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Production and Sanitary Profiles Evaluation of Complementary Fortified Flours with *Moringa oleifera* Lam Varieties Cooked, Precooked and Dried in Niger Republic

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Abstract: The effects of processing technologies, fortification with local resources, microbial pathogens and anti-nutrients hindered quality of complementary flours and became harmful for consumption and health. The objectives of this study were aimed to evaluate the sanitary qualities of complementary flours fortified with varieties *Moringa oleifera* Local (*MoL*) and *Moringa oleifera* Periyakulam1 (*MoPKM1)* and to establish the relationships between the fortification, the thermal treatments and shadow drying, the varieties of *Moringa oleifera* and the flours quality. The methodology was based on the analyses of microbiological pathogens, anti-nutrients and of ANOVA. The results had shown that fortification with *MoL* and *MoPKM1* had antimicrobial and antifungal effects. The fortified flours FCMPSO, FCMLSO, FCMPCV, FCMLCV, FCMPPV, and FCMLPV were exempt of *Escherichia coli, Staphylococcus aureus* and Salmonella (0 CFU/g). Also, the total Yeasts and Molds value of 10.63×10^4 CFU/g for FCT (no fortified) decreased to < 20 CFU/g for FCMPSO and FCMLPV fortified. Moreover, the values of phytates and oxalates were decreased by cooking, precooking, shadow drying and fortification with *MoPKM1* and *MoL*. The variety *MoL* had higher content of phytates and oxalates while *MoPKM1* had higher content of total polyphenols. The fortification with *MoPKM1* had decreased the oxalate values from 21.2 µg/100 g for FCT to 11.6; 12.3; 13.1 and 14.7 µg/100 g for FCMPPE, FCMPCV, FCMPSO and FCMPCE respectively. The fortification with *MoPKM1* had increased the values of total polyphenols from 89 to 131 µg/100 g respectively for FCT and FCMLCV. Whereas, the total polyphenol values of 131; 129 and 126 µg/100 g for FCMLCV, FCMLPV, FCMLCE fortified with *MoL* were higher than 91; 98; 100; 112 µg/100 g for FCMPSO, FCMPPE, FCMPCV, FCMPPV fortified with *MoPKM1* ($p < 0,05$) respectively. Steam cooking and precooking preserved polyphenols better than shadow drying and water cooking. The microbiological pathogens and anti-nutritional properties for *MoPKM1*, *MoL* and fortified flours FCMPPV, FCMLPV, FCMPSO, and FCMLSO were conformed to sanitary safety standards and safe for human consumption. The varieties *MoPKM1* could be used as vectors for quality fortification of complementary flours. Results valorization could enhance food and nutrition security, competitiveness of complementary flours, prevention and fight against anemia, malnutrition and poverty in developing countries.

Keywords: *Moringa oleifera* Varieties, Thermal Treatments, Shadow Drying, Malnutrition, Complementary Fortified Flours, Sanitary Profiles, Niger

1. Introduction

Moringa oleifera is a legume well utilized in the food feeding and sociocultural habits of Niger Republic ethnic groups [1-4]. The factors determining the choice of food in poor countries are defined by price, satiate, organoleptic quality of products and consumption habits [5, 6]. Moringa

leaves are rich in macronutrients, vitamins and minerals, and the enrichment of foods with Moringa powder increases the values in iron, protein and served for the prevention of malnutrition, the treatment of malnourished children, pregnant women and breastfeeding women [7, 8]. Moringa leaves were also reported to contain anti-nutritional properties such as phytates, oxalates and oligosaccharides [9-11].

The formulation of complementary foods with local raw materials satisfying nutritional and sanitary qualities are alternatives to industrial flours enriched from vitamins and mineral costly imported [12]. Complementary foods fortified with *Moringa oleifera* permit adequate supply of energy, proteins, lipids, carbohydrates, essential fatty and amino acids, vitamins and minerals that cover the nutritional needs of young children [8, 10]. But, a complementary flour even of excellent nutritional quality, may not be adequate for feeding if its sanitary qualities did not meet the sanitary safety standards. AFASS and Guiro *et al.* [13, 14] have revealed feeble bioavailability of iron in feeding foods due to the presence of inhibitors, phytates, polyphenols and fiber causing anemia. Complementary fortified flours must therefore be exempt of pesticide residues, anti-nutrients, pathogens and toxins produced by molds that lead to recurrent problems [15, 16]. The microbial control of foods is a crucial question to food nutrition and security [17, 18]. *Escherichia coli* can cause pathologies such as moderate intoxications, severe colitis hemorrhages, Salmonella are responsible of typhoid fever while Yeasts and Molds could compromise the sustainable storage of flours [19, 20].

