



NUTRITIONAL AND PHYTOCHEMICAL EVALUATION OF SOUMBARA (*Parkia biglobosa*) SEEDS POWDER AND *Moringa oleifera* LEAF FORMULATIONS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/JAFSAT/2022/v9i47943

Received: 20 September 2022

Accepted: 26 November 2022

Published: 30 November 2022

Original Research Article

ABSTRACT

Background: In the face of the numerous nutrition and public health problems experienced in many parts of the world, particularly in the poorest regions, food formulation is an excellent means of enhancing the nutritional value of foodstuffs and preventing certain pathologies.

Aim of the Study: This study was conducted to determine the biochemical and phytochemical composition and nutritional value of the formulations of *Néré soumbra* and *Moringa oleifera* leaves.

Materials and Methods: The different formulations were produced from powder from *Néré (Parkia Biglobosa)* and powder from leaves of *Moringa oleifera*.

Results: The results obtained revealed that the formulations made from *Néré soumbra* and *Moringa oleifera* leaves have a very appreciable nutritional potential. Thus the content of fat, protein, fiber, iron, and calcium was between 3.49 and 37.95%; 31.67 and 36.56%; 10.32 and 13.54%; 11.37 and 172.56 mg/100g and 1123.31 and 1586.73 mg/100g, respectively. The content of total flavonoids, condensed tannins, and total polyphenols was respectively between 4.66 and 37.22 mg EQ/g; between 3.20 and 7.17 mg EC/g DM; and between 53.42 and 102.14 mg EAG/g DM. In addition, The formulations S20M80 and S30M70 showed high antioxidant activity, with respective values of 82.44% and 83.36%.

Conclusion: From all the formulations analyzed, S20M80 and S30M70 had the highest levels of nutrients and phytochemicals.

Keywords: *Moringa oleifera* leaves; *Néré soumbra (Parkia biglobosa)*; food formulation.

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1. INTRODUCTION

In the most underdeveloped regions of developing countries, malnutrition and its related inadequacies are among the most serious public health issues [1]. The causes of this condition are multiple but mainly nutritional, characterized by low micronutrient intake. The development of micronutrient-rich plant types is one method for addressing micronutrient deficiencies. One sustainable food strategy to address nutrition-related public health issues among vulnerable groups is to investigate local food resources [2]. Indeed, rapid urbanization in West Africa has contributed to the development of artisanal sectors for processing and marketing local agricultural products [3]. This dynamic is akin to a "traditional food revolution," brought about by the rise of an urban class that is increasingly supplementing its diet with local products such as soumbara from *Nere* (*Parkia biglobosa*) and *Moringa oleifera* leaves. Soumbara, which is made from the fermentation of *Nere* (*Parkia biglobosa*) seeds, is a popular product not only among the Ivorian population but also in neighboring countries such as Mali, Guinea, and Senegal [4,5]. Soumbara is an important source of nutrients [6,7]. The therapeutic properties of this condiment are said to regulate blood pressure, which is encouraging its increasing consumption [8]. *Moringa oleifera* is commonly used in folk medicine and food in African and Asian societies [9]. Indeed, its leaves are commonly consumed throughout West Africa. Many virtues are attributed to this plant [10]. In addition, Fresh *Moringa oleifera* leaves analysis from the Dakar region, gave the following composition per 100 g: water (74.7%), protein (8.1%), lipids (0.6%), total carbohydrates (14.1%), cellulose (2.13%), ash (2.5%), calcium (531 mg), iron (11.7 mg), vitamin C (220 mg), thiamine (0.23 mg), riboflavin (0.77 mg), niacin (2.66 mg), and a vitamin A equivalent of 5000 g/100 g [10]. The richness of the leaves in certain nutrients, notably protein, calcium, and iron, led to the introduction of *Moringa oleifera* products in their program to combat malnutrition.

This work aimed to contribute to the reduction of deficiency malnutrition through the formulation of food from Soumbara (*Parkia biglobosa*) and *Moringa oleifera* leaves. For this, biochemical, physicochemical, mineral phytochemical, antioxidant activity and microbiological characteristics during storage at temperature ambient of different formulations were investigated.

2. MATERIALS AND METHODS

2.1 Materials

The samples (500g) of *Néré Soumbara* (*Parkia biglobosa*) were collected from female farmers in

Korhogo (the northern region of Ivory Coast), and *Moringa oleifera* leaves collected from fields in Port Bouet city (District of Abidjan, Ivory Coast). These samples were collected according to a random sampling plan.

2.2 Methods

2.2.1 Powders production and formulations

The leaves were freed with dredging, sorted, washed water, and drained. They were then oven-dried at 45°C for 72 hours and ground using a microgrinder equipped with 500 µm mesh sieves to obtain the dried leaf powder.

The Soumbara seeds have been roasted and crushed to produce the powder. The formulations (w/w %) have been produced as follows :

- Soumbara (*Parkia biglobosa*) (20%) + *Moringa oleifera* (80%)
- Soumbara (*Parkia biglobosa*) (30%) + *Moringa oleifera* (70%)
- Soumbara (*Parkia biglobosa*) (50%) + *Moringa oleifera* (50%)
- Soumbara (*Parkia biglobosa*) (70%) + *Moringa oleifera* (30%)
- Soumbara (*Parkia biglobosa*) (80%) + *Moringa oleifera* (20%)
- Soumbara (*Parkia biglobosa*) (100%) Control
- *Moringa oleifera* (100%) Control

The different formulations were conditioned in plastic boxes and stored at room temperature for further analyses.

2.2.2 Analyses

2.2.2.1 Physico-chemical analysis

The pH of different samples was directly determined using a pH-meter (pH-meter modèle Jenway, France) after calibration with standard buffers. Total titratable acidity was determined by titrating samples against 0.1 M NaOH using 1% phenolphthalein as indicator as described by AOAC [11] and expressed as percentage of lactic acid. According to the AOAC method [11], moisture was evaluated by drying the samples (10g) at 105°C for twenty-four (24) hours. The samples were then weighed after cooling in desiccators. To determine the samples moisture content, the weight loss was calculated as a percentage (%) of their initial weight. The measurements were carried out in triplicate.

