







## Biochemical characterization of three yellow cassava cultivars grown in the Republic of Congo

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### ABSTRACT

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Cassava exists in two varieties: sweet and bitter. However, the Congolese population considers all yellow-fleshed cassava varieties as sweet, and they are eaten raw or boiled. A variety is considered sweet if its hydrocyanic acid content is less than 50 mg/kg. The objective of this study is to characterize the three most consumed yellow-fleshed cassava varieties in Congo, namely Mboto, Dikondi, and Nkaba yellow varieties. Hydrocyanic acid and beta-carotene were measured by spectrophotometry. Water, dry matter, crude ash, lipid, titratable acidity, and pH were determined according to the Association of Official Analytical Chemists. Total crude fiber and protein were measured by the Weende and Kjeldahl methods, respectively. The analyses carried out on the three cultivars "Mboto, Dikondi, Nkaba yellow" revealed the following contents: hydrocyanic acid: 33.49, 42.75, and 121.24 mg.kg<sup>-1</sup>; water: 89.33, 70.02, and 58.96%; dry matter: 10.74, 29.98, and 41.04%; ash: 2.53, 1.68, and 2.78%; protein: 2.48, 2.01, and 1.98%; lipids: 0.27, 0.59, and 2.89%; fiber: 10.8, 2.4, and 2.4%. The  $\beta$ -carotene contents: 1.58, 0.39, and 0.84  $\mu$ g/g; energy values: 35.00, 123.73, and 171.90 kcal/100g. These results show that among the three varieties, the yellow Nkaba is a bitter variety and cannot be eaten raw.

**Contribution/Originality:** This characterization study of the three most consumed yellow cassava cultivars in Congo has helped the population to no longer believe that "Nkaba jaune" from Loudima, which is a yellow variety, is sweet; it is a bitter variety and should no longer be consumed raw.

## 1. INTRODUCTION

Cassava is one of the world's most important food crops, particularly in the tropics [Technologies for African Agricultural Transformation \(TAAT\) \(2022\)](#). Scientifically known as *Manihot esculenta* Crantz, cassava is an excellent source of carbohydrates. Its tuberous roots have white and yellow flesh or pulp. Yellow cassava contains a significant amount of  $\alpha$ -carotene, a precursor of vitamin A, and contains less cyanide than white varieties ([Whankaew et al., 2011](#)). It comes in two varieties: sweet (less than 50 mg kg<sup>-1</sup> of hydrocyanic acid) and bitter (greater than or equal to 50 mg kg<sup>-1</sup> of hydrocyanic acid) ([Janssens, 2001](#)). The adaptability and profitability of these yellow cassava varieties enable groups that use cassava as a staple food to combat malnutrition. In the Republic of Congo, cassava is

grown in all departments. The tuberous roots of cassava, with their white and bitter flesh, are processed into chikwangue and fufufu, which are staple foods, while those with yellow flesh are eaten raw like carrots or boiled.

However, the Congolese population considers all yellow-fleshed cassava varieties as sweet, and consumes them raw or boiled. The overall objective of this study is to characterize the three most consumed yellow-fleshed cassava cultivars in Congo.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

The plant material used in this study consists of the yellow tuberous roots of *Manihot esculenta* Crantz (yellow cassava). Three cultivars were harvested: "Mboto" from the National Institute of Agronomic Research in Brazzaville; "Dikondi" from the Pool Department in the Boko District; and "Nkaba Jaune" from the Bouenza Department in the Loudima District. Figures 1, 2, and 3 present the different yellow cassava cultivars harvested.



Figure 1. Yellow cassava cultivar "Mboto".



Figure 2. Yellow cassava cultivar "Dikondi".



Figure 3. Yellow cassava cultivar "Nkaba jaune".

## 2.2. Hydrocyanic Acid Determination

Hydrocyanic acid was determined using the picric acid method. A reddish-orange complex forms in the presence of picric acid and hydrocyanic acid. This complex, called isopurpurin, is used to identify hydrocyanic acid.

The harvested cassava tuberos roots are cleaned with water, peeled, then cut into very small pieces. Ten (10) grams of these pieces are ground, and 100 ml of distilled water are added to the ground material. We then mixed, filtered, and measured the volume of the filtrate. A 0.5 ml sample of the filtrate and 4.5 ml of picric acid (6.25 g/L, pH = 10) were placed in a test tube; a control was prepared as follows: 0.5 ml of distilled water and 4.5 ml of picric acid (6.25 g/L, pH = 10) in a test tube. The tubes were homogenized and brought to a water bath at 95°C for 5 minutes. Then they were cooled, and a reading was taken with a spectrophotometer at a wavelength of 490 nm.

A calibration curve (Figure 4) was established from solutions of 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 mg/mL, whose absorbance was measured at a wavelength of 490 nm. These solutions are obtained by successive dilution of 1 mg/mL of cyanide from a KCN solution.