Based on the above situation, complementary flours were produced from local raw materials and fortified with 2 varieties *Moringa oleifera* Local (*MoL*) and *Moringa oleifera* Periyakulam 1 (*MoPKM1*) cooked, precooked and shadow dried under ventilation. To ensure the hygienic quality and safety at the consumption of the elaborated complementary flours; it is useful to carry out analyses on microbiological pathogens and anti-nutritional properties through appropriate methods. The main objectives of this study were to produce complementary flours, to determine the microbiological profiles; to characterize the anti-nutritional properties and to establish the relationships between the thermal treatments and shadow drying, and the sanitary quality of complementary flours.

2. Materials and Methods

2.1. Materials

The vegetative materials were composed of fresh matured leaves from 2 varieties *MoL* and *MoPKM1* obtained from Mr Abdoulaye Amadou Moringa garden trees, village of Karégorou, Kollo department, Tillaberi region. The grains of millet (*Pennisetum glaucum*) HKP variety, soybeans (*Glycine max*), groundnut (*Arachis hypogea*) variety 55-437 and Tigernut bigger yellow tubers (*Cyperus sculentus*) were purchased from Wadata cereal market in Niamey.

2.2. Methodology

2.2.1. Preparation of Moringa oleifera Leaves

Fresh Moringa leaves were thinned out of petals, cleaned by removing stems, damaged leaves, discolored leaves and other unwanted parts. The quality sorted leaves were washed with distilled water to remove dirt particles and microbial loads. The excess water was allowed to drain out from leaves each washing. The water was changed 3 times and the process was repeated 3 times. The water was allowed to drain out completely from the cleaned Moringa leaves for 30 minutes at room temperature.

2.2.2. Cooking Techniques of MoL and MoPKM1 Leaves

(i). Water Cooking of Moringa oleifera Leaves

Prepared *MoL* and *MoPKM1* leaves were introduced separately into inox cooking pots containing 250 ml of water in ebullition and cooked at 90^{o}C for 45 minutes, until water has finished. The cooked leaves were spread on inox trays covered with thin white cotton mousseline tissue and left to cool for 30 minutes under ventilation at room temperature.

(ii). Steam Cooking of Moringa oleifera Leaves

A water quantity of 1 500 ml were poured into the first compartment of an inox steam cooking pot. Moringa leaves from each variety *MoL* and *MoPKM1* were separately packaged into white thin cotton mousseline tissue and introduced into the second perforated compartment of an inox pot when water reached ebullition. They were left for steam cooking at 90^{o}C for 25 minutes. The steam cooked leaves from *MoL* and *MoPKM1* were then spread on inox trays covered with thin white cotton mousseline tissue and left to cool for 30 minutes under ventilation at room temperature.

2.2.3. Precooking Techniques of MoL and MoPKM1

(i). Water Precooking of Moringa oleifera Leaves

Prepared leaves from *MoL* and *MoPKM1* varieties were introduced separately into inox precooking pots containing 250 ml of water in ebullition and cooked at 80°C for 30 minutes, until water has finished. The precooked leaves were spread on inox trays covered with thin white cotton mousseline tissue and left to cool for 30 minutes under ventilation at room temperature.

(ii). Steam Precooking of Moringa oleifera Leaves

A water quantity of 1 500 ml were poured into the first compartment of an inox steam precooking pot. Prepared Moringa leaves from each variety *MoL* and *MoPKM1* were separately packaged into white thin cotton mousseline tissue and introduced into the second perforated compartment of an inox pot when water was in ebullition. They were left for steam precooking at 80^{o}C for 15 minutes. The steam precooked leaves from *MoL* and *MoPKM1* were then spread on inox trays covered with thin white cotton mousseline tissue and left to cool for 30 minutes under ventilation at room temperature.

2.2.4. Shadow Drying Under Ventilation of Moringa Leaves

The fresh, cooked and precooked leaves of *MoL* and *MoPKM1* varieties were spread separately on drying trays covered with white thin mousseline tissue and kept under ventilation of a processing laboratory room temperature (34°C) and time (20-25 hours) until constant weight. The leaves were turned over and weighed every two hours. Precautions were taken to ensure that there was no fungal growth on the leaves and dark leaves were sorted.

