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# Determination of β-carotene by High Performance Liquid Chromatography in Six Varieties of Mango (*Mangifera indica L*) from Western Region of Burkina Faso

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**Abstract** Six varieties of mangoes (Amelia, Brooks, Kent, Keitt, Lippens, Springfield)harvested from different pedological areas of the western region of Burkina Faso (Bobo-Dioulasso, Orodara, Banfora) were analyzed by high performance liquid chromatography in order to determine the β-carotene content. The study revealed a variability in β-carotene content between different varieties and regions. The Amelia variety is the richest in β-carotene content and is a good source of provitamin A.

***Keywords:*** *β-carotene, mangoes, liquid chromatography, vitamin A*

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## 1. Introduction

Micronutrient deficiency is a major worldwide public health problem. It is estimated that more than two billion people of all ages are affected indifferent degrees by micronutrient deficiencies, particularly iron, vitamin A, iodine and zinc *Le Bihan et al., (2002), Von Braun (2005)*. According to WHO estimations, about 250 million children of school age are deficient in vitamin A and almost 500,000 become blind every year *Bendech et al., (2000)*. In French speaking countries in West Africa alone, it is estimated that vitamin A deficiency (VAD) contributes to 57, 000 deaths among children aged from 6 to 59 months *(MC Laren and Frigg 2002*). Burkina Faso is one of the most affected countries due to gradually deteriorating nutritional status (*Department of Nutrition 2005)*, and is classified by WHO as one of the 39 countries in which vitamin A deficiency is a major public health problem *(WHO / UNICEF / IVACG 1998*). Recent studies conducted in Burkina Faso showed that vitamin A deficiency (VAD) is still a public health problem *Nana et al., (2005), Hotz et al., (2012)*. One of the strategies to alleviate VAD is the promotion and the consumption of foods rich in vitamin and provitamin A. Animal sources are inaccessible to population due to the increasing poverty. Vegetable sources are more available and seasonal. So consumption of vegetables is more prone to success and is favored by governments and NGOs.

The burden of VAD in Burkina Faso is tackled mainly by the distribution of vitamin A capsules during National immunization days and fortification of staple foods. It should be noted that distribution programs targeted populations of children aged from 0 to 5. Edible, fortified oils and foods are not affordable to target populations due to poverty in our countries. Promoting the production of foods rich in carotenes and consuming these are considered as a sustainable strategy in a country such as Burkina Faso. Provitamins Aare found in dark-green vegetables orange-fleshed potatoes, red palm oil and especially in seasonal fruits. Several studies have shown their interest in the care and prevention of vitamin A deficiency *Zagréet al* (2003), *Millset al*., (2009), *Nana et al*., (2005), *Zéba et al., (2006)*. Moreover, in Africa 70-90% of dietary vitamin A comes from carotenoids *Bendech et al., (2000).* Among the provitamin A carotenoid, beta-carotenes are by far those that contribute most to the vitamin A activity of plants *(MC Laren and Frigg 2002*).

The mangoes are seasonal fruit of the tropics which are consumed in harvest times. Compared to other sources of vitamin A, mango is characterized by a low production cost, a significantly high content in β-carotene, improved bioavailability of β-carotene compared to vegetables *(Chen and Chen 1993)* and direct consumption even without being cooked. In Burkina Faso, more than thirty varieties of mangoes have already been identified.

The purpose of this study is to compare the carotenoid content of six (6) varieties of mangoes in three different

pedological characteristicregions namely Bobo-Dioulasso, Orodara and Banfora.

# 2. Materials and Methods

## 2.1. Materials and HPLC Conditions

Analytical standards of lycopene (LYCO), zeaxanthin (ZEA), cryptoxanthin (CRYP), echinenone (ECHI) and β-carotene (BCAR) were obtained either from Sigma (Germany), or a generous gift from Hoffmann-La Roche (Basel, Switzerland). HPLC grade of methanol, acetonitrile, dichloromethane and hexane were from Sigma (France).

