

# ANTIOXIDANTS CHARACTERISTICS OF TRADITIONAL CULTIVARS OF RAINFED RICE (*Oryza glaberrima*) FROM GOH-DJIBOUA AND MOUNTAINS DISTRICTS IN CÔTE D'IVOIRE

## ABSTRACT

The antioxidant compounds in foodstuffs are of great interests for the nutritional and health safety of consumers, given the preponderant role of food in the overall health. The current work focusses the main antioxidant compounds and activities from 27 traditional varieties of rainfed rice. Qualitative tests were performed to check the presence of the antioxidant compounds, namely total polyphenols, flavonoids, tannins, alkaloids, saponins, sterols and terpenes, probed and measured from raw brown rice or whole grains using standard methods. In addition, the antioxidant activity was investigated using standard ABTS and DPPH methods. Only total polyphenols and, to a lesser degree, flavonoids, were qualitatively found from the studied rice samples. The total polyphenols contents (43.83 to 194.60 mg GAE/100g) and flavonoids contents (16.08 to 124.83 mg QE/100g) displayed significant differences from the traditional rainfed rice cultivars investigated ( $P < 0.05$ ), recording higher values in the grains of coloured pericarp rice compared to the other varieties. The greatest ABTS content is found in the varieties *Sipricri* (357.51  $\mu\text{M}$  Trolox Eq/g) and *Jbröko* (137.85  $\mu\text{M}$  Trolox Eq/g) which respective percentages of degradation of ABTS radicals are 52.95% and 20.09%. Regarding DPPH assay, the lowest EC<sub>50</sub> is found from the variety *Sipricri* (36.07  $\mu\text{M}$  Trolox Eq/g) and the highest with the rice *Glawlon* (3445.84  $\mu\text{M}$  Trolox Eq/g). The antioxidant trend of the rainfed rice is nutritionally favorable to support a safety food diet using rice as common local food stuff for populations.

*Keywords: Traditional rainfed rice, small scale farming, antioxidants, ABTS, DPPH*

## 1. INTRODUCTION

Antioxidant compounds as global polyphenols are usually found in a wide variety of foods, especially fruits, vegetables, and grains [1]. Many factors are involved for the variation of the antioxidant compounds from foods [2]. Therefore, the type and amount of antioxidant absorbed by diets depend on the foods usually consumed.

Cereal grains are some capital agriculture resources for human nutrition, thanks to their various nutrients contents as the significant caloric value and micronutrients. Among cereals, rice is of the most consumed product with appreciated nutritional and economic interests since rice is generally included in the basic diet of about 60% population over the world [3]. With such significant involvement in the global food diet, rice could be a quite mean for the antioxidant intakes from food consumption to strengthen the nutritional safety and health of populations. Indeed, antioxidants are biomolecules known as secondary metabolites from plants kingdom, and record great presence in numerous plant foods as fruits, vegetables, herbs, and other crops. Akbari *et al.*[4] summarized several herbs and fruits reported by

many researchers as significant sources of antioxidants intake against various health concerns deriving from the oxidative stress in the body. The main biomolecules recorded as engaging antioxidant activities are silibinin, flavonoids, terpenoids, iridoids, polyphenols, ascorbic acid, anthocyanidins, alkaloids, and glycosides [5, 6].

Several antioxidant compounds have already been identified in rice grains, mainly phenolic acids and anthocyanins [7, 8, 9]. Brown rice, also known as whole rice grain, results from the elimination of the inedible husk and, unlike ordinary white rice, includes the germ, bran and endosperm. Number of health and nutraceuticals properties are associated with the consumption of the whole rice grain [10]. Hallfrischet *al.* [11] observed a significant drop of the cardiovascular risks resulting from the increase of the whole food grains consumption in daily diets. Many researches documented health benefits of the brown rice associated with the presence of polyphenols among the essential components. The presence of phenolic compounds in rice ensures a value addition for this food which usual consumption results in both nutritional and health benefits [3]. As a local main food crop, rice represents 1% to 2% of the Ivorian Gross Domestic Product. The national average of rice consumption is yearly set at 70 kg per capita [12,31,32,33], greatly overcoming other local common staple foods. In Côte d'Ivoire, the rainfed rice or African rice (*Oryza glaberrima*) varieties are cultivated on over 80% sown lands. In many regions, the traditional rice varieties are generally produced on small farming lands using traditional and rough means for household consumption [13, 14]. Investigations about the global nutritive traits of the traditional rainfed rice have shown significant differences between varieties [15]. However, there's scanty works about the micronutrients of the local rice varieties. The ongoing study targets the antioxidants from the rainfed rice, since these bio-function compounds are major micronutrients accounting for the nutritional food interests. The research could strengthen the global struggle against micronutrients deficiencies and promoting the traditional rainfed rice as socio-cultural product for food fortification to provide healthy diet to population. The study focuses some traditional varieties of rainfed rice from Côte d'Ivoire, especially from Goh-Djiboua and Mountains Districts.