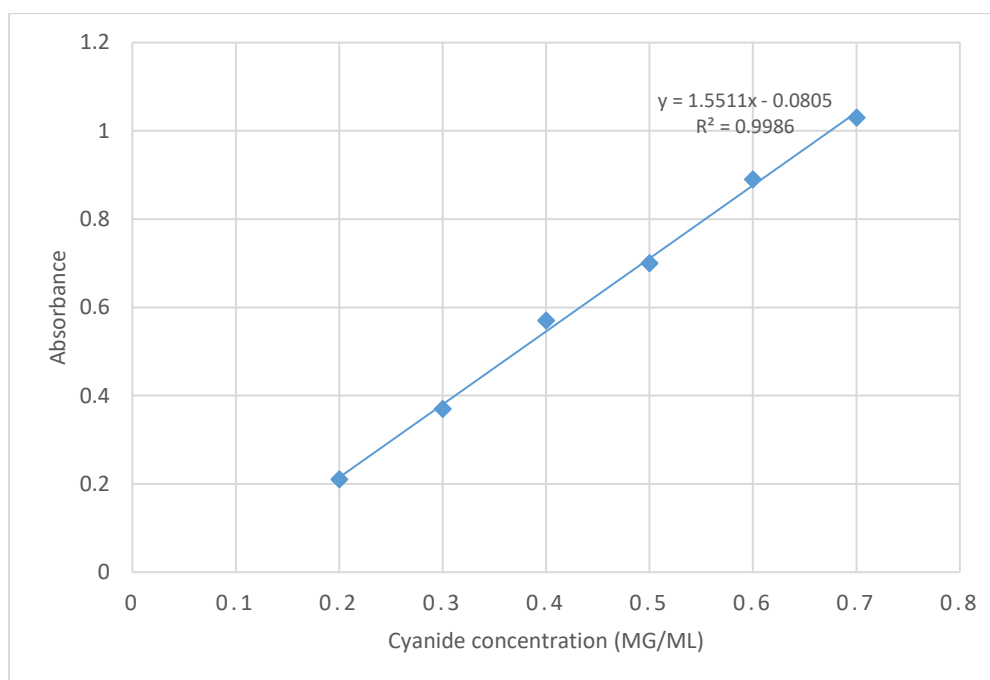


Figure 4. Calibration curve using KCN solution.

## 2.3. Determination of Water and Dry Matter Content

The determination of water content (% H) was carried out according to the AOAC (1995) based on the measurement of mass loss of samples after drying until complete elimination of free water and volatile matter. A mass of 70 g of samples (M) was dried at a temperature of  $105 \pm 2$  °C for 2 days while weighing regularly until the masses no longer varied.

The water content is expressed as a percentage and is determined by the mathematical expression below (Equation 1):

$$\%H = \frac{M - M_1}{M} \times 100 \quad (1)$$

The dry matter content (% DM) is given by the following Equation 2.

$$\%DM = \frac{M_1}{M} \times 100 \quad (2)$$

With: % H: Water content; M: Mass of fresh roots; M1: Mass of dry roots.

#### 2.4. Total Crude Ash Content

The crude ash content of dry matter is determined according to the AOAC (1995). Crude ash is the residue obtained after incineration at 550 °C in an electrically heated muffle furnace to a practically constant mass. Ash content is one of the characteristics used to judge the purity of a product. It also allows the mineral content in the sample to be assessed.

A mass (M) of 5 g of sample was placed in a previously cleaned and dried porcelain crucible. Then, the crucible was placed in a muffle furnace for incineration at 550°C for 8 hours. The crude ash content (% C) was determined from the following mathematical formula (Equation 3):

$$\%C = \frac{M_2 - M_1}{M} \times 100 \quad (3)$$

With: % C: Ash content; M: mass of the sample; M<sub>1</sub>: mass of the empty crucible; M<sub>2</sub>: mass of the crucible containing the ash.

#### 2.5. Protein Assay (AOAC, 1995)

Protein determination is carried out using the Kjeldahl method with a VELP SCIENTIFICA device. This is an indirect method of protein determination by measuring the amount of nitrogen in proteins in their mineral form.

In a Matra, we weighed 0.5 g of crushed plant sample, previously dried overnight in an oven at 105°C, in which we added a spatula tip of mineralization catalyst and 10 ml of concentrated sulfuric acid. Subsequently, we added some glass beads. We placed the tubes on the mineralization ramp and let them mineralize cold for 30 min, then turned on the heating (at thermostat 7) and let them mineralize hot for 2 hours. After obtaining a clear supernatant solution and a white residue, we let it cool and then added a solution containing 20 ml of water and about 30 ml of sodium hydroxide at 400 g/L (until the solution turns brown). The mixture was steamed by collecting the distillate in a 500 ml test tube containing 20 ml of boric acid and a few drops of colored indicator (bromocresol green and methyl red) until a volume of 125 ml was obtained. This solution was titrated with N/20 sulfuric acid until the indicator changed from green to pink. The total nitrogen content is obtained by the following mathematical expression (Equation 4).

$$\%N = \frac{V(H_2SO_4) \times 0.07}{M} \quad (4)$$

Where:

N (%): Total nitrogen content; V(H<sub>2</sub>SO<sub>4</sub>): Volume of sulfuric acid solution required to obtain the color change; M: Mass of the sample.

The corresponding total protein content is obtained by multiplying the total nitrogen content by the conversion factor 6.25 (Equation 5).

$$\%P = \%N \times 6.25 \quad (5)$$

With: (%) P: protein content in percentage; N (%): nitrogen content (%); 6.25: nitrogen to protein conversion coefficient.