2.2.5. Grains Preparation

The grains of millet (*Pennisetum glaucum*) HKP variety, soybeans (*Glycine max*), groundnut (*Arachis hypogea*) variety 55-437 and Tigernut yellow tubers (*Cyperus sculentus*) were prepared according to the methods of CAC, Codex Alimentarius Commission [21]. The grains were weighed, sorted, cleaned, washed, drained and dried until constant weight. They were then roasted (140°C, 10 mn) and allowed to cool at the laboratory room temperature (34°C). The raw materials were roasted so that to eliminate or reduce the activities of microorganisms, to reduce the anti-nutritional properties content, and to increase the dry matter content. The prepared and roasted grains and tubers were immediately used for grinding, formulation, and production of complementary flours.

Figures 1, 2 and 3 present the flow chats for the production of dried Moringa leaves *«Sashen El Makka» improved*, dried cooked Moringa leaves «*Bakin Bouzou El Makka*» improved and dried precooked Moringa leaves «*Farin Bouzou*» improved.

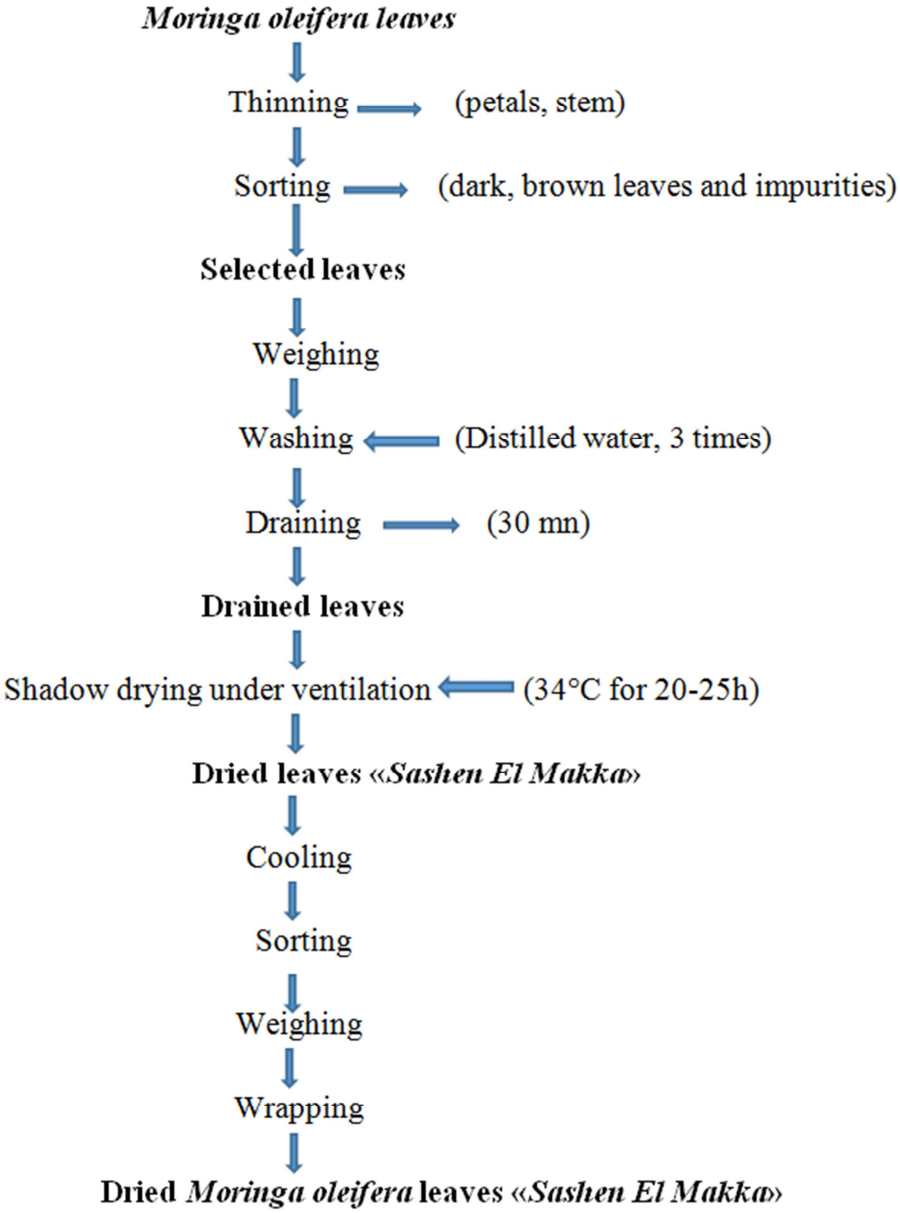

Figure 1. *Flow chat for the production of dried Moringa leaves «Sashen El Makka» improved.*