## 2. MATERIAL AND METHODS

### 2.1 Plant material.

The plant material was constituted of rainfed rice grains deriving from 27 traditional varieties (table 1) and collected between January and June 2017 in the western Côte d'Ivoire, namely in locations from Goh- Djiboua and Mountains regional Districts.

**Table 1:** List of the traditional rainfed rice cultivars assessed

Districts	Traditional rice cultivars spellings	Total
Gôh-Djiboua	<i>Abê, Akita, Azi red, Aziko, Biti-bitî, Boumabou, Dagnon, Danané precocious, Dananébelating, Dikouè, Gbêkléazi, Glawlon, Jbröko, Kôlôtchè white, Kôlôtchè red, Lepouleu, Loêgnini, Môgôssi, Nathalié, No nono, Present, Sipricri, Yoroukouagnêzê, Zonhonkloumin white, Zonhonkloumin black</i>	25
Mountains	<i>Danané 1, Danané fowl</i>	2
	<b>Total</b>	<b>27</b>

## 2.2 Sampling.

Samples of the 27 traditional rainfed rice varieties were collected from 450 farmers in both departments visited and from the Africa Rice centre. Amounts of 200 g of shelled rice were perceived per variety, conditioned and labelled, leading to a total sampling of 5.4 kg of traditional rice. The full rice volume was then conveyed to laboratory for analysis. Thus, 100 g were taken per rice variety and dried at 50 °C for 72 h in an oven (Memmert, Germany). The dried rice samples were then ground using a metallic grinder (Heavy Duty), sealed into polyethylene bags and kept in desiccator till analyses.

## 2.2 Determination of antioxidant compounds

### 2.2.1 Phytochemical screening

The detection of the presence of certain secondary metabolites (polyphenols, alkaloids, saponins) in the extracts was carried out according to the method described by **Bagre et al. [16]**, **N'guessan et al. [17]**, and **Bruneton [18]**.

- **Alkaloids test:** An extract aliquot of one (1) mL was added with 5 drops of Dragendorff reagent. Thus, the alkaloids presence is revealed by the occurrence of a precipitate or orange colouring into the test tube.

- **Flavonoids test:** Five (5) mL of rice extract were added with 5 mL of hydrochloric alcohol mixture prepared with 10 mL of 96% ethanol, 10 mL of distilled water and 10 mL of 12.1 N hydrochloric acid. Add 2 to 3 magnesium shavings and observe (orange-pink or purplish colouring). Adding 1 ml of isoamyl alcohol intensifies this colouring and confirms the presence of flavonoids.

- **Tannins test:** Five (5) mL of rice extract were added with 1 mL of ferric chloride solution ( $\text{FeCl}_3$ , 2%). Tannins were then revealed by a greenish or blue-blackish colour.

- **Sterols and terpenes test:** One (1) g rice sample were macerated for 24 h in 20 mL of hexane added with 1 mL of acetic anhydride and 1 mL of chloroform. The total mixture was then equally shared in two tubes (control + test). Using a pipette, 1 mL of concentrated sulfuric acid reagent (96%) was injected into the test tube allowing the observation of a brownish red or purple ring in the contact area of both liquids. The change in the supernatant to green or purple was quite indication of sterols and terpenes.

### 2.2.2 Determination of antioxidants contents

The phytochemical screening allowed the quantification of antioxidants as the significant secondary metabolites of the traditional rainfed rice varieties. Thus, the antioxidants were first extracted from the raw rice samples before their quite quantification and functioning analyses.

#### 2.2.2.1 Extraction of main antioxidants

The extraction was performed according to the methods described by **Walter et al. [1]** and **Huang and Lai. [19]**, with slight modifications. One gram (1 g) of whole rice flour was treated with 20 mL of 80% methanol at room temperature for 1 h with continuous stirring. The mixture was centrifuged at 3000 rpm for 10 min and the supernatant was collected. To the remaining residue, 20 mL of 80% methanol were added for the same operations of agitation, centrifugation and supernatant collection. Then, 20 mL of 70% acetone were

added to the second residue, undergoing the same operations described above. Overall supernatants were gathered as crude phenolic extract (CPE) to be used for the assessment of the total polyphenol compounds, total flavonoids and the main antioxidant activities.