#### 2.6. Lipid Extraction (AOAC, 1995)

Lipid extraction was performed using the Soxhlet method. 20 grams (M) of dried samples were placed in a Whatman cellulose cartridge. This cartridge was placed in the Soxhlet apparatus, above which was a condenser and below which was a flask of mass M<sub>1</sub> containing 200 ml of extraction solvent (N-hexane). The flask was heated using a heating mantle to a temperature allowing the solvent to boil. After several siphonings (6 hours of extraction), the extraction was stopped, and the flask containing the oil and solvent was removed. The solvent was then separated from the oil; the excess solvent in the fat was removed by placing the flask in the oven. Finally, the flask containing the oil was weighed, and a new mass M<sub>2</sub> was recorded.

The fat yield was determined as follows:

$$\%MG = \frac{M_2 - M_1}{M} \times 100 \quad (6)$$

With:

% MG: Oil (lipid) content; M: mass of the sample; M<sub>1</sub>: mass of the flask; M<sub>2</sub>: mass of the flask containing the extracted oil

### 2.7. Determination of Crude Fiber Content

A mass of 1 g of flour sample (M) is brought to a boil with 50 ml of sulfuric acid (0.25 N) for 30 minutes, and then 50 ml of sodium hydroxide (0.31 N) is added while continuing to heat for 30 minutes. The residue obtained is transferred into a previously weighed cup (P<sub>1</sub>) and then dried in an oven at 105 °C for 8 hours, then allowed to cool in a desiccator, and a new weighing is carried out (P<sub>2</sub>). The dried residue is then incinerated in an oven at 550 °C for 3 hours, followed by another weighing (P<sub>3</sub>). To determine the crude fiber content, the following formula was used (Equation 7):

$$\%F = \frac{(P_2 - P_1) - (P_3 - P_1)}{M \times DM} \times 100 \quad (7)$$

With: % F: Fiber content (%); P<sub>1</sub>: mass of the empty crucible; P<sub>2</sub>: mass of the crucible containing the residue after removal from the oven; P<sub>3</sub>: mass of the crucible containing the residue after removal from the furnace; M: mass of the sample; DM: dry matter content.

### 2.8. Dosage of $\beta$ -Carotene

Carotenoids were analyzed by UV-visible spectrophotometry using the method described by [Sass-Kiss, Kiss, Milotay, Kerek, and Toth-Markus \(2005\)](#). A mass of 50 mg of the powdered sample was placed in the centrifuge tube while adding a volume of 10 ml of the solvent mixture obtained from 5 ml of hexane, 2.5 ml of acetone, and 2.5 ml of ethanol. After shaking the tubes for 15 minutes and then centrifuging at 4500 rpm for 15 minutes, the supernatant was collected; the pellet underwent a second centrifugation under the same conditions as before. A reading with the UV-VISIBLE spectrophotometer at 450 nm was taken. The standard curve ([Figure 5](#)) below was made from the different  $\beta$ -carotene solutions.

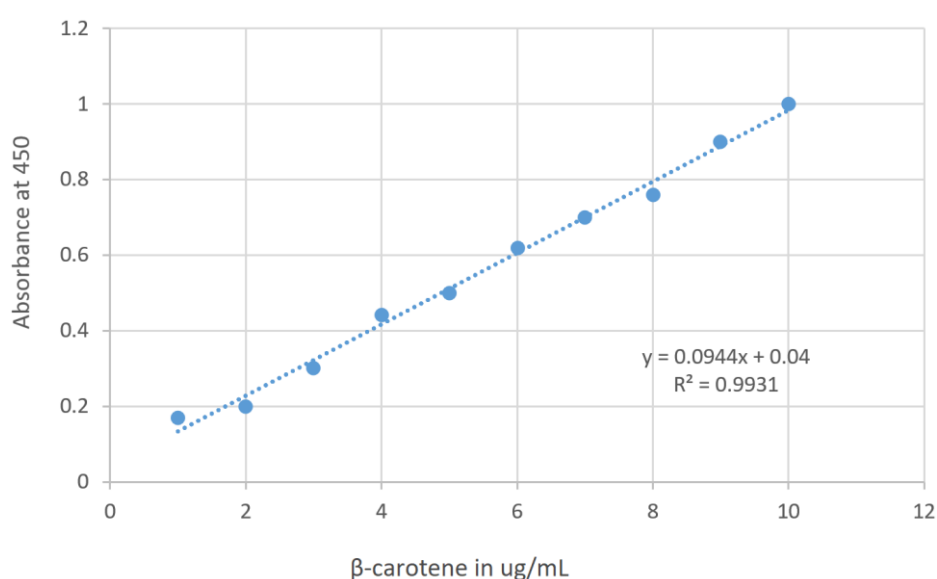


Figure 5.  $\beta$ -carotene Calibration curve.

### 2.9. Data Processing and Analysis

Data processing was performed using Excel 2016 software, which allowed us to determine means and standard deviations and to create graphs for each parameter studied.

## 3. RESULTS AND DISCUSSION

### 3.1. Hydrocyanic Acid Content

Figure 6 gives the hydrocyanic acid contents of the three yellow cassava cultivars studied.

