Indeed, formal studies showed that the exposure of CPO on red, green or blue light (that incidence distance is short compared to sunlight) leaded to high increase of FFA compared to exposure to sunlight [20]. Moreover, other authors remarked that exposure of olive oil on UV light and on white light resulted to high reduction of β-carotene and high increase of PV compared to exposure to sunlight [14]. One could explain this result by high content and diversity of natural antioxidants of CPO (carotenoids, tocopherol and tocotrienol). This hypothesis is supported by the results of the study of Zeb et al. [14] who noted, contrary to our study, that exposure of olive oil to sunlight during 14 hrs resulted to a significant decrease of β-carotene and high increase of PV. This contradictory result could be explained by the fact that antioxidants content of olive oil is lower compared to CPO. Moreover, Schroeder et al. [21] showed the synergic mechanism of antioxidants during frying with CPO.

Study of the heating effect on CPO was done in two parts. The first part on the effect of direct heating (bleaching) and the second part on the effect of heating in the matrix of food (case of maize cake). We noted that direct heating resulted to significant decrease of carotenoids content and significant increase of PV. The reduction of carotenoids could be attributed directly to the effect of heating or indirectly to the effect of reactive oxygen species (singlet oxygen, superoxide radical, hydroxyl radical) issued from lipids oxidation. In fact, these compounds react with polyene chain of carotenoids to generate carotenoids-adducts radicals [22,23]. This work confirms the synergic effect of time and temperature of heating on the lipids oxidation. These results corroborate with those of Ahmed et al. [13] who had the same observations during heating of red palm olein. Likewise, Zeb et al. [14] showed a significant decrease of β-carotene and a significant increase of PV in the olive oil samples heated at 72°C compared to those stored at 4°C or at ambient temperature (25°C). As Ahmed et al. [13], we observed that at high temperature, PV increased at the beginning of heating and decreased at high heating time. In fact, the primary products of lipid oxidation are hydroperoxides, therefore the result of PV give a clear indication of oxidation. The reduction of PV at elevated temperature could be attributed to the rapid decomposition of hydroperoxides to secondary oxidation product [13]. Opposing to carotenoids content and PV, we noted that heating did not significantly affect FFA. This result is in accordance with those of Ahmed et al. [13]
and Okiy and Oke [24] who had similar observations. Some authors noted a significant increasing of FFA of oils with time and temperature during frying [25]. The difference between the two studies is due to the fact that in the present study there was no frying process. Frying food results in increasing of the water content of the oil and subsequently increases the hydrolysis and FFA content. The bleaching of CPO carried on a significant oxidizing activity (proportional to heating time and temperature) resulting not only to the reduction of carotenoids content, but also to the formation of dangerous compounds (hydroperoxides) which may react with cellular components such as proteins, nucleic acids, lipids, carbohydrates and poly-unsaturated acids [26,27].

The results of heating of CPO in the matrix of foods on the carotenoids content are in accordance with the observations done during the direct heating. In fact, during cooking one noted a significant diminution of carotenoids content of maize cake with CPO and of controls (maize cake without oil and CPO) according to cooking time. It was remarkably noted that independently of cooking time, carotenoids losses in maize cake with CPO were significantly lower compared to the control maize without oil and higher compared to the control CPO. This result agrees with the study of Ahmed et al. [28] who showed that during storage, cakes made with palm oil resisted better to oxidation reactions compared to those made with ordinary margarine. As previously, this could be explained by high content and diversity of natural antioxidants of CPO (carotenoids, tocopherol and tocotrienol).

Conclusions and Recommendations

We can conclude that although sunlight does not have a significant effect on carotenoids content of CPO at the short term, it carries on a small pro-oxidant activity. Contrary, heating of CPO carried on a significant oxidizing activity (proportional to heating time and temperature) resulting not only to the reduction of carotenoids content, but also to the formation of hydroperoxides which are dangerous compounds. Likewise, the heating of CPO in the matrix of foods results to significant reduction (proportional to heating time) of carotenoids content. Thus, we recommend to traders to avoid exposing CPO in open air during distribution. To housewives, we recommend to avoid bleaching of CPO, to cook foods requiring uncolored oils, they could use less colored oils (cotton oil, olive oil, soya oil, groundnut oil or refined palm oil) or blend them with CPO. Also they could develop the strategies to reduce maximally carotenoids losses during cooking of foods in which CPO is mixed with the matrix of food by varying for example some cooking parameters as cooking time, quantity of oil used or kind of package.

Acknowledgements

The authors acknowledge BioAnalyt GmbH (Teltow, Germany) for gracefully provided us the photometer (ichekTM carotene) for carotenoids analysis.

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