#### 2.2.2.2 Determination of total polyphenols content

Appropriated volume of the CPE (80  $\mu$ L) was diluted in 2 mL of distilled water and mixed with 200  $\mu$ L of 0.25 N Folin- ciocalteu reagent. Then, 3 mL of 7.5% sodium carbonate were added and the mixture was incubated protected from light at room temperature for 2 h. Thereafter, the absorbance was read at 765 nm using a spectrophotometer [1]. Total polyphenols contents were recovered with a calibration using gallic acid solutions as standard polyphenolic compound and the results were casted in mg gallic acid equivalent (mg GAE/100g).

#### 2.2.2.3 Determination of total flavonoids content

Hundred (100) mL of the CPE were mixed with 400  $\mu$ L of distilled water and 30  $\mu$ L of 5% sodium nitrite, then successively treated with 30  $\mu$ L of 10% aluminium chloride, 400  $\mu$ L of NaOH 1N, and 240  $\mu$ L of distilled water. The absorbance was read at 510 nm using a spectrophotometer [19]. The total flavonoids contents were recovered with a calibration using catechin solutions as standard flavonoid compound and the results were expressed in mg of quercetin equivalent (mg QE/100 g).

### 2.3 Assessment of the antioxidant activities

Antioxidants bio-activities were investigated according to standard 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic (ABTS) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) reagents and biological models.

#### 2.3.1 Antioxidant activity using ABTS reagent

The 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic (ABTS) in radical cation solution (ABTS<sup>•+</sup>) was prepared by mixing a solution of 8 mM of ABTS and a solution of 3 mM of potassium sulphate peroxide (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>). The resulting mixture was incubated protected from light for 16 h at room temperature (25 $\pm$ 2  $^{\circ}$ C). Thereafter, the radical ABTS<sup>•+</sup> solution was diluted with methanol till a 734 nm spectrophotometric absorbance of 0.7 $\pm$ 0.02 was resulted. To 100  $\mu$ L of rice extract sample, 3.9 mL of diluted final ABTS<sup>•+</sup> solution were added and the deriving mixture was homogenized, incubated protected from light for 6 minutes. Thus, the spectrophotometric absorbance of the tests solutions was measured at 734 nm against a blank solution consisting of methanol. The antioxidant activity (AOA) of the rice extract, consisting in the ability of rice antioxidants to scavenge the stable radical cation ABTS<sup>•+</sup>, was finally calculated as below:

$$AOA(\%) = \frac{(A_0 - A_1) * 100}{A_0}$$

With: A<sub>0</sub>, absorbance from the control test, A<sub>1</sub>, absorbance from the rice extract test

#### 2.3.2 Antioxidant activity using DPPH reagent

The antioxidant activity was also quantified by measuring the trapping activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals [20, 1]. Prior to the reaction, the spectrophotometer was masked with methanol and the DPPH solution was diluted with methanol till reaching an absorbance of 1.1 $\pm$ 0.02 at 515 nm. Then, 3.9 mL of the DPPH solution was added to 100  $\mu$ L of the rice extract. A blank solution was prepared

simultaneously with 100  $\mu$ L of methanol. The reactive mixture was incubated for 30 min at room temperature ( $25\pm 2$  °C) protected from light to complete the reaction, and the spectrophotometric absorbance measured at 515 nm targeting absorbance values over 0.2. The AOA was estimated using standard Trolox (6-hydroxy-2,5,7,8-Tetramethylchroman-2-carboxylic acid) reagent and the results were expressed in  $\mu$ M Trolox equivalent per g of product using the effective concentration for inhibiting 50% of oxidative activity (EC50).

## 2.4. Statistical analysis

The investigations were carried out in triplicate and the data analysis was processed using SPSS 22.0 and Statistica 7.1 softwares at 5% significance. The statistical evaluation consisted in a one-way analysis of variance (ANOVA-1) using the traditional rice varieties as analysis factor. The means were compared using Student-Newman-Keuls post-hoc test. Multivariate analysis (Principal Components Analysis and Ascending Hierarchical Clustering) were also achieved to set the correlations between rice varieties with the investigated antioxidant traits.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical screening

The phytochemical screening revealed a quite presence of total polyphenols and at a lesser degree, flavonoids, whereas any obvious presence of tannins, alkaloids, sterols and terpenes is accounted from the overall rice samples, as shown in Table 2.

### 3.2 Antioxidant compounds and antioxidant activities of the rice samples

The averages of the antioxidant's contents and the antioxidant activities by ABTS and DPPH radicals of the rice samples investigated are recorded in Tables 3.

The total polyphenols significantly differentiated the traditional rainfed rice varieties (P-value  $< 0.001$ ). With general mean of 113.64 mg GAE/100g, the total polyphenols display lower content from the traditional rice appellations *Glawlon*, *Zonhonklouminwhite*, and *Yoroukouiagnêzê* (68.36, 78.86, 79.24 and 79.99 mg GAE/100g, respectively). However, the varieties *Present*, *Jbröko*, *Môgôssi*, *Azi red* (130.86, 141.08, 143.66 and 150.25 mg GAE/100g) and especially *Sipricri* (303.59 mg GAE/100g) are greatly provided in polyphenols.