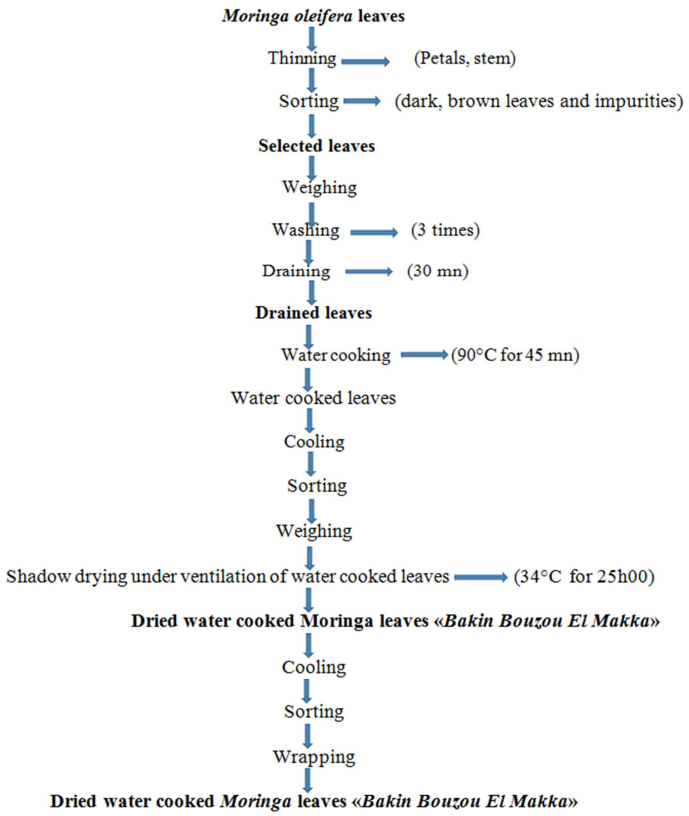

Figure 2. *Flow chat for the production of dried cooked Moringa leaves «Bakin Bouzou El Makka» improved.*

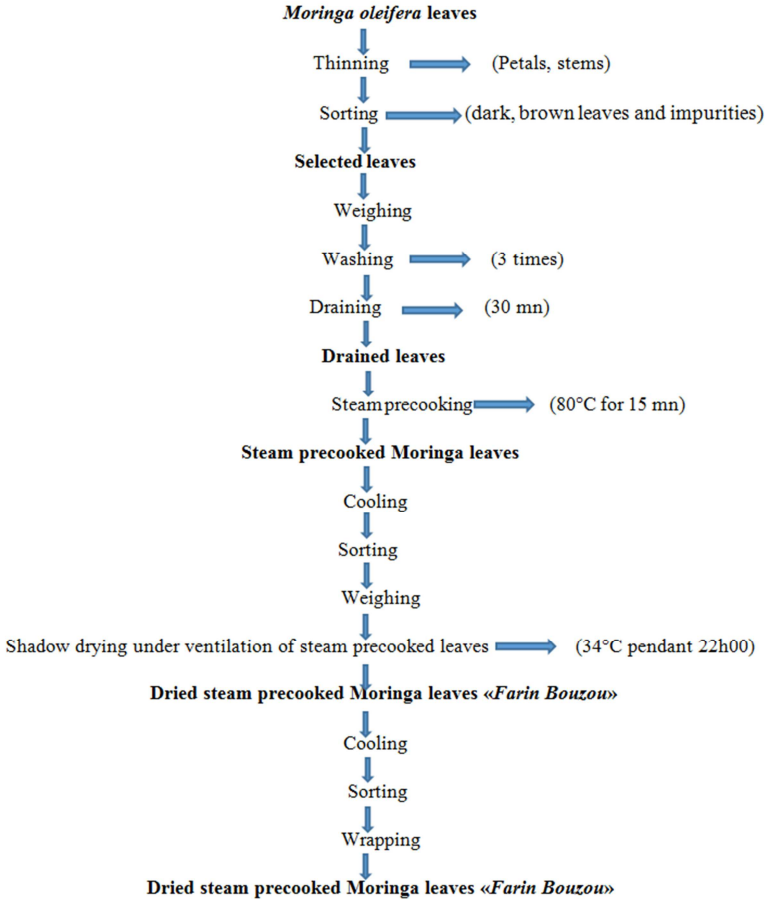

Figure 3. *Flow chat for the production of dried precooked Moringa leaves «Farin Bouzou» improved.*

2.2.6. Grinding and Sieving of Ingredients

The *MoL* and *MoPKM1* leaves cooked, precooked and dried and the prepared grains of millet, soybeans, groundnut and Tigernut yellow tubers were ground separately in an inox mill (Type 3375-E20 Thomas Scientific™, USA), containing sieves with narrow holes to get a composite flour ≤ 0.4 mm. The flours were then homogenized in thinner particles with a Kenwood blender and sieved to 200 µm of diameter in very thin particles.