2.2.2.2 Biochemical analysis

The macronutrient composition (protein, fat, and carbohydrates) was determined according to the AOAC [11] and FAO [12] methods, respectively. Fat contents were determined after extraction with hexane in Soxhlet. Protein contents were determined according to the Kjeldhal method. The ash contents were determined by the ratio of the weight of the sample after incineration at 550°C for 24 h to the weight of the sample before incineration [11]. The carbohydrate contents were determined by calculating the difference between the ratios for protein, fat, ash, and moisture. The energy value was calculated using Atwater's specific coefficients for protein, fat, and carbohydrates [12]. The AOAC [11] method was used to determine the crude fiber content in the different formulations. The determination of minerals (calcium, phosphorus, potassium, sodium, iron) was carried out according to the method by using an atomic absorption spectrophotometer with air-acetylene flame (SAA 20 type, Varian).

2.2.2.3 Phytochemical and antioxidant activity analysis

After ethanolic extraction, the extracts were used for phytochemical and antioxidant activity analysis. The determination of condensed tannin content in the different extracts was carried out according to the method described by Heimler et al. [13], and the absorbance was read at 500 nm. The results were expressed as μg of catechin equivalent per mg of extract. The determination of total flavonoids was performed according to the method of Hariri et al. [14]. A volume of 2 mL of each extract was diluted to 1/10th and mixed with 100 L of Neu reagent. The absorbance was read at 404 nm and compared to that of quercetol taken as standard (0.05 mg/mL), diluted under the same conditions, and treated with the same amount of reagent. The total polyphenol content was determined by colorimetric method by using the Folin-Ciocalteu reagent [15]. To 1 mL of each extract diluted to 1/10th with distilled water, 1.5 mL of Na_2CO_3 (17% w/v) and 0.5 mL of Folin-Ciocalteu reagent (0.5N) were added, and the absorbance was read at 760 nm 1 hour later. Total phenols were determined as gallic acid equivalents per mL (μg GAE/mL), and the values are presented as means of triplicate analyses.

The antioxidant activity was determined by colorimetric method by using of DPPH ((2,2-diphenyl-1-picryl-hydrazyl), and the absorbance was read at 715 nm [16].

2.2.2.4 Microbiological analysis

During storage at temperature ambient, each two (2) weeks, the samples were collected for microbiological analyses.

2.2.2.4.1 Samples preparation

The formulations to be analyzed were prepared in the proportions (g/mL) described in the standard NF EN ISO 6887-V08-010-6 [17]. Ten (10 g) of the formulation to be analyzed were homogenized in 90 mL of sterile peptone water using a stover. From these stock suspensions, successive decimal dilutions (10^{-1} to 10^{-5}) were made in sterile peptone water. Inoculation of 0.1 mL of the stock solutions and prepared dilutions was done in duplicate, according to the microbiological analysis method described by Mensah et al. [18], by surface spreading on sterile agar culture media in Petri dishes.

2.2.2.4.2 Microbial enumeration

Mesophilic aerobic bacteria (MAG) were determined on Plate Count Agar (PCA) medium (Fluka BioChemica 70152), which was incubated aerobically at 30°C for 72 hours [19].

Man Rogosa Sharpe agar was plated and incubated at 30°C for 72 hours under anaerobic conditions to enumerate lactic acid bacteria [20].

Total and faecal coliforms were enumerated on crystal violet and neutral red lactose agar (VRBL agar) at 37°C for 24 hours for total coliforms and 44°C for faecal coliforms [21].

Sabouraud + chloramphenicol agar was plated and incubated at 25°C for 5 days to enumerate yeasts and moulds.

Mossel media was inoculated and incubated at 30 °C for 24 hours. For the enumeration and identification of red-appearing *Bacillus*. The catalase and pH 3 growth tests, as well as incubation at 50 °C and gelatin and starch hydrolysis, were performed.

Baird Parker agar was used for the detection and enumeration of *Staphylococcus aureus*, incubation was done at 37°C for 24 hours.

RAPID' agar was used for the detection and enumeration of *Escherichia coli*. Incubation was at 37°C for 24 hours.

The medium used for the detection and enumeration of *Salmonella* was Salmonella-Shigella agar after pre-enrichment and enrichment on Rappaport de Vassiliadis broth. Each enrichment culture was streaked onto Shigella-Salmonella (SS) agar. Incubation is done at 37 °C for 24 h on SS agar.

2.2.2.5 Statistical analysis

With the use of the R.3.01 software, an analysis of variance was carried out, and Tukey's test was used to identify differences between mean values ($P < 0.05$). Principal component analysis (PCA) was performed using XLSTAT (Version 2016 ; Adinosoft Inc.) in order to visualize relationships among variables represented by formulations compounds. Through Pearson correlation analysis, the relationships between antioxidant activity and the presence of phenolic compounds were investigated using XLSTAT software.

3. RESULTS

Table 2. Mineral content of different formulations.

3.1 Physicochemical and Biochemical Characteristics of Formulations

The values of physicochemical and biochemical parameters of formulations were consigned in Table 1. The moisture and dry matter content did not varied significantly between the formulations ($P > 0.05$). *Soumbara* (S) had the highest dry matter content ($94.2 \pm 0.01\%$) and the lowest moisture content ($5.79 \pm 0.01\%$), whereas the S70M30 formulation has the lowest dry matter content ($93.35 \pm 0.49\%$) and the highest moisture content ($6.65 \pm 0.48\%$). The pH and titratable acidity of the samples varied in the opposite direction. From this observation, the *Moringa* leaf powder (M) had the highest pH ($5.61 \pm 0.05\%$) and the lowest titratable acidity ($1.98 \pm 0.02\%$); however the pH of the S80M20 formulation was the lowest ($5.23 \pm 0.02\%$) and its titratable acidity was the highest ($2.21 \pm 0.03\%$).