Mangoes were harvested from April to July. The following varieties of mangoes (Anacardiaceae) have been studied: Mangiferaindica var. Amelia, Mangiferaindica var. Brooks, Mangiferaindica var. Kent, Mangiferaindica var. Keitt, Mangiferaindica var. Lippens, Mangiferaindica var. Springfield. All the varieties are not found in all the pedologicalregions. The samples of mangoes were more or less similar as regards their maturity status in the different sites and were taken to the laboratory to be kept refrigerated (4-8°C) until they are analysed.

The chromatographic system consists of a JASCO PU-980 pump (Tokyo, Japan) equipped with a 20 µL loop injection, a chromatographic column Supelcosil LC-18 (Bellefonte, USA) of 25 cm length, 4.6 mm in diameter and a particle size equal to 5 µm. The mobile phase used is a ternary mixture consisting of acetonitrile (70% v/v), methanol (20% v/v) and dichloromethane (10% v/v). The mobile phase flow rate was set at 2 mL per minute. The carotenoid detection was carried out at 450 nm with a UV detector JASCO 975 (Tokyo, Japan). The system is coupled with a computer system and data processing software (Galaxy WorkStation). The measurement of the optical density (OD) of standard solutions was done with a UV-visible spectrophotometer A-160 Type CECIL (UK).

## 2.2. Methods

### 2.2.1. Extraction of Carotenoids from Samples of Mangoes

Mangoes weighed, then butchered, crushed and kneaded with a mixer grinder (Moulinex®). The extracts for analysis were prepared as follows: one (1) g of the grounded product is mixed with 1 mL hexane, 1 mL of sodium chloride (3M) and 1 mL ethanol; a stirrer (Vortex®) is used to stir the whole for 2 min. The mixture was then centrifuged at 3000 rpm / min for 5 min at -5°C (Jouan®) to separate the hexane phase containing carotenoids and the aqueous phase. A pipette is used to remove and collect the hexane phase in a small Pasteur test tube. A second extraction is done through the same procedure. The hexane phases are mixed, homogenized and 1 mL mixture is removed and evaporated under nitrogen flux. The residue obtained after evaporation is dissolved in 1 mL of acetonitrile and injected into the chromatograph after being thoroughly mixed. Two injections are performed and the average area value for the calculation of β-carotene content is considered *Somé et al (2004), Mills et al (2009).*

### 2.2.2. Chromatographic Analysis

**Table 1. Regression parameters obtained for five (5) carotenoids validation**

| Carotenoids | Regression parameters | | | |
|---|---|---|---|---|
| | Concentration range (pmol/ uL) | Slop eaverage estimated(SD) | Interceptaverage (SD) | Coefficient of determination |
| **Zéazanthine** | 0.25-1.50 | 2892(107) | 641 (2098) | 0.997 |
| **β-Cryptoxanthine** | 0.25-1.50 | 3808(119) | -277(2319) | 0.996 |
| **Échinénone** | 0.25-1.50 | 2644 (93) | 991 (1807) | 0.995 |
| **Lycopene** | 0.25-1.50 | 4370 (262) | -660(5105) | 0.986 |
| **β-carotene** | 0.25-1.50 | 3217(121) | 1233 (2367) | 0.994 |

The numbers in brackets are standard deviation (SD) for the parameter estimation (n = 3).
Linear regression model: y = a x + b.

**Table 2. Recovery rate (in percentages) for different concentrations measured, compared to linear regression line**

| | Concentration (pmol/uL) | | | | | |
|---|---|---|---|---|---|---|
| | 0.25 | 0.50 | 0.75 | 1.00 | 1.25 | 1.5 |
| ZEA | 98.33 (4.66) | 100.07 (6.24) | 104.37 (4.33) | 96.20 (4.79) | 99.28 (9.87) | 101.87(0.46) |
| CRYP | 97.50 (350) | 103.98 (2.45) | 97.57 (4.44) | 102.74 (2.86) | 102.07 (2.03) | 98.41 (1.52) |
| ECHI | 101.16 (8.88) | 101.06 (4.61) | 93.55 (5.89) | 102.16(2.90 | 101.93 (2.69) | 98.06 (0.86) |
| LYCO | 100.59 (3.60) | 107.69 (3.45) | 87.01 (7.30) | 104.32(1.27) | 103.56(1.42) | 97.97 (2.25) |
| BCAR | 96.73 (1.25) | 101.04 (9.46) | 101.30 (8.73) | 94.24(1.60 | 100.06(11.68) | 104.6 (2.12) |