2.2.7. Formulation of Complementary Flour Samples

The mixture of proportions of ingredients powders and flours from millet, soybeans, groundnut, Tigernut yellow tubers, iodine and *MoPKM1* and *MoL* dried, cooked or precooked were formulated into composites flours and fortified flours. A total of Eleven (11) flour samples were formulated among them one (1) sample formulated with millet, soybeans, groundnuts, iodine salt; five (5) fortified flour samples with 26% *MoPKM1* and five (5) fortified flour samples with 26% *MoL* cooked, precooked and dried in the same conditions. Samples were immediately taken to the laboratories for analyses. Table 1 summarizes the different samples formulations of complementary flours.

Table 1. *Composition of formulated flours.*

Types of Flours	Composition of flours (%)							
	Millet	**Tigernut**	**Soybeans**	**Groundnut**	**Iodized salt**	***MoPKM1***	***MoL***	**Total (%)**
FCT	39	13,5	33	13,5	1	-	-	100
FCMPSO	29	10	24,5	10	0,5	26	-	100
FCMPCE	29	10	24,5	10	0,5	26	-	100
FCMPCV	29	10	24,5	10	0,5	26	-	100
FCMPPE	29	10	24,5	10	0,5	26	-	100
FCMPPV	29	10	24,5	10	0,5	26	-	100
FCMLSO	29	10	24,5	10	0,5	-	26	100
FCMLCE	29	10	24,5	10	0,5	-	26	100
FCMLCV	29	10	24,5	10	0,5	-	26	100
FCMLPE	29	10	24,5	10	0,5	-	26	100
FCMLPV	29	10	24,5	10	0,5	-	26	100

Key: FCT = flour composite + 0% Moringa *(*no fortified) FCMLSO = FCT + 26% *MoL* shadow dried FCMLCE = FCT + 26% *MoL* water cooked FCMLCV = FCT + 26% *MoL* steam cooked FCMLPE = FCT + 26% *MoL* water precooked FCMLPV = FCT + 26% *MoL* steam precooked FCMPSO = FCT + 26% *MoPKM1* shadow dried FCMPCE = FCT + 26% *MoPKM1* water cooked FCMPCV = FCT + 26% *MoPKM1* steam cooked FCMPPE = FCT + 26% *MoPKM1* water precooked FCMPPV = FCT + 26% *MoPKM1* steam precooked

2.2.8. Methods for Microbiological Analyses

(i). Preparation of Initial and Decimal Solutions

The initial and decimal solutions were prepared according to ISO 6887-V0-010-6 [22]. Ten (10 g) of each sample of flour stocked at 4°C were taken aseptically with sterile spatula and put into sterile plastic stomachers, and diluted into 90 ml peptone salted water, then homogenized into a sample mixer to obtain the mother dissolution (10^{-1}). The sterilization was done at 121°C for 15 minutes. Nine Milliliters (9 ml) of EDS were added to 1 ml of the initial dilution to obtain a series of decimal dilution (10^{-2} to 10^{-9}). The lower concentrated dilutions (10^{-5}; 10^{-6}; 10^{-7}) were used for the research of specific bacteria. For the surface culturing, the culture left to cool was used and the solution was spread with a sterile spreader. The microbiological isolation was done based on the specific cultures, time and incubation temperature leading the formation of specific colonies and the appropriate methods for the enumeration of each microorganism. On each box, the origin of the analysis, the culture used and the corresponding dilution was registered. The colonies were counted with colony counter into the boxes containing between 30 and 300 Colony Forming Units (CFU) [23-25].

(ii). Determination of Escherichia Coli

Escherichia coli were determined according to the horizontal method ISO 7251 [26]. The germs were renumbered in *Violet Red Bile Gelose agar* environment sterilized at 121°C after 48 hours of incubation at 44°C.