As quite part of polyphenols, flavonoids contents also significantly differed (P-value  $< 0.001$ ) from the overall rice samples investigated. Flavonoids value below 20 mg QE/g are recovered from varieties *Yoroukouiagnêzê* (16.08 mg QE/100g) and *Glawlon* (19.94 mg QE/g). On the other hand, numerous varieties are provided in higher flavonoids contents, namely *Azi Red*, *Aziko*, *Boumabou*, *Danane Fowl*, *Danane Precocious*, *Jbröko*, *Kolotchê*, *Loêgnini*, *Mogossi* (from 81.71 to 88.82 mg QE/g) and the appellation *Sipricri* displays the most significant total flavonoids content ( $124.83\pm 2.94$  mg QE/100g).

**Table 2.** Qualitative profile in antioxidant compounds of the traditional varieties of rainfed rice

Rainfed rice local spellings	Total Polyphenols	Total Flavonoids	Tannins	Alkaloids	Saponins	Sterols & terpenes
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<i>Dikouè</i>	+++	+	-	-	-	-
<i>Danané precocious</i>	+++	+	-	-	-	-
<i>Aziko</i>	+++	+	-	-	-	-
<i>Lepouleu</i>	+++	+	-	-	-	-
<i>Gbêkléazi</i>	+++	+	-	-	-	-
<i>Present</i>	+++	+	-	-	-	-
<i>Loégnini</i>	+++	+	-	-	-	-
<i>Dagnon</i>	+++	+	-	-	-	-
<i>Boumabou</i>	+++	+	-	-	-	-
<i>Kôlôтчè white</i>	+++	+	-	-	-	-
<i>Glawlon</i>	+++	+	-	-	-	-
<i>Jbröko</i>	+++	+	-	-	-	-
<i>No nono</i>	+++	+	-	-	-	-
<i>Akita</i>	+++	+	-	-	-	-
<i>Abé</i>	+++	+	-	-	-	-
<i>Kôlôтчè red</i>	+++	+	-	-	-	-
<i>Sipricri</i>	+++	+	-	-	-	-
<i>Zonhonkloumin black</i>	+++	+	-	-	-	-
<i>Azi red</i>	+++	+	-	-	-	-
<i>Môgôssi</i>	+++	+	-	-	-	-
<i>Yoroukouiagnézè</i>	+++	+	-	-	-	-
<i>Biti-biti</i>	+++	+	-	-	-	-
<i>Dananébelating</i>	+++	+	-	-	-	-
<i>Nathalié</i>	+++	+	-	-	-	-
<i>Danané 1</i>	+++	+	-	-	-	-
<i>Danané fowl</i>	+++	+	-	-	-	-
<i>Zonhonkloumin white</i>	+++	+	-	-	-	-

++ +: strong presence; +: trace; -: absence

Regarding the antioxidant capacity of the traditional rice, various values are recovered from both ABTS and DPPH methods. The highest ABTS rate is found from the rice appellations *Sipricri* (357.51  $\mu\text{M TE/g}$ ) and *Jbröko* (137.85  $\mu\text{M TE/g}$ ), resulting in respective percentages of ABTS radical degradation of 52.95% and 20.09%. Oppositely, the traditional rice *Red Kôlôтчè* (35.33  $\mu\text{M TE/g}$ ) and *Gbêkléazi* (36.30  $\mu\text{M TE/g}$ ) deal with lower antioxidant activity by ABTS and allowed respective degradation rate of only 5.17% and 5.36% ABTS radicals.

About the DPPH antioxidant activity, the lowest EC50 value results from the traditional rice *Sipricri* (36.07  $\mu\text{M TE/g}$ ) and *DananéBelating* (50.83  $\mu\text{M TE/g}$ ), and the highest values are revealed by *Glawlon* (3445.84  $\mu\text{M TE/g}$ ) and *Azi Red* (2218.81  $\mu\text{M TE/g}$ ). Other varieties as *Môgôssi* and *Yoroukouiagnézè* are also provided in significant DPPH EC50 values (807.83 and 732.25  $\mu\text{M TE/g}$ , respectively).