The numbers in brackets are standard deviation (SD) for the parameter estimation over 3 days ‘experiments (n = 3).

The HPLC method has been previously validated for β-carotene and other carotenoid content determination *Somé et al (2004).* Tables 1 and Table 2 present the performance criteria of the validated method performed over three (3) days with several independent series. An excellent linear range was obtained for the five carotenoid between 0.25 à 1.5 pmol/µL. The recovery rate was in the range of 100±10 % and fidelity study showed that the coefficient of variation was less than 10%.

### 2.2.3. β-carotene Content Determination in Samples

β-carotene peak was identified and measured on the chromatogram on the basis of the retention time for the specific standard located around six (6) min. Figure 1 also illustrates the chromatograms displayed for the six (6) varieties. For each sample of mango, three (3) tests were performed. For each test, the injections were duplicated and the average area of the two (2) resulting injections was subsequently considered for calculation. The data were analyzed and processed by Microsoft Office Excel 2003. A calibration mixture including an internal standard with defined concentrations was injected; a calibration factor is then calculated for each peak as follows:

$$Fi = \frac{Auce \times Ns}{Ne \times Aucs}$$

Fi: calibration factor; *Auce*: Area under curve for the sample; *Aucs*: Area under curve for the standard; Ns: number of pmol injected for β-carotene standard and Ne: number of pmol for injected sample in the following formula.

$$Ne = \frac{Auce \times Ns}{Fi \times Ascs}$$

The calculation of β-carotene content was done with the average of 3 samples weigh tests according to the following formula:

$$T = \frac{Ne \times F \times Pm \times Auce \times 10^{-6}}{Aucs \times Pe} \times 100$$

with T the content of β-carotene (µg/100 g), Pm is the molecular weight for vitamin A standard in g, and Pe is the sample weight test in g.