The ash content in the *soumbara* (S) powder ($13.21 \pm 0.08\%$) was higher than in the other samples, and the ash content of *soumbara* powder decreased. Thus, the ash contents of the formulations S80M20 ($12.86 \pm 0.22\%$) and S70M30 ($12.50 \pm 0.33\%$) were higher than those of other formulations. The fat content of the *Nere soumbara* (S) ($37.95 \pm 0.04\%$) was higher than the *Moringa* leaves (M) ($3.49 \pm 0.01\%$), and this fat content in formulations depended of amount of *soumbara*. Among all the formulations, S80M20 ($34.25 \pm 0.02\%$) and S70M30 ($32.03 \pm 0.03\%$) have the highest fat contents. In contrast, fat and protein contents were higher in *Moringa* (M) leaves ($36.56 \pm 0.57\%$) than in *Soumbara* (S) ($31.67 \pm 0.57\%$). The formulations S20M80 ($33.75 \pm 0.25\%$) and S30M70 ($33.12 \pm 0.48\%$) were higher in protein than other formulations. The fiber content of *soumbara* (M) powder ($10.32 \pm 0.01\%$ DM) was lowest, while the fiber content of *Moringa* leaves powder was higher ($13.54 \pm 0.11\%$ DM). Regarding

the formulations, the fiber content was higher in S20M80 ($12.08 \pm 0.05\%$ DM) and S30M70 ($11.95 \pm 0.11\%$ DM) compared to the other formulations. The statistical analysis showed a significant difference between formulations ($P < 0.05$).

The carbohydrate content of the samples was significantly different ($P < 0.05$) except for the formulations S20M80 ($39.34 \pm 0.11\%$) and S30M70 ($39.39 \pm 0.48\%$). Then *Moringa* leaves were richer in carbohydrates ($43.39 \pm 1.24\%$) than *soumbara* (S) ($11.68 \pm 0.62\%$). As for the energy value, it was higher in *soumbara* (S) (513.73 ± 0.54 Kcal/100g) than in *Moringa* leaves (M) (351.19 ± 2.82 Kcal/100g). The formulations S80M20, S70M30, and S50M50 had a high energy value of 493.61 ± 2.31 Kcal/100g, 483.55 ± 1.32 Kcal/100g, and 428.73 ± 3.13 Kcal/100g, respectively.

3.2 Mineral Composition of Different Formulations

The Table 2 showed the mineral contents of different formulations. A significant difference in calcium, phosphorus, and iron content was observed between the formulations ($P < 0.05$). The highest calcium content was obtained with *Moringa* leaves (M) and the S20M80 and S30M70 formulations at 1586.73 ± 0.22 mg/100g; 1496.21 ± 0.05 mg/100g, and 1460.52 ± 2.78 mg/100g respectively. For phosphorus, its content was higher in *Moringa* leaves (M) and formulations S20M80 and S30M70, respectively, by 70.04 ± 0.12 mg/100g, 61.02 ± 2.05 mg/100g, and 61.03 ± 2.78 mg/100g. For phosphorus, its content was higher in *Moringa* leaves (M) and formulations S20M80 and S30M70, respectively, by 70.04 ± 0.12 mg/100g, 61.02 ± 0.04 mg/100g, and 45.20 ± 0.02 mg/100g. For iron, the highest content was obtained with *Moringa* leaves (M) and the formulations S20M80 and S30M70, respectively 172.56 ± 0.45 mg/100g, 166.97 ± 0.22 mg/100g, and 151.61 ± 0.01 mg/100g. On the other hand, *soumbara* (S) has the lowest calcium, phosphorus, and iron content at 1123.31 ± 1.1 mg/100g, 24.99 ± 0.02 mg/100g, and 11.37 ± 0.04 mg/100g, respectively.

With regard to sodium and potassium content, a significant difference ($p < 0.05$) was observed between the samples. Also, the sodium content was higher in *soumbara* (S) (319.51 ± 0.03 mg/100g) and the formulations S80M20 (309.04 ± 0.01 mg/100g) and S70M30 (301.31 ± 0.04 mg/100g). The potassium content was high in *soumbara* (1590.7 ± 0.43 mg/100g), S80M20 (1495.99 ± 0.11 mg/100g) and S70M30 (1402.35 ± 5.96 mg/100g). While *Moringa oleifera* leaves had low sodium and potassium contents of 228.59 ± 0.07 mg/100g and 1137.56 ± 0.49 mg/100g respectively.

Table 1. Physicochemical and biochemical parameters of different formulations

Parameters	Formulations						
	S20M80	S30M70	S50M50	S70M30	S80M20	S	M
Dry Matter (%)	93.50±0.32 ^a	93.39±0.36 ^a	93.75±0.88 ^a	93.35±0.49 ^a	93.45±0.40 ^a	94.21±0.01 ^a	93.74±0.71 ^a
Moisture (%)	6.50±0.32 ^a	6.61±0.36 ^a	6.25±0.88 ^a	6.65±0.48 ^a	6.55±0.40 ^a	5.79±0.01 ^a	6.26±0.71 ^a
pH	5.57±0.01 ^b	5.55±0.01 ^{bc}	5.54±0.01 ^c	5.39±0.01 ^e	5.23±0.02 ^f	5.49±0.01 ^d	5.61±0.05 ^a
Titrateable acidity (%)	2.01±0.01 ^d	2.03±0.07 ^{cd}	2.10±0.02 ^{bc}	2.16±0.06 ^{ab}	2.21±0.03 ^a	2.12±0.03 ^b	1.98±0.02 ^d
Ash (%)	10.52±0.01 ^{ef}	10.75±0.03 ^e	11.41±0.10 ^d	12.50±0.33 ^c	12.86±0.22 ^b	13.21±0.08 ^a	10.3±0.01 ^f
Fat (%)	9.88±0.09 ^f	10.12±0.05 ^e	19.88±0.13 ^d	32.03±0.03 ^c	34.25±0.02 ^b	37.95±0.04 ^a	3.49±0.01 ^g
Protein (%)	33.75±0.25 ^b	33.12±0.48 ^{bc}	32.80±0.21 ^c	32.51±0.32 ^{cd}	31.80±0.47 ^{de}	31.67±0.57 ^e	36.56±0.57 ^a
Fibers (%)	12.08±0.05 ^b	11.95±0.11 ^b	11.31±0.24 ^c	11.24±0.02 ^c	11.03±0.04 ^d	10.32±0.01 ^e	13.54±0.11 ^a
Carbohydrates (%)	39.34±0.11 ^b	39.39±0.48 ^b	29.64±0.96 ^c	16.30±0.67 ^d	14.52±0.69 ^e	11.38±0.62 ^f	43.39±1.24 ^a
Energy value (Kcal/100g DM)	381.25±1.71 ^f	381.15±1.43 ^e	428.73±3.13 ^d	483.55±1.32 ^c	493.61±2.31 ^b	513.73±0.54 ^a	351.19±2.82 ^g

S20M80 : 20% soumbara and 80% moringa leaf powder; S30M70: 30% soumbara and 70% moringa leaf powder; S50M50: 50% soumbara and 50% moringa leaf powder; S70M30: 70% soumbara and 30% moringa leaf powder; S80M20: 80% soumbara and 20% moringa leaf powder; S: soumbara; M: moringa leaf powder. Values are expressed as the mean±standard deviation for three independent measurements. The mean values with a different letter on a line are significantly different based on Tukey's test at the 0.05 level

Table 2. Mineral content of different formulations (mg/kg d.w.)