**Table 3.** Antioxidant characteristics of the 27 traditional rainfed rice varieties studied

Rice appellations	TPC (mg GAE/100g)	TFC (mg QE/100g)	ER50-DPPH ( $\mu\text{M TE/g}$ )	ABTS ( $\mu\text{M TE/g}$ )
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<i>Abé</i>	98.05±0.46 <sup>gh</sup>	50.20±3.34 <sup>e</sup>	355.51±2.00 <sup>n</sup>	62.06±3.13 <sup>ghi</sup>
<i>Akita</i>	89.03±0.29 <sup>i</sup>	48.90±2.22 <sup>e</sup>	388.02±3.08 <sup>l</sup>	60.61±1.07 <sup>ghi</sup>
<i>Azi red</i>	150.25±4.58 <sup>b</sup>	88.82±1.94 <sup>b</sup>	2218.81±1.75 <sup>j</sup>	58.22±2.66 <sup>d</sup>
<i>Aziko</i>	108.35±1.17 <sup>f</sup>	81.71±1.11 <sup>bcd</sup>	434.84±2.76 <sup>b</sup>	94.07±3.88 <sup>hi</sup>
<i>Biti-Biti</i>	130.44±3.15 <sup>d</sup>	76.57±2.23 <sup>d</sup>	517.09±0.01 <sup>g</sup>	85.78±0.95 <sup>de</sup>
<i>Boumabou</i>	98.05±0.46 <sup>gh</sup>	84.30±4.86 <sup>bc</sup>	189.84±2.13 <sup>q</sup>	85.78±5.30 <sup>de</sup>
<i>Dagnon</i>	79.99±2.20 <sup>j</sup>	21.87±1.11 <sup>fgh</sup>	237.86±1.97 <sup>p</sup>	69.08±12.43 <sup>efghi</sup>
<i>Danané 1</i>	98.55±0.42 <sup>gh</sup>	23.80±1.11 <sup>fg</sup>	165.1±1.78 <sup>r</sup>	78.36±10.71 <sup>defgh</sup>
<i>DananéBelating</i>	103.01±2.41 <sup>i</sup>	50.20±3.34 <sup>e</sup>	50.83±1.45 <sup>t</sup>	58.45±1.09 <sup>hi</sup>
<i>Danané Fowl</i>	122.07±2.40 <sup>fg</sup>	81.71±1.11 <sup>bcd</sup>	168.43±0.13 <sup>r</sup>	80.21±3.50 <sup>defg</sup>
<i>Danané Precocious</i>	90.28±2.07 <sup>e</sup>	84.32±4.89 <sup>bc</sup>	516.76±1.76 <sup>g</sup>	97.39±5.02 <sup>d</sup>
<i>Dikouè</i>	115.52±3.43 <sup>e</sup>	79.79±1.11 <sup>cd</sup>	588.26±2.32 <sup>e</sup>	84.16±5.65 <sup>def</sup>
<i>Gbêkléazi</i>	92.36±1.01 <sup>hi</sup>	27.03±3.35 <sup>f</sup>	463.65±3.01 <sup>h</sup>	36.30±4.90 <sup>j</sup>
<i>Glawlon</i>	68.36±4.95 <sup>k</sup>	19.94±2.23 <sup>gh</sup>	3445.84±4.83 <sup>a</sup>	64.84±11.58 <sup>fghi</sup>
<i>Jbröko</i>	141.08±9.28 <sup>c</sup>	87.53±1.17 <sup>b</sup>	165.94±0.57 <sup>r</sup>	137.85±1.77 <sup>b</sup>
<i>Kôlôtchè Red</i>	121.88±2.56 <sup>f</sup>	84.32±4.89 <sup>bc</sup>	365.36±0.05 <sup>m</sup>	35.33±4.20 <sup>j</sup>
<i>Kôlôtchè White</i>	106.99±0.85 <sup>e</sup>	84.32±4.89 <sup>bc</sup>	168.52±1.18 <sup>r</sup>	85.50±12.52 <sup>de</sup>
<i>Lepouleu</i>	119.70±1.18 <sup>e</sup>	77.86±1.11 <sup>cd</sup>	550.49±0.08 <sup>f</sup>	73.73±3.81 <sup>efgh</sup>
<i>Loègnini</i>	120.96±1.15 <sup>e</sup>	81.72±2.95 <sup>bcd</sup>	155.56±0.04 <sup>s</sup>	87.96±1.54 <sup>de</sup>
<i>Môgôssi</i>	143.66±5.21 <sup>c</sup>	83.65±1.12 <sup>bc</sup>	807.83±2.38 <sup>c</sup>	50.10±4.40 <sup>ij</sup>
<i>Nathalié</i>	90.87±0.94 <sup>i</sup>	48.27±0.02 <sup>e</sup>	450.39±0.11 <sup>i</sup>	73.24±5.58 <sup>efgh</sup>
<i>No NoNo</i>	99.14±5.16 <sup>gh</sup>	25.99±3.59 <sup>fg</sup>	336.54±0.44 <sup>o</sup>	69.56±7.94 <sup>efghi</sup>
<i>Present</i>	130.86±3.02 <sup>d</sup>	76.57±1.11 <sup>d</sup>	169.12±0.10 <sup>r</sup>	125.19±15.25 <sup>c</sup>
<i>Sipricri</i>	303.59±5.48 <sup>a</sup>	124.83±2.94 <sup>a</sup>	36.07±0.02 <sup>u</sup>	357.51±10.01 <sup>a</sup>
<i>Yoroukouïagnêzê</i>	79.24±2.19 <sup>j</sup>	16.08±1.11 <sup>h</sup>	732.25±0.08 <sup>d</sup>	52.87±5.62 <sup>ij</sup>
<i>Zonhonkloumin Black</i>	87.18±0.63 <sup>j</sup>	20.59±2.23 <sup>fgh</sup>	422.81±1.14 <sup>k</sup>	40.58±8.37 <sup>j</sup>
<i>Zonhonkloumin White</i>	78.86±2.55 <sup>i</sup>	23.16±0.02 <sup>fg</sup>	165.83±0.41 <sup>r</sup>	78.36±10.71 <sup>defgh</sup>
F-value	526.140	370.630	469107.380	193.471
P-value	<0.001	<0.001	<0.001	<0.001