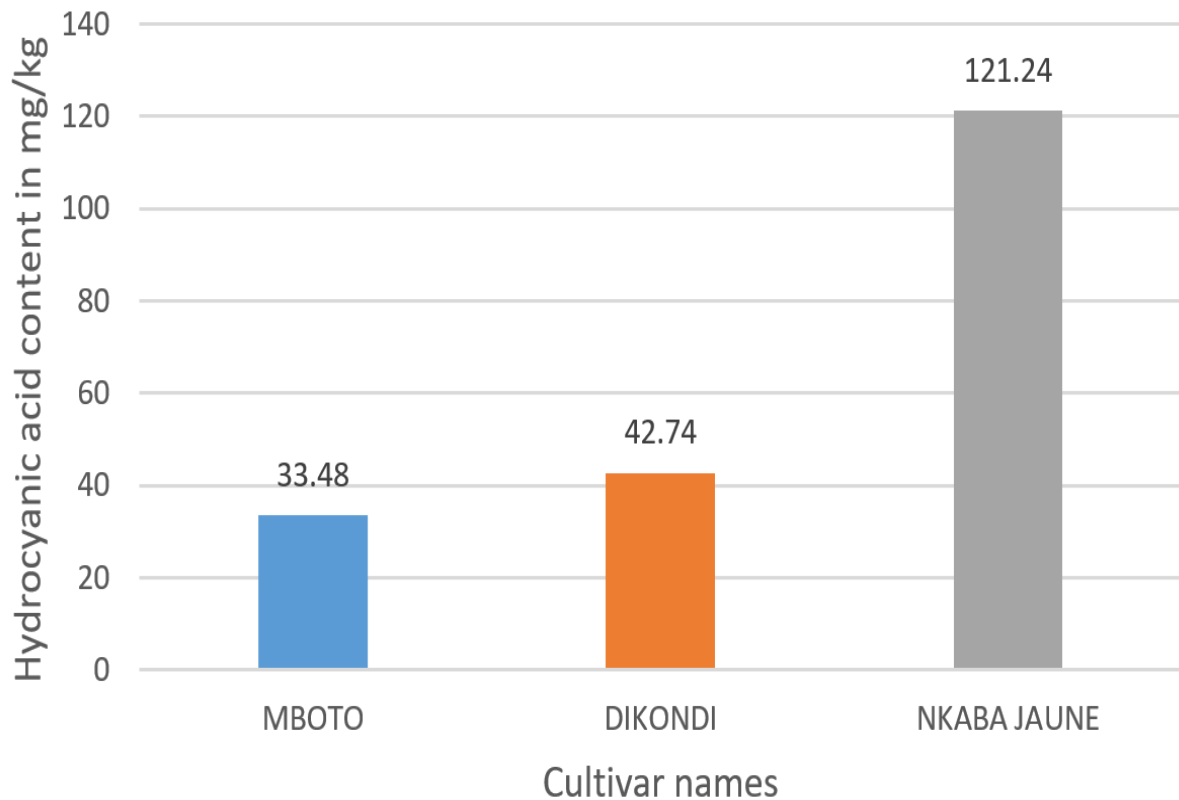


Figure 6. Cyanide content of the studied cultivars.

Figure 6 reveals that the yellow cassava cultivars studied are characterized by cyanide levels ranging from 33.48 to 121.24 mg/kg. Data analysis revealed differences between cultivars with respect to these values. Cultivars with levels lower than 50 mg HCN•kg<sup>-1</sup> are called sweet varieties and cultivars with levels greater than or equal to 50 mg HCN•kg<sup>-1</sup> are called bitter varieties (Cumbana, Mirione, Cliff, & Bradbury, 2007; Janssens, 2001). Thus, two groups of varieties were distinguished. The first group consists of the cultivars “Mboto” and “Dikondi,” which, with respective levels of 33.48 mg/kg and 42.74 mg/kg, are sweet varieties and therefore harmless, i.e., with less than 50 mg HCN•kg<sup>-1</sup>. The cyanide levels of these two cultivars were lower than those of the “Nkaba yellow” cultivar 121.24 mg/kg, which is bitter because its content is higher than 50 mg HCN•kg<sup>-1</sup> and according to Kobawila, Louembe, Keleke, Hounhouigan, and Gamba (2005) the varieties whose content is 100 mg HCN•kg<sup>-1</sup> are highly toxic cultivars, cannot be eaten raw, and must be detoxified before any processing and consumption.

### 3.2. Water Content

The results of the water content of the yellow cassava cultivars studied are presented in Figure 7.