Mineral mg/100g	Formulations						
	S20M80	S30M70	S50M50	S70M30	S80M20	S	M
Calcium	1496.21±0.05 ^b	1460.52±2.78 ^c	1345.16 ± 0.57 ^d	1224.50±0.15 ^e	1134.62±0.15 ^f	1123.31±1.10 ^g	1586.73±0.22 ^a
Phosphorus	61.02±0.04 ^b	45.20±0.02 ^c	34.02±0.03 ^d	31.84±0.04 ^d	26.95±5.24 ^e	24.99±0.02 ^e	70.04±0.12 ^a
Sodium	236.66±0.09 ^f	256.49±0.36 ^e	275.65±0.81 ^d	301.31±0.04 ^c	309.04±0.01 ^b	319.51±0.03 ^a	228.59±0.07 ^g
Potassium	1146.56±5.60 ^f	1228.23±0.80 ^e	1330.34±0.40 ^d	1402.35±5.96 ^c	1495.99±0.11 ^b	1590.7±0.43 ^a	1137.56±0.49 ^f
Iron	166.97±0.22 ^b	151.61±0.01 ^c	80.85±0.67 ^d	65.60±0.55 ^e	32.52±0.48 ^f	11.37±0.04 ^g	172.56±0.45 ^a

S20M80 : 20% soumbara and 80% moringa leaf powder; S30M70: 30% soumbara and 70% moringa leaf powder; S50M50: 50% soumbara and 50% moringa leaf powder; S70M30: 70% soumbara and 30% moringa leaf powder; S80M20: 80% soumbara and 20% moringa leaf powder; S: soumbara; M: moringa leaf powder. Values are expressed as the mean±standard deviation for three independent measurements. The mean values with a different letter on a line are significantly different based on Tukey's test at the 0.05 level

The S20M80 formulation has a more interesting mineral profile than the other formulations.

3.3 Phytochemical Composition of Different Formulations

The polyphenol total, flavonoids, and condensed tannins content of the formulations was presented in Table 3. The S20M80 formulation showed a significant variation and had highest polyphenol content (102.14 ± 1.63 mg EAG/g DM). Then, the formulations S30M70 and S50M50 did not showed significant differences ($P > 0.05$) between them, while the formulations S80M20, S70M30, M, and S were statistically similar. The total Flavonoids in Moringa leaf extract (M) showed a significant difference and exhibited highest content (37.22 ± 1.79 mg EQ/g DM) ($P > 0.05$). The formulations S20M80, S30M70, S50M50, and S70M30 were not statistically different from each other. In addition, the formulations S80M20, M, and S do not showed significant

variation ($P > 0.05$) between them. Condensed tannin content, no significant difference ($P > 0.05$) was observed. The S20M80 formulation had a high tannin content (7.17 ± 1.89 mg EC/g DM) and the *Néré soumbara* (S) the lowest (3.20 ± 0.95 mg EC/g DM).

3.4 Antioxidant Activities of Different Formulations

The change in antioxidant activity of different formulations from several concentrations of ethanolic extracts of powders was depicted in Fig. 1. All the extracts at 2 mg/ml except the *soumbara* extract showed an antioxidant activity above 50%. The S20M80 formulation showed the highest antioxidant activity (83.36%), followed by S30M70 (82.44%) and *Moringa* leaf extract (78.78%). *Soumbara* extract (41.42%) had the lowest antioxidant activity. Statistical analysis showed significant difference between the value between formulations ($P < 0.05$).

Table 3. Phytochemical compounds contents of formulations

Formulations	Phytochemical compounds		
	PT (mgEAG /gDM)	FT (mg EQ /gDM)	TC (mgEC /gDM)
S20M80	102.14 ± 1.63^a	29.95 ± 3.01^b	7.17 ± 1.89^a
S30M70	74.36 ± 1.66^b	28.36 ± 4.62^b	6.49 ± 1.82^a
S50M50	69.34 ± 0.57^b	26.02 ± 1.43^b	4.97 ± 0.41^a
S70M30	56.68 ± 0.69^c	25.94 ± 5.99^b	4.94 ± 0.71^a
S80M20	54.38 ± 6.49^c	5.23 ± 1.50^c	4.51 ± 0.05^a
S	53.42 ± 6.10^c	4.66 ± 0.43^c	3.20 ± 0.95^a
M	57.43 ± 6.66^c	37.22 ± 1.79^a	6.25 ± 1^a

S20M80 : 20% soumbara and 80% moringa leaf powder; S30M70: 30% soumbara and 70% moringa leaf powder; S50M50: 50% soumbara and 50% moringa leaf powder; S70M30: 70% soumbara and 30% moringa leaf powder; S80M20: 80% soumbara and 20% moringa leaf powder; S: soumbara; M: moringa leaf powder. Values are expressed as the mean $\pm$ standard deviation for three independent measurements. The mean values with a different letter in the same column are significantly different based on Tukey's test at the 0.05 level