(iii). Determination of Staphylococcus Aureus

Staphylococcus aureus were determined according to the horizontal method ISO 6888-2 [27]. A surface culturing was done on the environment of Chapman followed by an incubation at 37°C during 24 hours. The colonies of yellow color were considered as *Staphylococcus aureus* (fermentation of mannitol and turning of red of phenol).

(iv). Determination of Salmonella

The Salmonella were determined according to the horizontal method and renumbered following the miniaturized techniques most probable number according to ISO 6579-1 [28].

(v). Determination of Yeast and Molds

Yeast and Molds were determined according to ISO 21527-1 [29]. The Gelose Glucose to the extract of Yeast and to Chloramphenicol sterilized at 121°C served as the culture

for the isolation of the flora. The surface sowing was used followed by an incubation at 25°C for Yeast and 30°C for Molds during 3-5 days.

2.2.9. Methods for Anti-Nutritional Properties and Polyphenols Analyses

(i). Determination of Oxalates

The determination of oxalates in the flour samples was done according to the method described by Yao N'zué *et al.* [30]. One (1) g of the sample flour was homogenized into 75 ml of sulfuric acid (H2SO4) to 15 N under magnetic agitation during 1 hour. The mixture obtained was filtered on Whatman n° 1 paper filter. Twenty five (25 ml) of the filtrate was hot titrated with a solution of potassium permanganate (KMnO4) (0.05 M) until appearance of a feeble rose pale coloration that insisted for 30 seconds.

(ii). Determination of Phytates

The dosage of phytates was done according to the method of Latta and Eskin [31] by using the reagent of Wade. One (1) g of the sample was dried and ground then homogenized into 20 ml of HCl (0.65 N) under agitation, for 12 hours at ambient temperature. After centrifugation of the mixture at 12 000 rds. /min for 40 min, 5 ml of the floating liquid was withdrawn and added to 3 ml of the Wade reagent. It was then left to rest for 15 min and read at 490 nm using the Spectrophotometer against the blank. A range of calibration curve readings with the phytates of 10 µg/ml was done in the same conditions as the standard test was used.

(iii). Determination of Total Polyphenols

The method of Singleton and Lamuela-Raventos [32] was used to determine the total polyphenols. The reagent constituted from a mixture of phosphotungstic acid (H3PW12O40) and phosphomolyddic acid (H3PMo12O40) was reduced, during the oxidation of phenols, in a mixture of blue oxides of tungsten and of molybdenum. The coloration produced was proportional to the quantity of polyphenols present in the vegetable extract. After incubation of the solutions at ambient temperature for 2 hours, in the dark and the absorbance of mixture reaction was measured at 760 nm against a methanol blank (70%) by using a Spectrophotometer. A range of calibration curve readings were established from a mother solution of Gallic acid (1 mg/ml) in the same conditions as the standard test was used.

2.2.10. Statistical Analyses

The results from microbiological and anti-nutritional properties analyses were calculated using Word and Excel 2013. They were then subjected to descriptive statistical analyses and Analyses of Variance (ANOVA) using SPSS Statistics 20. Least Significant Difference and Duncan Multiple Range Tests were used to compare and separate the means and the homogenous under-sets. A value ($p < 0.05$) was used for the statistical signification.

3. Results and Discussion

3.1. Results

3.1.1. Microbiological Profile of Fortified Complementary Flours

The results on the microbiological composition of complementary flours were presented in Table 2. The values in *Escherichia coli* for FCMPSO, FCMPCV, FCMPPE, FCMPPV, FCMLSO, FCMLCE, FCMLCV, FCMLPE, and FCMLPV were nil and equal to that of FCT (0 CFU /g). The fortification with *MoL*, the steam cooking and precooking, the water precooking and shadow drying under ventilation did not have significant effects on *Escherichia coli* ($p < 0.05$).

Table 2. *Effects of water cooking and precooking, steam cooking and precooking, shadow drying under ventilation and fortification with MoL and MoPKM1 leaves on the microbiological composition (CFU/g) from fortified complementary flours.*

Types of flours	Microbiological composition of complementary flours (CFU/g)			
	Escherichia coli^{W}	*Staphylococcus aureus*^{X}	Salmonella	Yeats and Molds^{Z}
FCMPSO	0^{b}	0^{c}	0^{a}	$< 20^{h}$
FCMPCE	$< 10^{a}$	$< 30^{a}$	0^{a}	$0{,}33\times10^{4e}$
FCMPCV	0^{b}	0^{c}	0^{a}	30^{f}
FCMPPE	0^{b}	$< 20^{b}$	0^{a}	$0{,}43\times10^{4d}$
FCMPPV	0^{b}	0^{c}	0^{a}	$0{,}50\times10^{4c}$
FCMLSO	0^{b}	0^{c}	0^{a}	$< 30^{g}$
FCMLCE	0^{b}	0^{c}	0^{a}	$< 30^{g}$
FCMLCV	0^{b}	0^{c}	0^{a}	$0{,}57\times10^{4b}$
FCMLPE	0^{b}	0^{c}	0^{a}	$10{,}54\times10^{4a}$
FCMLPV	0^{b}	0^{c}	0^{a}	$< 20^{h}$
FCT	0^{b}	0^{c}	0^{a}	$10{,}63\times10^{4a}$