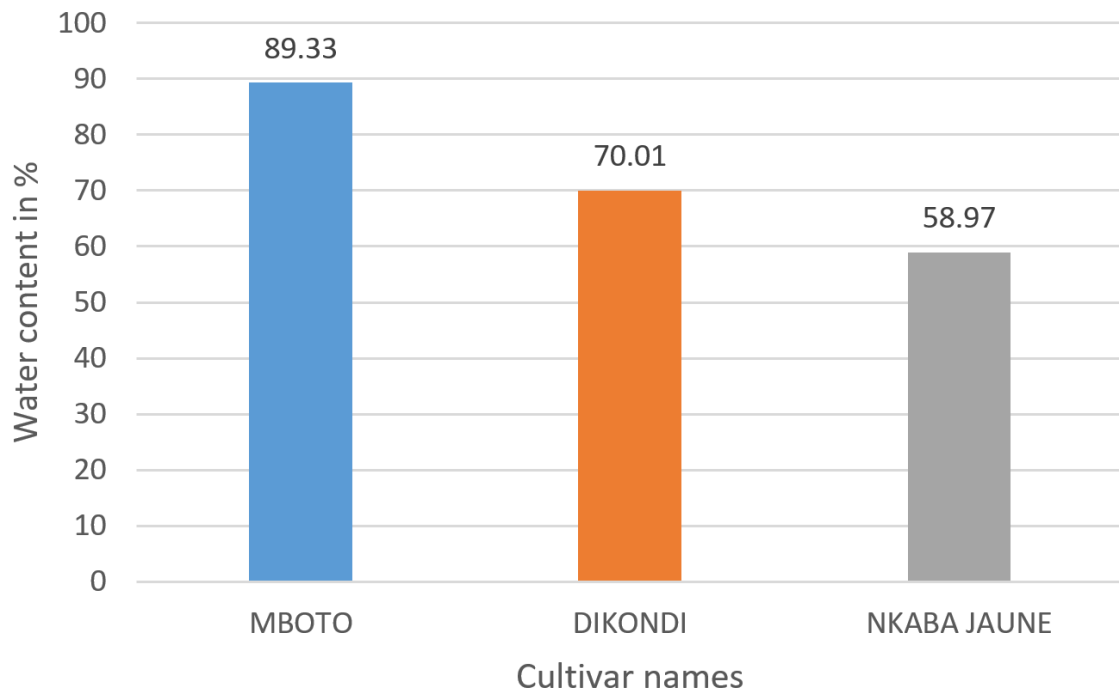


Figure 7. Water content of the studied cultivars.

The results presented in Figure 7 show that the samples have water contents of 89.33% for "Mboto", 70.01% for "Dikondi" and 58.95% for "Nkaba jaune" respectively. From the analysis of these data, it appears that the highest water content, estimated at 89.33%, was recorded in the Mboto cultivar. This content is high compared to the water content of Mboto harvested at the Odziba Nord agricultural site, which is 60.82% (Kiminou Ngounga, 2020). Nevertheless, both yellow and white cassava tuberous roots contain high water contents (Kiminou Ngounga, 2020), which makes cassava tuberous roots perishable and cannot be stored beyond ten days after harvest.

### 3.3. Dry Matter Content

The dry matter contents of the samples studied are presented in Figure 8.

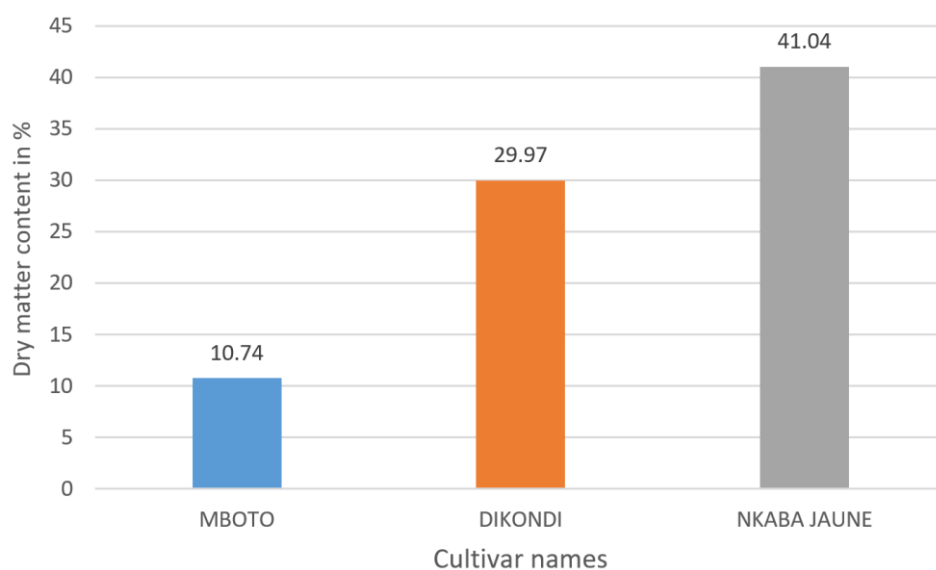


Figure 8. Dry matter content of the studied cultivars.



Figure 8 shows that the samples have a dry matter content of 10.74% for the cultivar “Mboto”; 29.97% for the cultivar “Dikondi”; and 41.04% for the cultivar “Nkaba jaune”. From the analysis of these data, it appears that the highest dry matter content is 41.04%, recorded at the level of the cultivar “Nkaba jaune”, which is quite rich in dry matter. This value is higher than those of the other varieties, “Mboto” and “Dikondi”, respectively. The cultivar “Nkaba Yellow and Dikondi” has high dry matter contents, which are greater than or equal to 30% (Kouakou et al., 2015; Mahungu, Tatahangry, Bidiaka, & Frangoie, 2014) the cultivar “Mboto” has a low dry matter content, which is due to non-compliance with the cultivation cycle of 10 to 12 months, instead of 24 months, which is the age of the plant at the time of collection. The cultivar Mboto harvested at 8 months in the agricultural site of Odziba Nord has a dry matter content of 39.18%. “Dikondi” from Ntoula and Loukoko, collected at 12 months, have dry matter contents of 35.53% and 43.17%, respectively. Another yellow variety harvested in Loumo, both in the forest and in the savannah, shows dry matter contents of 38.2% and 40.28%, respectively (Kiminou Ngounga, 2020).