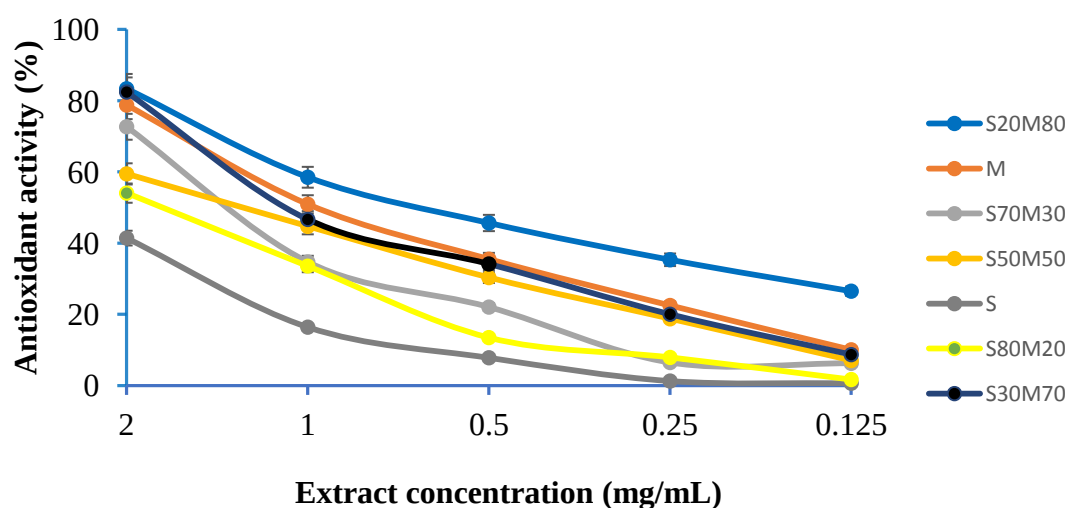


Fig. 1. Antioxidant activity of ethanolic extracts of formulations

3.5 Correlation Matrix between Phenolic Compounds and Antioxidant Activity

In order to explore a possible relationship between antioxidant activity and phenolic content, the Pearson correlation matrix was performed (Table 4). A strong and positive correlation was observed between antioxidant activity and the content of condensed tannins ($r = 0.950$, $P < 0.05$) and total flavonoids ($r = 0.872$, $P < 0.05$) in the formulations.

3.6 Microbial Load Evolution during Storage at Ambient Temperature

Figs. 2 and 3 showed the microbial load of the formulations during storage. From all microbial enumerated, only the presence of Mesophilic Aerobic Germs, moulds and *Staphylococcus aureus* in the analyzed samples had been revealed. The mould load was below 1 (Log CFU/g). However, total and faecal coliforms, *Escherichia coli*, lactic acid bacteria, *Salmonella*, and *Bacillus* were not found in the samples. During the first week, the AMGs had a load of 4.02 (Log CFU/g) for the *Moringa* leaf powder and 4.1 Log (CFU/g) for the S20M80 formulation. This microbial load evolved to 4,26 (log CFU/g) CFU/g at

week 4. Then the MAGs continued to grow up to 4.44 (Log CFU/g) at the sixth week. From week 6 to week 12, the microbial load of the MAGs did not changed significantly ($P > 0.05$) (Fig. 2). For *Staphylococcus aureus*, the load increased from week one 1.07 (Log CFU/g) to week eight (1.88 Log UFC/g) (Fig. 3). From week 8 to week 12, the microbial load did not increased significantly ($P > 0.05$).

3.7 Principal Component Analysis of Different Formulations

The principal component analysis (PCA) was used to compare formulations the measured variables. PCA allowed grouping the measured variables into new variables called “components” or “factors.” This grouping is based on the correlation of variables (Fig. 4). From the 20 measured parameters, a principal component analysis was performed and reduced to two principal components (F1 and F2). F1 and F2 expressed 87.15% of the total variance (Fig. 4); the first principal component F1 represented 72.44% of the observed variations, and the second principal component F2 represented 14.70%. The variables that mainly contributed significantly to the different axes

Table 4. Correlation matrix

Variables	Total Polyphenols	Condensed Tanins	Total Flavonoids
Antioxidant Activity	0.598*	0.949*	0.872*

*, $P < 0.05$.