TPC, total polyphenols content (mg Gallic Acid Equivalent/g); TFC, total flavonoids content (mg Quercetin Equivalent/g); DPPH, antioxidant activity by EC50 value using 2, 2-diphenyl-1-picrylhydrazyl reagent ( $\mu$ MTrolox Equivalent/g); ABTS, 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic ( $\mu$ MTrolox Equivalent/g)  
F-value, value of the statistical Fisher test, P-value, value of the probability test

### 3.3 Gathering of antioxidants parameters and rice samples by multivariate analysis

The principal components analysis (PCA) sets the data around four (4) factors (F1 to F4). However, both F1 and F2 factors, assuming 86.97% of the total variance, are considered to cast the gathering design and display the correlations between the rice samples and their antioxidant traits. Thus, the F1 component records an eigenvalue of 2.57, expresses 64.29% total variance, and is mainly built by the total polyphenols content (TPC), total flavonoids content (TFC) and the antioxidant activity by ABTS value. With the component F2 (29.71% total variance and 0.91 as eigenvalue), the significant correlation is provided by the EC50 value of DPPH antioxidant activity (Table 4).

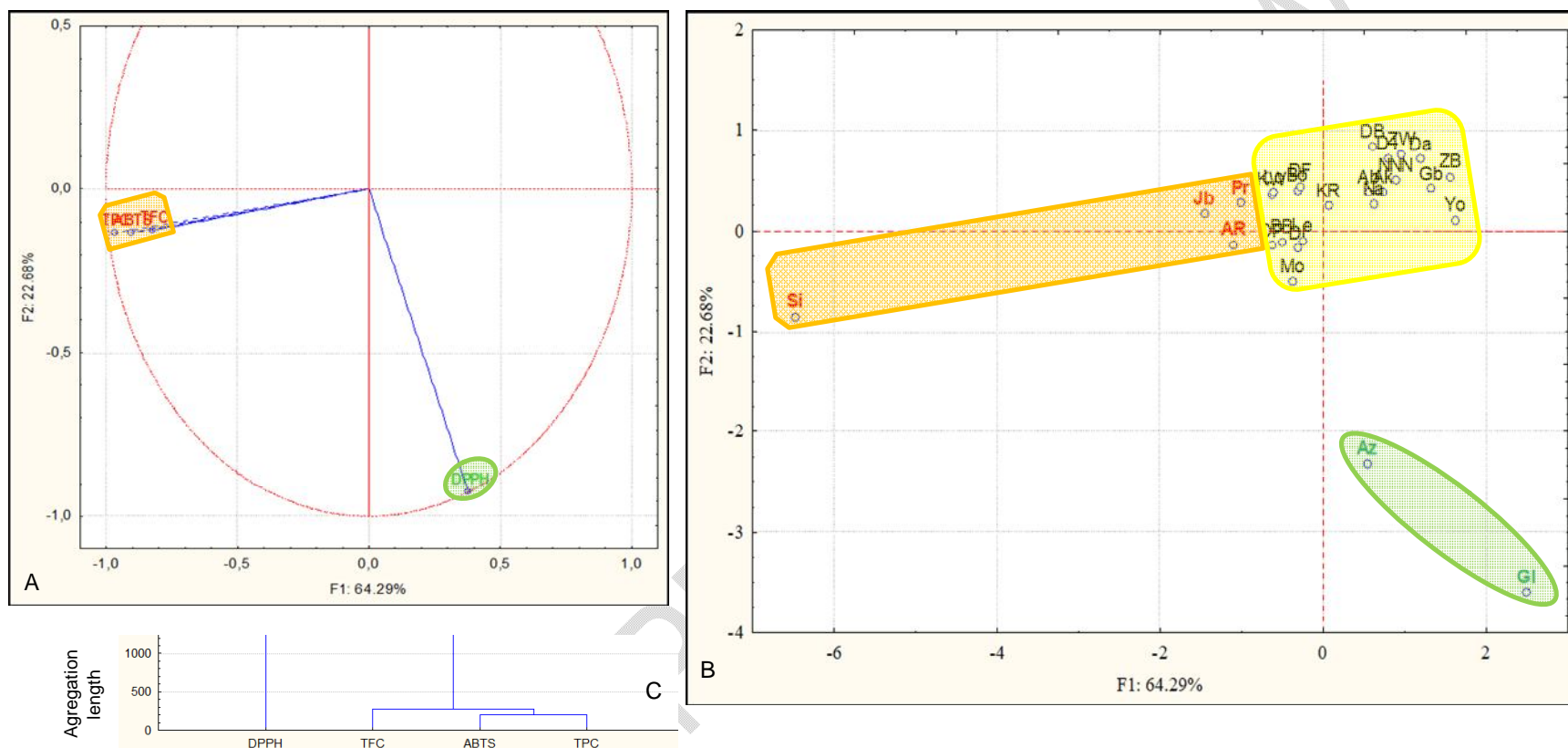
Three groups of traditional rice spellings are drawn from the F1-F2 factorial design of the PCA, with various antioxidant characteristics (Figure 1 A & B). The rice spellings *Sipricri*, *Present*, *Azi Red*, and *Jbröko* are correlated to the most significant TPC, TFC and ABTS values. But the rice samples spelled *Glawlon* and *Aziko* provide the most significant antioxidant value by DPPH analysis. The great extent of the traditional rainfed rice samples studied display intermediate antioxidant characteristics. Besides, the hierarchical ascending clustering show the obvious correlation between the TPC and TFC contents and the antioxidant activity by ABTS value (Figure 1 C), compared to the EC50 value of DPPH.