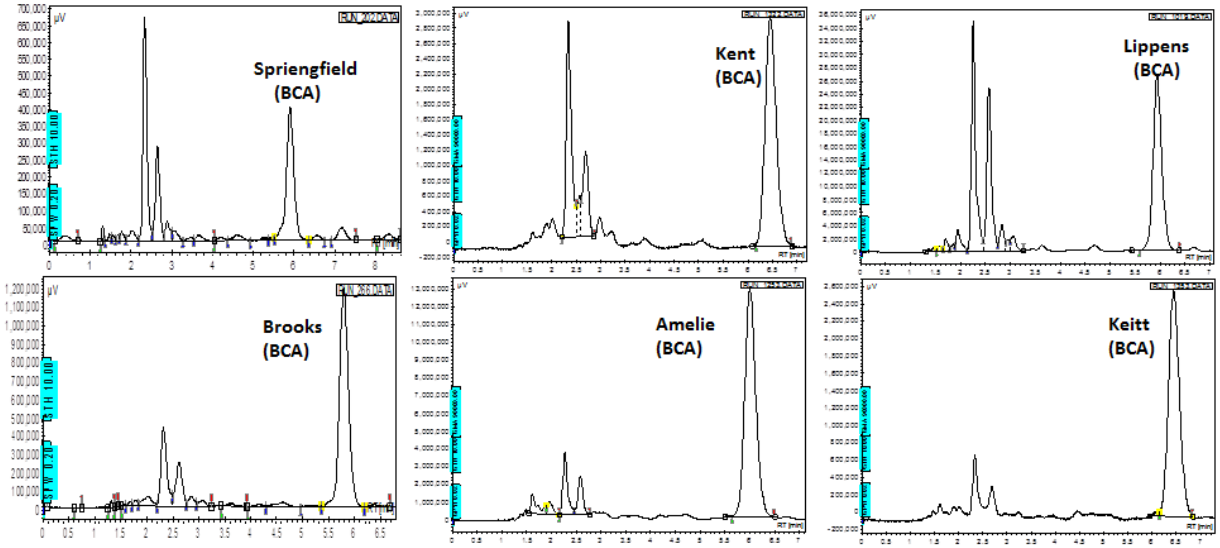


**Figure 1.** Chromatograms of the analysis of the six varieties of mangoes (BCA=β-carotene peak); the retention time is around 6 min

## 3. Results and Discussion

The weights and the percentage of pulp (w/w) for samples of mangoes from different varieties and pedological regions were estimated (Table 3). In the three areas, the *Springfield variety* is the weightiest one followed by the *Kent* and *Keitt* varieties. The *Lippens variety* presented the lowest weight. Except for *Lippens*, the mangoes from Banfora are heavier compared to the two other areas. However, the proportion of pulp was relatively homogeneous for all the samples ($p > 0.05$).

When we consider the three regions, we can find out that Orodara area has the highest frequency of varieties with the highest β-carotene content: three out of the six varieties (*Kent, Lippens, and Amelia).* The samples of these varieties from Orodara are more concentrated in β-carotene compared to those collected in Banfora and Bobo-Dioulasso. As regards the β-carotene contents of the three regions in mangoes the details are shown in Table 4. The *Amelia* variety is the most concentrated in β-carotene in Banfora and Orodara and the *Brooks* in Bobo-Dioulasso. In the region of Bobo-Dioulasso, the *Brooks* variety has the highest content. Samples of the *Brooks variety* harvested in this area are more concentrated in β-carotene than those from Orodara and Banfora. But, except for the *Brooks* variety, the region of Bobo-Dioulasso showed the lowest concentration in β-carotene for all the other varieties. The highest concentration was found for *Amelia* in Orodara.

**Table 3. Average weight of mangoes and their pulp (in bracket) from the 3 pedological regions**

Average weight of mangoes in gram (Pulps percentage in brackets*)*

| Mango variety | Bobo Dioulasso | Orodara | Banfora |
|---|---|---|---|
| *Springfield* | 628 ± 59 (74 ± 6) | 650±83 (74 ± 4) | 709±185 (81±2) |
| *Kent* | 398 ± 43 (75 ± 5) | 464 ± 47 (79 ± 1) | 560 ± 173 (80 ± 4) |
| *Keitt* | 391 ± 55 (81 ± 1) | 497 ± 8 (77±5) | |
| *Brooks* | 312 ± 33 (73 ± 4) | 298 ± 36 (67 ± 6) | 333 ± 48 (71 ± 1) |
| *Amélie* | 287 ± 69 (75 ± 3) | 287 ± 69 (76 ± 3) | 467 ± 87 (80 ± 1) |
| *Lippens* | 280 ± 19 (74 ± 5) | 251 ± 40 (75 ± 2) | 254 ± 27 (79 ± 4) |

**Table 4. β-carotene content in the six varieties of mangoes from the three pedological regions**

| | β-carotene content in mg / 100 g for mangoes samples | | |
|---|---|---|---|
| **Mango variety** | **Bobo Dioulasso** | **Orodara** | **Banfora** |
| *Springfield* | 0.421 ± 0.067 | 0.431±0.115 | 0.702±218 |
| *Kent* | 1.212 ± 0.258 | 2.044±0.637 | 1.703±0.665 |
| *Brooks* | 1.984 ± 0.423 | 1.512±0.439 | 1.297±0.212 |
| *Lippens* | 0.815 ± 0.502 | 1.344±0.242 | 0.850±0.365 |
| *Keitt* | ND | 0.577±0.153 | 0.763±140 |
| *Amelia* | 1.068 ± 0.109 | **5.442±0.800** | **3.952±0.654** |