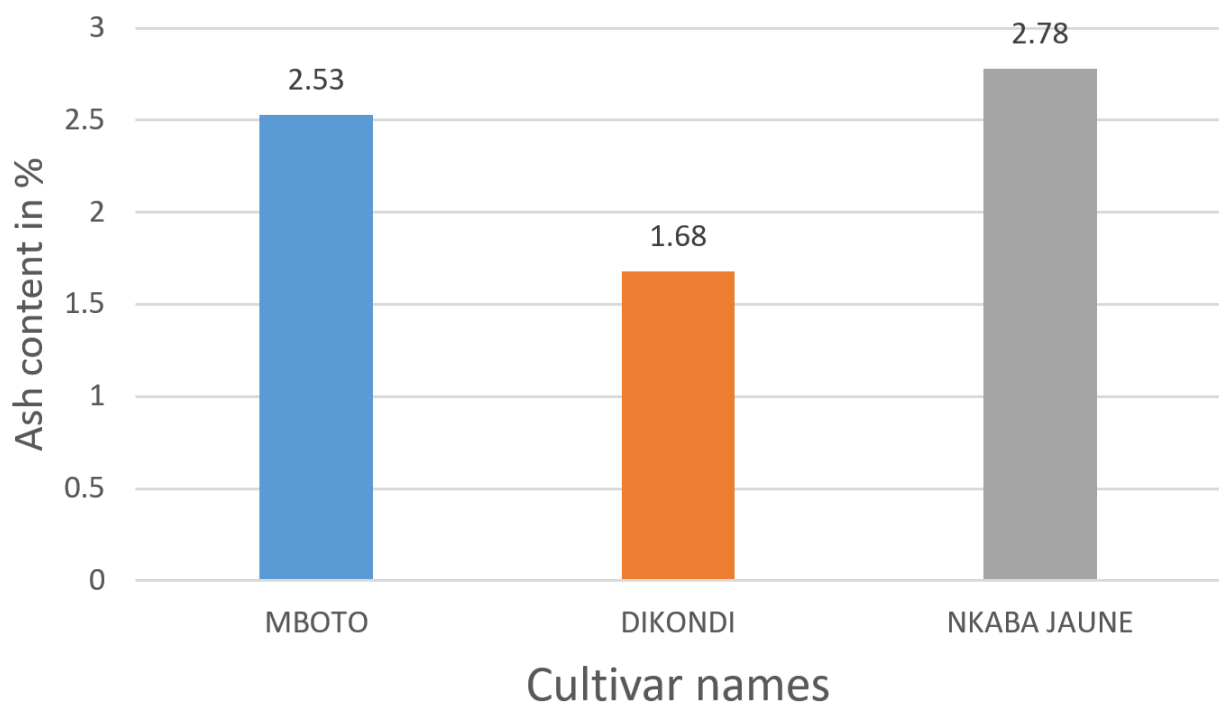


Figure 9. Total ash content of the studied cultivars.

### 3.4. Total Ash Content

The average ash content of the samples studied was 2.52% “Mboto”, 1.68% “Dikondi” and 2.78% “Nkaba jaune”. Data analysis showed that there are differences between the ash content values of the yellow cassava varieties studied (Figure 9). The rates varied between 1.68% and 2.78% for all varieties combined. The yellow Nkaba variety recorded the highest ash content, which was 2.78%. These differences could be explained by the fact that processing conditions, soil type, and ecological conditions of cultivation are all factors likely to influence ash content. After Nkaba jaune, the Mboto variety has an ash content of 2.47%. The other variety, Dikondi, recorded the lowest rate of 1.68%. Our results are close to the work reported by Assemand, Camara, Kouamé, Konan, and Kouamé (2012) working on the biochemical characteristics of two cultivars of plantain fruits (*Musa paradisiaca* L.), at different degrees of maturity, the values being 1.47% and 2.40%. The cassava varieties studied have a higher content compared to the data of FAO (1991), which is 0.54% and also higher than that obtained by Safo-Kantanka, Aboagye, Amartey, and Olaham (1984), which are 0.14 to 0.50% on yellow pigmented cassava.



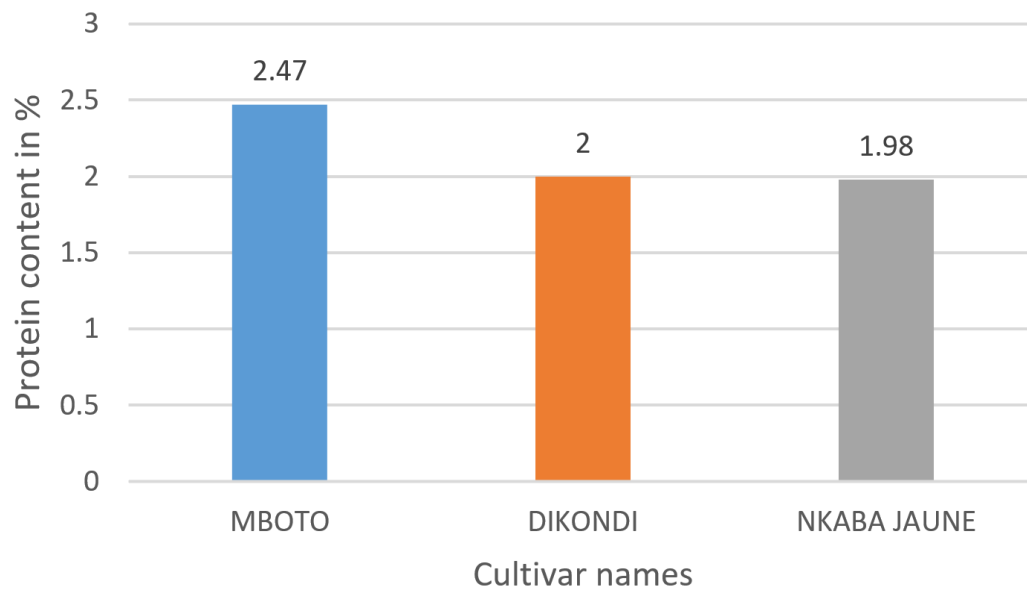


Figure 10. Protein content of the studied cultivars.