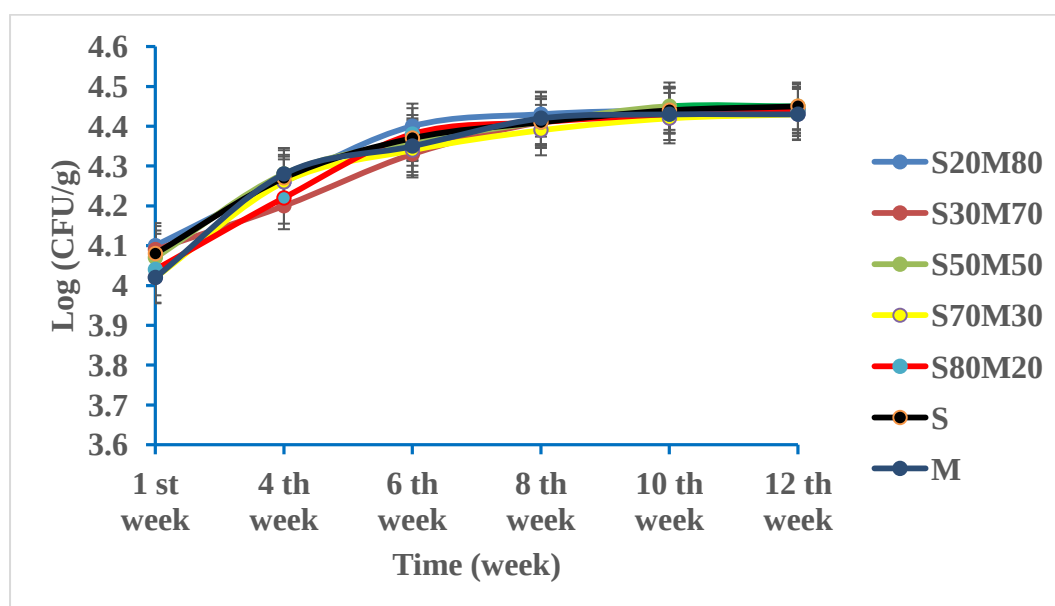


Fig. 2. Mesophilic Aerobic Germs load evolution during storage

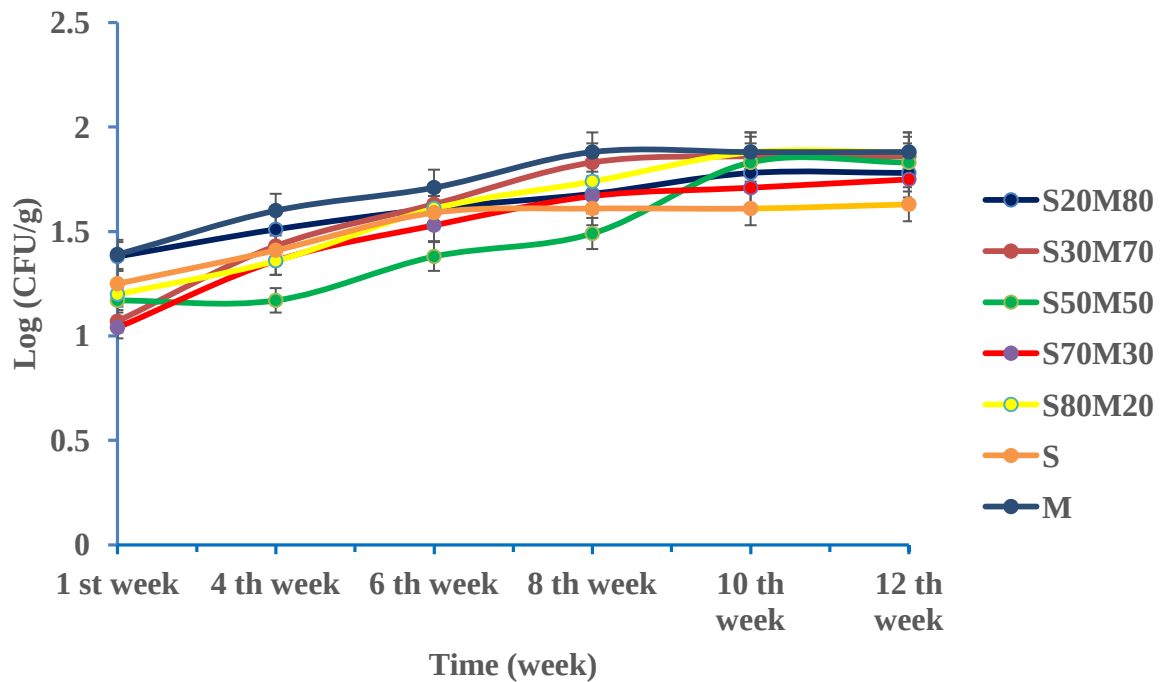


Fig. 3. *Staphylococcus aureus* load evolution during storage

Table 5. Rotated principal component loadings resulting by principal component analysis of different formulations

Parameters	F1	F2
Fat	0,983	0,114
Protein	-0,855	-0,183
Fiber	-0,920	0,012
Carbohydrates	-0,976	-0,123
Calcium	-0,989	-0,138
Fer	-0,994	0,000
Sodium	0,988	0,111
Potassium	0,994	-0,036
Phosphorus	-0,942	-0,146
Total polyphenol	-0,587	-0,025
Condensed Tannins	-0,938	0,225
Total Flavonoids	-0,913	0,031
MAG	0,467	-0,547
<i>Staphylococcus aureus</i>	-0,555	0,503
Antioxidant Activity	-0,899	0,336
Dry Matter	0,375	-0,906
Moisture	-0,371	0,907
Ash	0,984	0,091
pH	-0,705	-0,648
Energy Value	0,984	0,098
Titrateable Acidity	0,892	0,416

F1 and F2 had an absolute value coefficient > 0.8 (Table 5). The distribution of the formulations along the F1 and F2 axes allowed them to be classified into four (4) groups. The first group was composed of

sample S30M70, the second was composed of samples S70M30 and S80M20, the third group was composed of *soumbara* (S) and the formulation S50M50, and the fourth group was composed of

Moringa leaves (M) and the formulation S20M80. As shown in Fig. 4, the different formulations were closed. The S30M70 formulation fell in the upper right half of the graph due to condensed tannins, total flavonoids, iron, fiber, and DPPH content. Then the formulations S70M30 and S80M20 fell in the upper left part of the graph and were mainly distinguished by titratable acidity, fat, ash content, sodium and energy value. The S50M50 formulation and *soumbara* (S) fell to the bottom right of the biplot due to MAP, potassium, and dry matter. *Moringa* leaves and the S20M80 formulation are located in the lower right part of the graph and stood out due to carbohydrates, protein, calcium, phosphorus, pH, and total polyphenols.

4. DISCUSSION

Nere soumbara and *Moringa oleifera* leaves are commonly used in the human diet for a variety of reasons, including taste, flavor, and the ability of these foods to prevent certain diseases. This suggests that *Nere soumbara* and *Moringa oleifera* leaves have remarkable nutritional and therapeutic properties. Furthermore, the water content of a food is a very important indicator for its preservation. Moreover, a food rich in water is conducive to the growth of

microorganisms that will lead to its deterioration [22]. The water content of the *soumbara* and *Moringa* leaf formulations ranged from 5.79% for *soumbara* to 6.65% for the S70M30 formulation. These results were lower than those obtained by Camara et al. [23] in their work on *Nere soumbara* (15.35–23.86%) and soybean *soumbara* (27.53%). This difference could be explained by the drying of *Moringa* leaves in an oven and the roasting of *soumbara* beans before grinding. This low moisture of the samples was very useful as it would allow them to be stored at room temperature for a long period of time.

The pH of the different samples ranged from 5.23% for the S80M20 formulation to 5.61% for the *Moringa* leaf powder. These results were below those obtained by Houndji [24] in *Moringa oleifera* leaves. According to this author, the pH of *Moringa* leaves varied from 7.3% to 8.7%. This difference could be explained by the chlorophyll character of *Moringa oleifera* leaves, which tends to confer a basicity that gradually fades over time and evolves towards neutrality. The main role of fat was to provide energy to the body [25]. The fat content of the different formulations ranged from 9.88% for the S20M80 formulation to 34.25% for the S80M20 formulation.

