TOTAL CAROTENOIDS, TOCOPHEROLS AND FREE FATTY ACIDS LEVELS OF PALM OILS PRODUCED FROM SMALL SCALE MILLS IN OVIA - NORTH EAST LOCAL GOVERNMENT AREA OF EDO STATE-NIGERIA

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ABSTRACT
This research was conducted to determine the total carotenoids, tocopherols and Free Fatty Acid (FFA) levels of oils produced from small scale mills and to evaluate their antioxidant effects. The study areas were located in Ovia-North East Local Government area of Edo State. The methods of harvesting, processing of the fruits and the storage of the oils were observed because of the positive effects the latter have on quality. Samples of palm oil were collected in duplicates with 100ml screw cap glass sample bottles from the storage drums of the ten (10) small scale mills. The total tocopherols and carotenoids of the oil samples were determined by the AOCS methods. The moisture content of the oils was determined with an electronic moisture analyser. The Free Fatty Acid was determined by the titrimetric method of sodium hydroxide. The results indicated that most of the oils were prone to oxidation and hydrolysis as their moisture (between 0.15 ± 0.01% and 0.53 ± 0.03%), tocopherols (between 19.50 ± 0.10 mg/100g and 51.0 ± 0.00 mg/100g) and carotenoids (between 24.40 ± 0.10 mg/100g and 65.0 ± 0.20 mg/100g) values suggest. Statistical analysis revealed that significant difference exists between the mean of each of the parameters of the oil sample analyzed. The FFA results indicated that the oils were oxidized which suggested that the the carotenoids and tocopherols did not play their expected roles because their antioxidant limit exceeded.

Keywords: Carotenoids, Tocopherols, Moisture, Free Fatty Acids, Small Scale Mills, Palm oil

INTRODUCTION
Carotenoids are made-up of a pigment family containing over 700 different species, consisting of a C-40 polyene backbone with conjugated double bonds. Their structure could be modified at one or both ends, that is, cyclization or the introduction of oxygen to yield different species. The presence of the latter functions establishes the basis for a general classification into oxygen containing (xanthophylls) and monoxigen containing carotenoids (carotenes) (Briton, 1995; Eric et al., 2010). The carotenoids give the palm oil its characteristic colour (Edem, 2002). The major carotenoids found in palm oil are the beta-carotenes. The carotenoids in red palm oil are the most important minor constituents. When the carotene molecule is slit by the addition of the elements of water to both halves, vitamin A is produced. In beta-carotene, the two halves are identical, but in the other carotenones they are not, only one-half being of the constitution is necessary for vitamin A formation (Hartley, 1977).

All plants and many micro-organisms (bacteria and fungi) are able to synthesize carotenoids, but animals and humans are not able. Consequently, animals and humans have to depend on dietary intake for these phytonutrients. Apart from palm oil, other dietary sources include coloured vegetables and fruits (Miller, 1997), eggs (Schlatterer and Breithaupt, 2006) and some fish (Ytrestoyl et al., 2004).
Vitamin E was first discovered as a nutritional factor preventing death and resorption of foetus in pregnant rats (Bramley et al., 2000; Giovanna and Anna, 2011). No characteristic disease state or disorder has been linked to vitamin E deficiency in human, but there are clear literature indications that they are associated with high risk of atherosclerosis and degenerative diseases (Giovanna and Anna, 2011).

The increased cultivation of the oil palm in the area where this study was conducted has led to private ownership of small scale mills which culminated in the indiscriminate production of the palm oil. Arising from the above background, this study was carried out to determine the levels of carotenoids, tocopherols and the Free Eatty Acid in oils processed from small scale mills and to evaluate the antioxidant effects of the tocopherols and the carotenoids. Additionally, the harvesting, processing and storage methods of the processors were examined and related to the quality of the oils.

**MATERIALS AND METHODS**

**Harvesting, processing and storage of finished products.**

The oil palm processors harvested their fruits from both personal and hired fields by making use of contract harvesters. A combination of ripe and not fully ripe fruit bunches were harvested. Majority of the fruit bunches were bruised. The fruit bunches were left to 'ferment' for one week and some of the processors had to cut the bunches into two halves or more in order to enhance the process of fruit loosening. Thereafter, the fruits were transferred into a metal drum with water where they were cooked /sterilized for hours and the extraction of the oils was done with a hand screwed press. The mesocarp residues were heated in the processing drum with water for hours in order to re-extract any remaining oil. After cooling to room temperature, the oil was separated from the water by manual method. Repeated visits of more than ten times were done to the sites to monitor the above processes before and during the experimental periods.

**Sample collection**

The oils were stirred for homogeneity with a wooden stirrer before 100ml of the oil samples were collected in duplicates into screw cap glass bottles from the storage drums and labeled accordingly. It was from the storage drums the oils were dispensed for sale to the public for consumption.

**Laboratory Analysis.** The AOCS methods for the determination of tocopherols and carotenoids were adopted for these studies (AOCS, 1995). The moisture content was determined by using an electronic moisture analyzer, Model M-L50 made by Cole Palmer USA. Five grams of oil sample was weighed, transferred into the moisture analyzer and switched on. Within few minutes the moisture content was displayed and recorded accordingly. The High Pressure Liquid Chromatographic (HPLC) method which permitted the analysis at low temperature in order to preserve the tocopherol was used. The sample was first derivatized to form the trimethylsilyl (TMS), with low boiling point, and was subsequently analyzed and quantified. The Free Fatty Acid was determined by the titrimetric method of sodium hydroxide.