**Table 4.** Matrix of Eigen- values, variances, and correlations between the PCA components and the antioxidant parameters of the studied traditional rainfed rice samples

Components	F1	F2	F3	F4
Eigen Value	2.57	0.91	0.46	0.06
Total variance (%)	64.29	22.68	11.58	1.45
Cumulated variance (%)	64.29	86.97	98.55	100.00
TPC	<b>-0.97</b>	-0.13	-0.12	0.18
TFC	<b>-0.82</b>	-0.13	0.55	-0.06
ABTS	<b>-0.91</b>	-0.13	-0.38	-0.14
DPPH	0.38	<b>-0.93</b>	0.00	0.00

TPC, total polyphenols content (mg Gallic Acid Equivalent/g); TFC, total flavonoids content (mg Quercetin Equivalent/g); DPPH, antioxidant activity by EC50 value using 2, 2-diphenyl-1-picrylhydrazyl reagent ( $\mu$ MTrolox Equivalent/g); ABTS, 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic ( $\mu$ MTrolox Equivalent/g)

The antioxidants are assessed to fit the global nutritional interest of the traditional rainfed rice which main nutrients were previously achieved by **Aka et al.[15]**. Various antioxidant traits are rated from the 27 traditional rice spellings. First, polyphenols appeared as the principal secondary metabolites of the rice samples, within flavonoids are significantly sounded, compared to tannins, alkaloids, saponins, sterols and terpenes. With such significant presence of antioxidants, the current investigation emphasizes the works of **Chen et al.[8]** claiming that rice could have due involvement in the daily intake of food antioxidants compounds. These authors identified numerous polyphenols compounds from rice.



**Figure 1:** Correlations between the F1-F2 factorial design of the PCA and the antioxidant traits (A) and samples (B) of the traditional rainfed rice varieties studied; and Hierarchical Ascending Clustering of the antioxidant parameters of the traditional rainfed rice (C)  
**TPC**, total polyphenol content; **TFC**, total flavonoids content; **ABTS**, antioxidant activity by 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic reagent; **DPPH**, antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl reagent.  
**Bo**, Boumabou; **Na**, Nathalié; **Pr**, Present; **Ab**, Abê; **DB**, Dananébelating; **Ak**, Akita; **KR**, Kôlôchè red; **ZB**, Zonhonkloumin black; **Gb**, Gbêkléazi; **NNN**, No NoNo; **Az**, Aziko; **Jb**, Jbrôko; **BB**, Biti-bititi; **Gl**, Glawlon; **DP**, Danané Precocius; **Mo**, Môgôssi; **Le**, Lepouleu; **AR**, Azi red; **Yo**, Yoroukouiagnêzê; **Di**, Dikouè; **KW**, Kôlôchè white; **ZW**, Zonhonkloumin white; **DI**, Danané I; **Si**, Sipricri; **Da**, Dagon; **Lo**, Loêgnini; **DF**, Danané fowl.