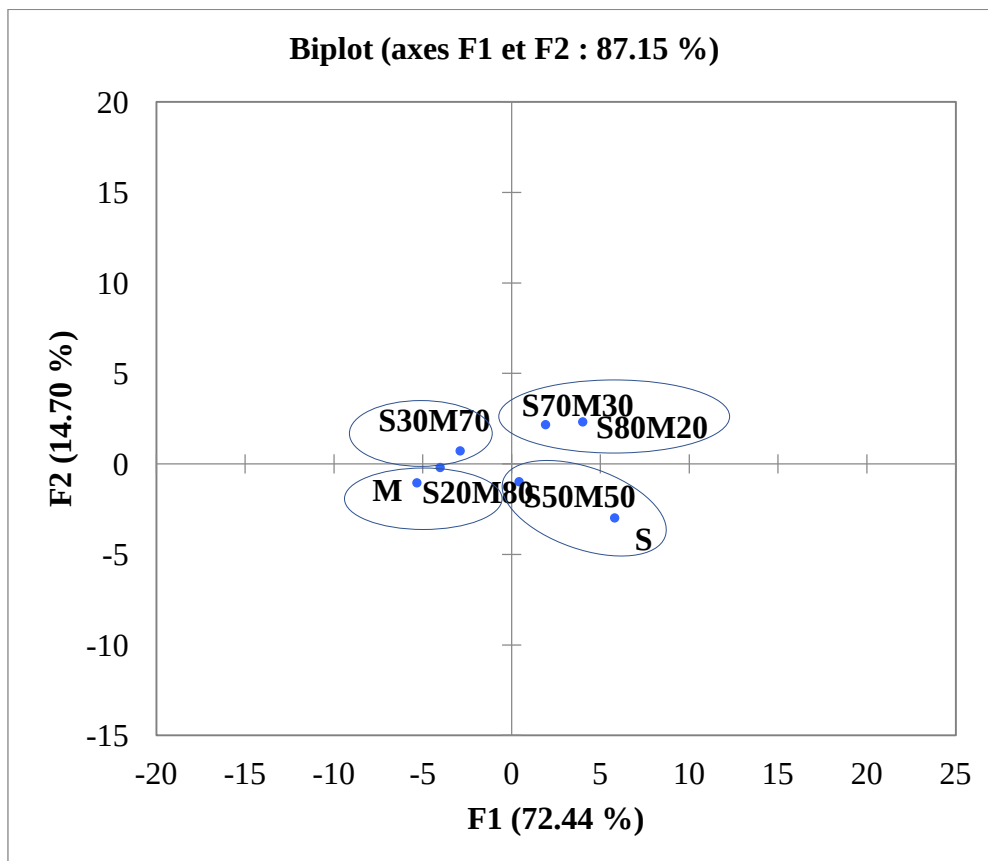


Fig. 4. Plot of the two principal components in PCA of formulations

The fat content of *soumbara* was 37.95% and 3.49% for *Moringa* leaf powder. Clearly, the increase in fat content in the formulations was due to the high fat content of the *soumbara* rather than the *Moringa* leaves. The fat content of *soumbara* determined in this study was similar to that reported by Camara et al. [23] for Korhogo *Nere soumbara* (37.13%). However, the fat content of *soumbara* obtained in this study was higher than those obtained by Koura et al. [26] on raw seeds of *Parkia biglobosa* (19.39 to 22.56%) in Benin. The difference in value between these two consumed products could be explained either by the processing techniques or by the variety of raw materials, but also by the composition of the soils from which these raw materials come. This high fat content showed that *soumbara* was a very energetic food and it conferred to the formulations made in this study an important nutritional asset that could improve the diets of the populations.

The protein content of *Moringa* leaf powder was 36.56%. This protein content was higher compared to the protein content in *Moringa* leaves from Algeria (22.8%) [27], Mexico (22.4%) [28], and Pakistan (22.2%) [29]. However, this protein content was similar to this reported by Rokhaya et al. [30] on dried *Moringa oleifera* leaves (35.03%). The protein content of *Nere soumbara* in this study was 31.67%. This protein content corroborates the results of Camara et al. [23] on Korhogo *Nere soumbara* (30.48%). Furthermore, these differences in protein content observed between the different studies could be explained not only by the origin of the plant but also by the difference in soil, climate, and age of the plant. These findings indicated that *soumbara* and *Moringa* leaves are excellent sources of plant protein because the obtained protein content was higher than 12%. However, 12% is the minimum recommended value for foods considered rich in protein [31]. The high protein content in the formulations made from these two (2) foods would be beneficial in the fight against protein-energy malnutrition, especially the S20M80 formulation. Because of its high protein content (33.75%), this food could be used to replace animal proteins in the food ration in certain regions and during hunger periods.

The carbohydrate content was high in *Moringa* leaves (43.39%) and in the S30M70 formulation (39.59%). These values were higher compared to those of Rokhaya et al. [30] on dried *Moringa* leaves (35.33%). The increase in carbohydrate content in *Moringa* leaves and the S30M70 and S20M80 formulations would be justified by the low fat content of *Moringa* leaves.

The energy value of *soumbara* (513.73 kcal) was similar to this found by Camara et al. [23] for

Korhogo *Nere soumbara* (512.96 kcal). The high fat content of *soumbara* can be the cause of the increase in energy value with the amount of *soumbara* in the formulations. As for the ash, which represents the total amount of mineral salts present in a sample [32]. The ash rate obtained varied from 13.21% for *soumbara* and 10.3% for *Moringa* leaves. These values were higher than those of Camara et al. [23] (2.81-4.93%). However, the ash content of *Moringa* leaves was lower than that reported by Rokhaya et al. [30] for dried *Moringa* leaves (11.39%). *Moringa* leaves and *soumbara* could be considered a source of minerals compared to other leafy vegetables like lettuce (0.4%) and spinach (0.7%) [33].