### 3.5. Protein Content

The average protein content of the cassava cultivars studied was 2.47% for "Mboto," 2% for "Dikondi," and 1.98% for "Nkaba jaune," respectively. The protein contents of the yellow cassava cultivars analyzed varied between 1.98% and 2.47% (Figure 10). The analysis showed that the protein content, 1.98% recorded in the Nkaba jaune variety, was the lowest. Next comes the Dikondi variety, with a content of 2%. As for the Mboto variety, the analysis revealed that it obtained the highest content, which was 2.47%. Regarding protein contents, our results indicated values between 1.98 and 2.47%. The average rate of the studied cultivars is 2.15%. These results are in agreement with those of Younoussa et al. (2013) who worked on Senegalese cultivars with protein contents varying between 0.6% and 2.6%, and also in agreement with the protein contents of the 16 white-fleshed cultivars from the Odziba Nord agricultural site, ranging from 1.11% to 2.5%, and the 28 cultivars from the Odziba Sud agricultural site, varying from 1% to 2.5%. The protein content of the "Mboto" cultivar obtained from this Odziba Sud site is lower (0.9%) than the "Mboto" found in Brazzaville (2.47%) (Kiminou Ngounga, 2020). Yellow-fleshed cassava cultivars have low protein contents, similar to white-fleshed cultivars.

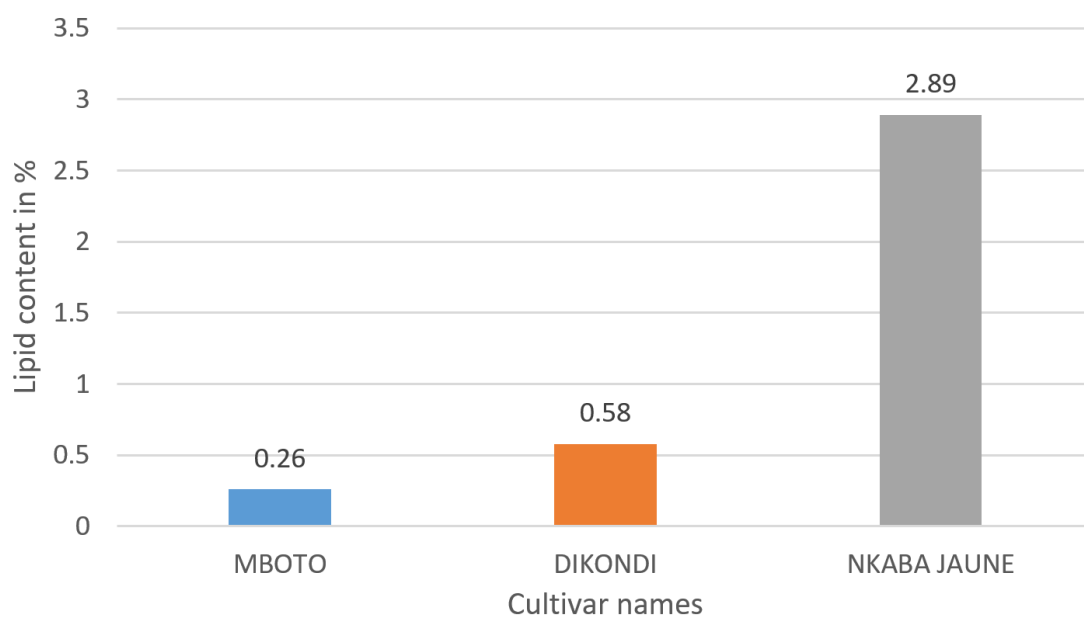


Figure 11. Lipid content of cassava cultivars.

### 3.6. Lipid Content

The results show that the samples have a lipid content of 0.26% for “Mboto,” 0.58% for “Dikondi,” and 2.89% for “Nkaba jaune” (Figure 11). The “Nkaba jaune” variety is therefore richer in lipids than the other two. This low-fat content could contribute to reducing the chances of rancidity and increasing the shelf life of cassava-based formulations.

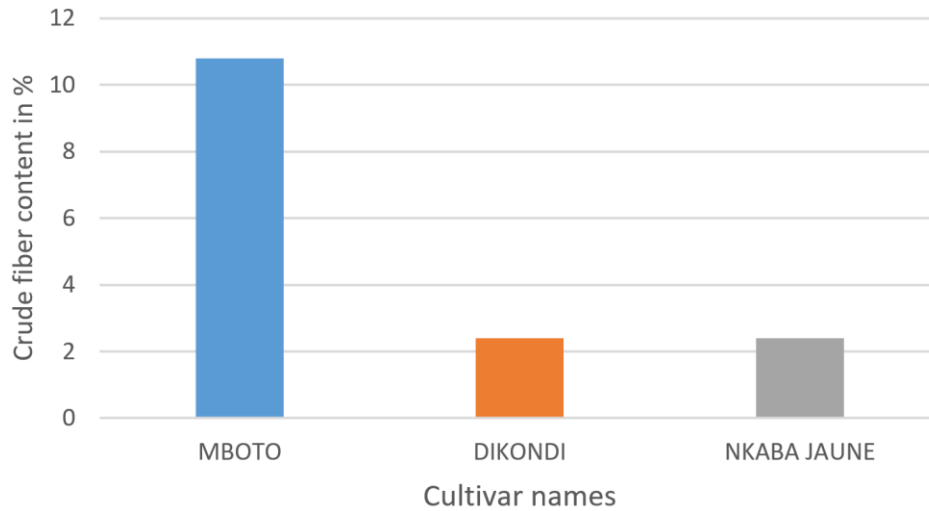


Figure 12. Crude fiber content of yellow cassava cultivars studied.