**Sample Moisture (%) Tocopherols Carotenoids Free Fatty Acids**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Tocopherols (mg/100g)</th>
<th>Carotenoids (mg/100g)</th>
<th>Free Fatty Acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.30 ± 0.01</td>
<td>21.00 ± 0.10</td>
<td>30.00 ± 0.30</td>
<td>6.90 ± 0.20</td>
</tr>
<tr>
<td>2</td>
<td>0.41 ± 0.01</td>
<td>19.50 ± 0.10</td>
<td>20.00 ± 0.10</td>
<td>7.20 ± 0.00</td>
</tr>
<tr>
<td>3</td>
<td>0.32 ± 0.00</td>
<td>26.00 ± 0.00</td>
<td>28.50 ± 0.30</td>
<td>6.50 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>0.15 ± 0.01</td>
<td>51.00 ± 0.00</td>
<td>55.00 ± 0.00</td>
<td>3.99 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.19 ± 0.02</td>
<td>40.00 ± 0.55</td>
<td>65.00 ± 0.20</td>
<td>3.59 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>0.23 ± 0.00</td>
<td>30.00 ± 0.10</td>
<td>27.00 ± 0.30</td>
<td>4.20 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>0.30 ± 0.00</td>
<td>20.00 ± 0.00</td>
<td>30.00 ± 0.00</td>
<td>6.90 ± 0.13</td>
</tr>
<tr>
<td>8</td>
<td>0.53 ± 0.03</td>
<td>25.00 ± 0.80</td>
<td>25.00 ± 0.50</td>
<td>7.90 ± 0.00</td>
</tr>
<tr>
<td>9</td>
<td>0.30 ± 0.01</td>
<td>22.00 ± 0.00</td>
<td>29.50 ± 0.40</td>
<td>6.80 ± 0.18</td>
</tr>
<tr>
<td>10</td>
<td>0.25 ± 0.00</td>
<td>29.50 ± 0.10</td>
<td>24.40 ± 0.10</td>
<td>6.60 ± 0.02</td>
</tr>
</tbody>
</table>
DISCUSSION
The high moisture contents of most of the oils resulted from the processing methods adopted. For example, the oils were separated from the water after processing by manual method, which led to a lot of moisture carried-over into the finished products and thus hydrolysis. The exposure of the oils in the open storage tanks during the day to the atmosphere/oxygen and light as was practiced by most of the processors resulted in the oxidation of the oils, thus culminating in the formation of volatile products of unsaturated lipid/fats, which causes rancidity/spoilage. Additionally, such practices result in the decomposition of the triglycerides (major component of the oil) and the formation of a wide range of carbonyl compounds, ketones and other by-products that could contribute to flavour deterioration (Frankel, 1980). The low concentration of carotenoids in most of the oils analyzed is associated with the prolonged heating the oils were subjected to during processing. The carotenoids give the yellow characteristics to the oil and are very sensitive to destruction by excessive heat and light (Contrell, 1991). The destruction of these yellow pigments during processing reduces their concentration in the oil. If their concentration falls below the minimum concentration, which is capable of protecting the oil against oxidation, their antioxidant role becomes negative. Additionally, the findings of earlier workers on carotenones which varied from bunch to bunch arising from the studies done on progenies in Nigeria would be relevant in explaining the cause of the low concentrations of the carotenoids. In one progeny it was observed that, thirty-eight out of fifty-one bunches had carotene contents in the low range of 250-500ppm and ten were in the range of 501-700ppm (Nwanze, 1961; Rao, 2000).
Over 80% of the carotenoids values of the oils were below the recommended range of 500-1000ppm. Prolonged sterilization is known to influence the stability of tocopherol in the extracted palm oil mesocarp since this phytoneutrient is biologically active and extremely sensitive towards heat and light. The low tocopherol values in the various oils was certainly due to the prolonged and uncontrolled heating of the oils. Additionally, the FFA analysis which is a measure of the oxidation that has taken place in any oil or fat has shown high values in most of the oils analysed. The implications of the FFA results, which served as a back check on the antioxidant effects of the carotenoids and tocopheroles, indicate that higher levels of these antioxidants correlated with low values of FFA and vice versa. In other words, since most of the oils had high FFA values and low carotenoids and tocopherols values the antioxidant roles were negative. The significances of the carotenoids and tocopherones (which are part of the minor constituents of the palm oil) in human nutrition have continue to attract research interest in recent times. Research has revealed about 600 carotenoids in nature. Amongst these, 13 have been detected in orange-red palm oil and 10% have pro-vitamin A activity. They could be converted to vitamin A, and are thus positive source for the treatment of vitamins A deficiency. They have also been implicated in such diseases like some cancer, arthritis, Alzheimer’s disease, atherosclerosis, cardiovascular disorders and in some eye pathogens(Cho et al., 2007; Dillard and German, 2000; Giovanna and Anna, 2011).

CONCLUSION
The dispersion of tocopherols and carotenoids in adequate concentrations in natural oils (palm oil) are meant to protect the latter against oxidation in storage and in human physiology. The low levels of the above phytonutrients in the oils would expose them to oxidation and thus spoilage. It is obvious from the concentrations of the carotenoids, tocopherols and the correlation between them and the FFA values, that the expected antioxidant roles were not achieved and thus they could not delay the time of oxidation of most of the oils. Human and animal trials on these antioxidants have revealed several health benefits, reducing the risk for development of certain human diseases, especially coronary heart diseases, some cancers and cataract.

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REFERENCES