The means with the same super scripts letters within the same colon are not significantly different ($p < 0.05$).
Key: Acceptable value limit: W = < 10 CFU/g; X = < 10^4 CFU/g; Y = absence/25 g; Z = < 10^4 CFU/g FCT = Composed flour + 0% Moringa oleifera (no fortified); FCMPSO = FCT + 26% MoPKM1 shadow dried under ventilation; FCMPCE = FCT + 26% MoPKM1 water cooked; FCMPCV = FCT + 26% MoPKM1 steam cooked; FCMPPE = FCT + 26% MoPKM1 water precooked; FCMPPV = FCT + 26% MoPKM1 steam precooked; FCMLSO = FCT + 26% MoL shadow dried under ventilation; FCMLCE = FCT + 26% MoL water cooked; FCMLCV = FCT + 26% MoL steam cooked; FCMLPE = FCT + 26% MoL water precooked; FCMLPV = FCT + 26% MoL steam precooked.

Whereas, water cooking and precooking had effect on *Escherichia coli* development, and FCMPCE had microbial load equal to the sanitary safety reference acceptable value (< 10 CFU/g). Otherwise, the no fortified flour FCT and

fortified flours FCMPSO, FCMPCV, FCMPPV, FCMLSO, FCMLCE, FCMLCV, FCMLPE and FCMLPV had nil values for *Staphylococcus aureus* (0 CFU/g). This means that fortification with *MoL*, steam cooked and precooked, and shadow dried under ventilation had no effects on *Staphylococcus aureus.* Meanwhile, FCMPCE and FCMPPE had minimal loads ranging from < 20 to < 30 CFU/g for *Staphylococcus aureus* respectively. This means that water cooking and precooking and the fortification with *MoPKM1*, had contributed in increasing *Staphylococcus aureus* levels.

The Salmonella were quasi-absent in the complementary flours (0 CFU/g). This result is in conformity with sanitary quality norms (absence/25 g). Therefore, the fortification, the varieties *MoPKM1* et *MoL,* shadow drying under ventilation, water cooking and precooking and the steam cooking and precooking had no significant effects on Salmonella growth ($p < 0,05$).

The fortification with 26% *MoPKM1* and *MoL* had antimicrobial and antifungal effects on fortified flours. The microbial loads in Yeasts and Molds varied decreasingly from < 20 for FCMPSO, FCMLPV fortified to 10.63×10^4 CFU/g for FCT no fortified. Also, the values in Yeasts and Molds of < 20, < 20, < 30 and 30 CFU/g for FCMPSO, FCMLPV, FCMLSO, and FCMPCV were smaller than 0.43×10^4 and 0.33×10^4 CFU/g for FCMPPE and FCMPCE respectively. Therefore, the microbial loads in Yeasts and Molds for complementary fortified flours were inferior to the acceptable sanitary safety standards reference limits (< 10^4 CFU/g). Unfortunately, the microbial loads of 10.63×10^4 and 10.54×10^4 CFU/g for FCT and FCMLPE were superior to the acceptable sanitary standards reference limits (< 10^4 CFU/g) and unsafe for human consumption. The fortification with *MoPKM1,* the shadow drying under ventilation and the steam precooking had decreased the microbial loads for Yeasts and Molds ($p < 0.05$).

3.1.2. Anti-Nutritional Profiles for Fortified Complementary Flours

The results on the anti-nutritional properties and total polyphenols composition for complementary flours were presented in Table 3. The phytates values of 188 µg/100 g for FCT were inferior to 196 and 217 µg/100 g for FCMLSO and FCMLCV, FCMLPV fortified with *MoL* but were higher than 172 µg/100 g for FCMPCE fortified with water cooked *MoPKM1.* Therefore, the fortification with *MoL* had more increased the phytates levels while the water cooking and shadow drying under ventilation had more reduced the phytates levels than the steam cooking and precooking.