### 3.7. Crude Fiber Content

The fiber contents of the yellow cassava cultivars measured range from 2.4 to 10.8% (Figure 12). The lowest fiber content of 2.4% was recorded for the Dikondi and Nkaba yellow cultivars. Conversely, the highest content of 10.8% was observed in the Mboto cultivar. The results for the Dikondi and Nkaba cultivars are similar to those proposed by Technologies for African Agricultural Transformation (TAAT) (2022) on high-quality cassava flour. These results are similar to the work of Wills, Lim, Greenfield, and Bayliss-Smith (1983), whose fiber content varied between 1.4% and 5.4% DM. The fiber content of the Mboto cultivar, 10.8%, is extremely high due to non-compliance with the cultivation cycle and excessively long post-maturity longevity.

### 3.8. $\beta$ -Carotene Content

The beta carotene contents of the studied cultivars are presented in the following Figure 13.

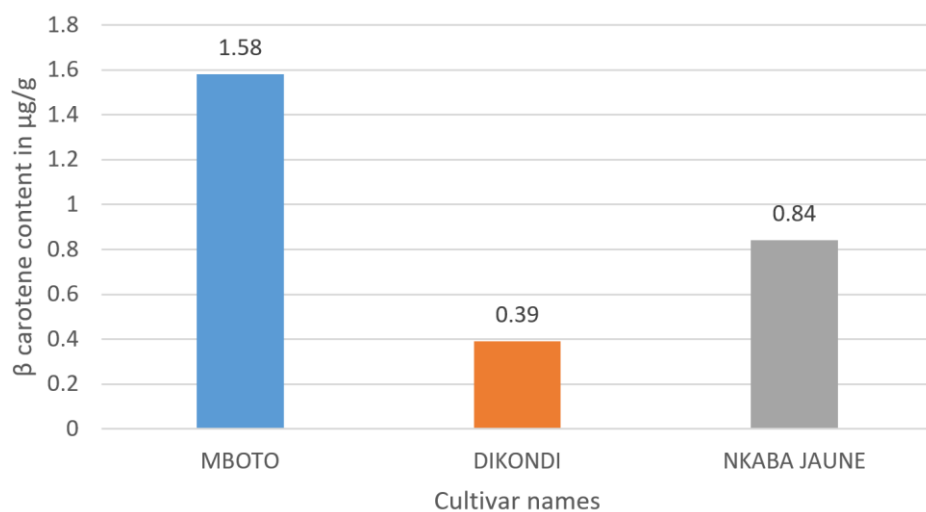


Figure 13.  $\beta$ -carotene content of yellow cassava cultivars studied.

The beta-carotene contents of the yellow cassava cultivars studied were 1.58 µg/g for "Mboto"; 0.39 µg/g for "Dikondi"; and 0.84 µg/g for "Nkaba jaune." The highest value, 1.58 µg/g, was recorded for the Mboto variety. This variety differs from the other varieties, "Dikondi" and "Nkaba jaune," which have values of 0.39 and 0.84 µg/g, respectively.

These values are lower than those obtained by Wembonyama et al. (2020) for the "Kindisa" variety, which are 6.9 µg/g; 6.2 µg/g; and 5.7 µg/g. and also lower than those found by Djinadou, Olodo, and Adjanohoun (2018), which are 9.82 µg/g; 9.7; 9.69 µg/g; 9.23 µg/g; 9.16 µg/g. These values are lower than those reported by other authors. This can be explained by the fact that the beta-carotene content is measured in a powder obtained after processing fresh yellow-fleshed tuberous roots, and processing can generate losses since provitamin A is sensitive and does not store well in heat and light. Its actual bioavailability at the end of the food chain is not guaranteed even when using biofortified varieties (Vernier, N'zué, & Zakhia-Rozis, 2018).

#### 4. CONCLUSION

This work contributed to the chemical characterization of three yellow cassava cultivars found in Congo. The results of this work show that two cultivars are sweet, namely "Mboto" and "Dikondi," and one cultivar is bitter and highly toxic, "Yellow Nkaba." This indicates that yellow cassava exists in both sweet and bitter varieties.

The high water content of the studied cultivars justifies the perishable nature of cassava tuberous roots. Indeed, the water content must be between 50 and 70% to have a solids content of at least 30%. Protein and lipid contents are low. All three cultivars have acceptable beta-carotene levels, which can meet the provitamin A needs of rural communities that depend on this crop as a staple food. To ensure a healthy and nutritious diet, the "Nkaba Jaune" cultivar should not be consumed directly but must be detoxified beforehand.

Since the soil is a food store for some producers, it is essential to respect the post-ripening cycle and longevity of each cultivar.

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**Transparency:** The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

**Competing Interests:** The authors declare that they have no competing interests.

**Authors' Contributions:** All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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