The total polyphenols contents are significantly different by the traditional rice varieties. Higher polyphenols contents are recorded from the rice appellations *Sipricri* (303.59 mg GAE/100g), *Azi Red* (150.25 mg GAE/100g), *Môgôssi* (143.66 mg GAE/100 g), *Jbrôko* (141.08 mg GAE/100g), and *Present* (130.86 mg GAE/100g). Oppositely, no rather polyphenols, below 80 mg GAE/100g, derive from the samples *Glawlon*, *Zonhonkloumin white*, and *Yoroukouignêzê*. Similar divergence was mentioned by **Goffman and Bergman[21]** who reported averages between 69.21 and 274 mg GAE/100 g rice grains deriving from various pericarp colours. Their works evidenced the polyphenols type and content in rice grains as mainly correlated with the pericarp colour. **Walter et al.[1]** also forecasted significant links between the rice polyphenols and the grains' pericarp. Rice grains produced from *Sipricri*, *AziRed*, *Môgôssi*, *Jbrôko* and **Present** display red pericarp and seem logically enclosing more phenolic compounds compared to the rice grains with light brown pericarp such as *Glawlon*, *Dagnon* and *Akita*. These results corroborate the main observations of **Tian et al.[22]** and **Zhou et al.[7]** who gathered the red and black rice grains engaging higher phenolic concentrations compared to the rice grains with light brown pericarp. As secondary metabolites, polyphenols are mainly produced in plants through shikimic acid and malonic acid [23]. The food diets accounting polyphenols are friendly with greater immune defence and global body healthy thanks to their oxidation resistance. Polyphenols in grains present a stronger antioxidant ability by bioactive effects and scavenging free radicals [24, 25]. Flavonoids were rated as significant part of polyphenols in the rice samples. As shown in figure 1, flavonoids are so correlated with polyphenols that both characteristics have similar trend from the rice grains.

The study showed antioxidant activity (AOA) diverging from the rice samples investigated, whether assessed using ABTS or DPPH methods. Both methods are of the major assays commonly worked for checking the AOA in foods products [26]. Our data display the rice spelling *Sipricri* as the least AOA EC50 value (36 µM TE/g) and the highest scavenging value of the radical cation ABTS<sup>•+</sup> (357.51 µM TE/g) resulting in the greatest ABTS<sup>•+</sup> degradation power. The rice *Jbrôko* was also significantly provided in such AOA by ABTS<sup>•+</sup> degradation (137.85 µM TE/g). Oppositely, other rice spellings as *Gbêklêazi* and *Kôlôchê Red* result in weaker antioxidant activity. These observations also corroborate the dependence of the rice' antioxidative trend from the whole grains versus polished grains. Using DPPH assay, **Goffman and Bergman[21]** concluded higher antioxidant activities for whole rice grains with red and black pericarp colours. In addition, **Shen et al.[27]** revealed comparable data with the ABTS<sup>•+</sup> degradation for AOA assessment in rice grains. The AOA is significantly correlated to antioxidant contents (total polyphenols and total flavonoids) as evidenced by the PCA analysis. **Zhang et al.[28]** have also observed similar positive correlation between the polyphenols content and the antioxidative ability of rice grains. It's therefore clear that polyphenols are of significant contribution in the antioxidant activity of grain foods. Number of researchers previously achieved similar observations from many other foods, such as blackberries, flax seeds, wheat, oats, and ginseng [29, 30]. Many documented health benefits of brown rice such as husked rice from have been linked to the presence of polyphenols in its chemical composition [3]. The presence of phenolic compounds in rainfed rice is an added value for this food whose inclusion in the daily diet is important not only for its nutritional content, but also its health benefits for populations.

#### 4. CONCLUSION

The study assessed the antioxidant contents in traditional rainfed rice grains and the main antioxidant activity dealing with the intake of this food usually consumed by rural populations. The data revealed appreciable occurrence of polyphenols and flavonoids, with significant divergence from the traditional rice spellings. Thus, the varieties *Sipricri*, *Azi Red*, *Môgôssi*, *Jbrôko* and *Present* were more provided in total polyphenols, with consistent part of flavonoids. The antioxidant activity assessed using ABTS and DPPH assays resulted in quite value of antioxidative ability of these rice grains. The study emphasizes and strengthens the suitable global nutritional traits of the traditional rice which sustainable production could ensure the food safety.

#### Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

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