Secondly, calcium is indicated as the most abundant mineral in *Moringa* leaves (1586.73 mg/100g) and the S20M80 formulation (1496.21 mg/100g). Given its close involvement in skeletal construction, consumption of *Moringa* leaves and the S20M80 formulation would contribute to the prevention of certain pathologies, such as osteoporosis in adults and rickets in children. Also, when calcium was combined with phosphorus, it promotes the proper functioning of bones, teeth, and muscles [34]. According to Turan et al. [35], a diet could be considered beneficial when the calcium to phosphorus (Ca/P) ratio is greater than 1 and poor when it is less than 0.5. In the case of this work, the Ca/P ratio of the *Moringa* leaf and *soumbara* formulations was greater than 1. This ratio could mean that the *Moringa* leaf and *soumbara* formulations were satisfactory. The sodium-to-potassium (Na/K) ratio is of great interest in the prevention of hypertension. It was recommended that this ratio be less than 1 [36]. However, for formulations, the Na/K ratio is less than 1. Therefore, the consumption of foods formulated with *soumbara* and *Moringa* leaves could contribute to the prevention of hypertension. Therefore, these formulated foods should be recommended to anyone who is susceptible to this disease.

Iron is responsible for oxygen transport in the body [37]. Iron is therefore essential in the prevention of anaemia, which affects more than one billion people worldwide. The significant amount of iron in *Moringa* leaves (172.56 mg/100g) and in the S20M80 formulation (166.97 mg/100g) suggested that these leaves and formulations would be ideal for iron deficiency anaemia.

Furthermore, the recommended nutritional intake of minerals is respectively: calcium (500-1200 mg/day); magnesium (80 - 420 mg/day); potassium (800 - 4000 mg/day); and iron (3.9 -21 mg/day) [38]. The residual amount of minerals in the *soumbara* and *Moringa* leaf formulations could contribute to the body's needs.

According to the results of the quantitative analysis, the formulations S20M80, and S30M70 have high polyphenol contents of (102.14 mg EAG/gDM) and (74.36 mg EAG/gDM), respectively. These results were higher than those obtained by Rani and Sharmelee [39] for uncooked (433 mg EAG/100g) and water-cooked (104.9 mg EAG/100g) spinach leaves.

For flavonoid content, it is 37.22 mg EQ/gDM extract in *Moringa* leaves and 29.95 mg EQ/gDM extract in the S20M80 formulation. These values obtained were higher than those of Khwairakpam et al. [40] for non-pre-cooked leaves (23.09 mg EQ/100g) and mustard slurry (23.10 mg EQ/100g DM). Polyphenols were secondary metabolites with antioxidant activity. Thanks to this activity, they play a beneficial role in human health. Flavonoids were effective scavengers of free radicals. They are essential to inhibit lipid peroxidation, to chelate redox-active transition metals, and to prevent the catalytic degradation of hydrogen peroxide [41].

The samples analyzed were rich in tannins which are involved in the protection against cardiovascular and metabolic diseases [42]. Secondly, they play an important role in the defense system as antioxidants. Given the importance of polyphenols and specifically flavonoids in the human body, it would be preferable to favor foods rich in these secondary metabolites such as leaves of all plants, vegetables, oil seeds and pulses [43]. In this study, the antioxidant activity was mainly due to condensed tannins and flavonoids with positive correlation coefficients (respectively, $r = 0.949$ and $r = 0.872$). These results were in agreement with some authors [44-46] who reported condensed tannins as responsible for the antioxidant activity. Indeed, according to Ferradji [47] polyphenols, flavonoids and tannins could be beneficial in the treatment of cardiovascular diseases and facilitate blood circulation. Thus, the formulated diets, especially the S20M80 formulation, which showed the highest antioxidant power, could help people suffering from metabolic diseases such as diabetes and hypertension.

The microbiological investigations revealed the level of microorganism contamination in the designed meals. The average value determined for the total aerobic mesophilic flora at the twelfth week of storage was 4,44 (log CFU/g). This value is lower than this obtained by Camara et al. [23] on dwarf and soybean *soumbara* 5,25 (log CFU/g). Also, this microbial load was lower than the 5,27 (log CFU/g) reported by Fall et al. [48] in Guedji, a condiment based on fermented and dried fish. This low load of mesophilic aerobic germs could be explained by the lack of exposure of

soumbara to sunlight and open air, as well as by the roasting process carried out before the grinding of the *Nere* grains [49-51]. In addition to this total aerobic mesophilic flora, there are certain molds that are linked to the plant kingdom by their cellular structure, as well as staphylococci presumed to be pathogenic.

These microbes, which are part of the spoiling flora, could cause products to lose their marketability by changing their taste, smell, or appearance [52]. However, the low microbial load of these bacteria in foods that are specifically formulated may reduce the risk of poisoning. According to the results, *Salmonella*, *Bacillus*, *Escherichia coli*, and coliforms were not present. However, *Staphylococcus* may be present during the fermentation of *Nere* seeds. In fact, Babacar et al. [53] suggested that the *Staphylococcus* strain would likely be in charge of the generation of the fragrance components during the fermentation of the *né-té-tu*. As the product develops and flourishes, this specific strain of *Staphylococcus sp.* excretes flavours. These substances are in charge of giving the finished goods (*soumbara*) their distinctive flavor, odor, and scent. As the product developed and ripened, this *Staphylococcus sp.* strain would generate flavorful substances. These substances were what give the finished goods (*soumbara*) their distinctive flavor, scent, and taste as well as being in charge of the antibiosis phenomena [54-56]. These antibiosis occurrences aid in removing pathogenic flora from the fermentation medium, enhancing the *soumbara*'s quality [57-58]. The absence of these microorganisms is in agreement with the results obtained by Yao et al. [57] in fermented beverages. The evaluation of the marketability of *soumbara* samples according to Directive 2005/2073/EC relating to microbiological criteria applicable to foodstuffs, showed an acceptable microbiological quality for all diets formulated with *soumbara* and *Moringa* leaves. In addition, the results showed a weak growth of mesophilic aerobic flora and *Staphylococcus aureus* from the eighth week until the twelfth week. This would be explained not only by the stabilization of the microbial load but also by the low moisture content of formulations which does not promote microbial growth.

5. CONCLUSION

Among all formulations studied, the S20M80 formulation excelled over the other studied formulations in terms of micronutrient and macronutrient content. Condensed tannins, flavonoids, and polyphenols were all present in significant concentrations in the S20M80 formulation, which also demonstrated the strongest antioxidant activity. These results revealed that the S20M80 formulation had significant phytochemical and

nutritional potential and might be used to prevent metabolic diseases. The formulations have an acceptable level of microbiological quality for the length of storage at room temperature, according to the ratio of bacteria found during microorganism enumeration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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