ND: Not Determined

These concentrations in β-carotene for the *Keitt* and *Springfield* varieties cultivated in Burkina Faso are lower than those reported by Mercadante in Brazil who found concentrations of 1.500 ± 0.200 mg / 100 g *(Mercadante and Rodriguez-Amaya 1998).* The pedological area therefore influences the quantitative composition in β-carotene. A significant difference between the levels of β-carotene can be found depending on the geographical origin in Kenya Muoki *et al., (2009)* and Brazil *(Mercadante and Rodriguez-Amaya 1998).*

The variation of β-carotene content between the different varieties was also revealed in this study. The *Amelia* and *Kent* varieties are the richest in β-carotene with respective values of 3.487 ± 1.932 mg/100 g and 1.653 ± 0.638 mg/100 g. The lowest values are found for the *Springfield* variety whose β-carotene content is 0.518 ± 0.195 mg/100 g. A statistical difference was found between varieties ($p < 0.05$) in β-carotene content. In previous studies, *Amelia* and *Brooks* varieties were found to have respective content of β-carotene of 1.260 ± 0.090 mg/100 g and 1.290 ± 0.220 mg/100 g in ripe fresh mangoes *Zagre et al., (2003)*, which values match those of this study (1.597 ± 0.464 mg/100 g). The content found in *the Springfield* variety in the three (3) regions seems to be comparable with those reported elsewhere *Muoki et al (2009)*. The β-carotene content of mango is related to several factors including the genetic, the stage of maturity, climate or geographic site production and cultivation techniques used (*Mercadante and Rodriguez-Amaya 1998*), *Muoki et al., (2009), (Rodriguez-Amaya 1997), Nestel et al., (2006*).

In comparison with other sources, the β-carotene content in the varieties was higher than those found in fruits like banana (0.04 mg – 0.1 mg/100 g), grapes (0.006 – 0.150 mg/100 g) and watermelon (0.228 – 0.324 mg/100 g) (*UNICEF 1998*). However, these contents were lower than the content reported by Tawata et al. (2003) in carott (*Daucus carota:* 7.8 mg/100 g*).* Furthermore, the *Kent* variety in Orodora and Banfora and the *Brooks* variety in Bobo-Dioulasso and Banfora had similar content compared to potato *(*1.9 - 2.3 mg/100 g) and spinach (2.2 mg/100 g). The concerned varieties for potato are: *Ipomoea batata var. Jewel, Ipomoea batata var. Narumintang, Ipomoea batata var. Caromex Niger Somé et al., (2004).*

Based on its β-carotene content, 100 g of the *Amelia variety* provides retinol equivalent (RE) of 582 µg; the *Springfield strain* is the one that provides the delivery of the least important retinol equivalent with 86 RE. The daily requirement is around 450-500 µg RE and the consumption of only 100 g of the *Amelia* variety mango can cover a daily requirement that provides efficient biotransformation from carotene to retinol is done.

Furthermore, no correlation between the weight of mango and β-carotene content was found. The *Springfield* variety with the lowest content of β-carotene has the most important weight in the 3 regions. On the other hand, the *Amelia* that is the richest one variety in β-carotene is found to have the lowest weight. The β-carotene content from a mango is not linked to its weight percentage of pulp.

# 4. Conclusion

The study showed that the β-carotene content depends on the varieties of mango and the area of growth. This variability is attributable to several factors including differences in plant genetic material, stage of maturity, climate and the geographical location of production and cultivation techniques that are used. The *Amelia* variety is the riches tone in β-carotene content and Orodara is the region with the richest varieties of mangoes. Promotion of culture and consumption of this variety of mango would enhance and meet the need for vitamin A in general and that of the region of Bobo Dioulasso in particular are the poorest one in β-carotene. However, The *Springfield* variety has the highest mass values. No correlation was found between the mass of the mango and carotenoid content. High acceptability of mango, especially for young children, is a definite advantage for the promotion of its consumption in the intervention programs.

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