The oxalate value of 21.2 µg/100 g for FCT was higher than 11.6; 12.3; 13.1 and 14.7 µg/100 g for FCMPPE, FCMPCV, FCMPSO and FCMPCE fortified with *MoPKM1* respectively. Hence, fortification with *MoPM1* had more increased the oxalates content. The variety *MoPKM1*, water cooking and precooking, shadow drying under ventilation had reduced the oxalate content than steam cooking and precooking. The values in polyphenols varied from 89 to 131 µg/100 g for FCT and FCMLCV respectively. The fortification with *MoPKM1* and *MoL* had increased the levels of total polyphenols of complementary fortified flours ($p < 0.05$). Meanwhile, the values in polyphenols of 131; 129 and 126 µg/100 g respectively for FCMLCV, FCMLPV, FCMLCE fortified with *MoL* were higher than 91; 98; 100; 112 µg/100 g for FCMPSO, FCMPPE, FCMPCV, FCMPPV fortified with *MoPKM1* respectively. The *MoL* variety had higher polyphenols content than *MoPKM1*. Results showed also that shadow drying under ventilation and steam precooking had more significantly decreased polyphenols levels ($p < 0.05$). Therefore, the variety *MoPKM1*, steam cooking and precooking had more preserved the total polyphenols contents.

Table 3. *Effects of water cooking and precooking, steam cooking and precooking, shadow drying under ventilation and fortification with MoL and MoPKM1 leaves on the anti-nutritional factors composition from fortified complementary flours.*

Types of flours	Anti-nutritional factors and polyphenols values (µg/100 g)		
	Phytates	Oxalates	Total Polyphenols
FCMPSO	176^{i}	$13,1^{h}$	91^{j}
FCMPCE	172^{j}	$14,7^{g}$	120^{d}
FCMPCV	182^{h}	$12,3^{i}$	100^{h}
FCMPPE	187^{g}	$11,9^{j}$	98^{i}
FCMPPV	201^{c}	$16,3^{f}$	112^{f}
FCMLSO	196^{e}	$19,6^{e}$	107^{g}
FCMLCE	213^{b}	$24,4^{b}$	126^{c}
FCMLCV	217^{a}	$26,8^{a}$	131^{a}
FCMLPE	199^{d}	$16,2^{f}$	113^{e}
FCMLPV	217^{a}	$23,4^{c}$	129^{b}
FCT	188^{f}	$21,2^{d}$	89^{k}

The means with the same super scripts letters within the same colon are not significantly different ($p < 0.05$). Key: FCT = Composed flour + 0% Moringa oleifera (blank); FCMPSO = FCT + 26% MoPKM1 shadow dried under ventilation; FCMPCE = FCT + 26% MoPKM1 water cooked; FCMPCV = FCT + 26% MoPKM1 steam cooked; FCMPPE = FCT + 26% MoPKM1 water precooked; FCMPPV = FCT + 26% MoPKM1 steam precooked; FCMLSO = FCT + 26% MoL shadow dried under ventilation; FCMLCE = FCT + 26% MoL water cooked; FCMLCV = FCT + 26% MoL steam cooked; FCMLPE = FCT + 26% MoL water precooked; FCMLPV = FCT + 26% MoL steam precooked.

3.2. Discussion

The fortification with 26% *MoPKM1* and *MoL* leaves had antibacterial and antifungal effects on fortified flours. As such, the fortified complementary flours FCMPSO, FCMPCV, FCMPPV, FCMLSO, FCMLCV, and FCMLPV had shown nil microbial loads (0 CFU/g) in *Escherichia coli, Staphylococcus aureus* and Salmonella. These results were in agreement with the recommended acceptable sanitary security standards limits respectively of < 10 CFU/g for *Escherichia coli* [26]; < 10^4 CFU/g for *Staphylococcus aureus* [27] and absence/25 g for Salmonella [28] as also shown from findings [16, 33]. These results were similar to that of [34] who revealed a reduction of *Escherichia coli* loads in the Moringa food regime. The fortification of flours with 26% *MoPKM1* and *MoL* had also decreased Yeasts and Molds values from 10.63×10^4 CFU/g for FCT to < 20 CFU/g for FCMPSO and FCMLPV fortified. Nevertheless, the

values in Yeasts and Molds for fortified flours were conformed to the recommended acceptable sanitary safety standards limits of < 10^4 CFU/g [29] while there levels were higher for FCT no fortified and superior to the acceptable sanitary safety standards limits [16, 33]. The absence of *Escherichia coli, Staphylococcus aureus,* Salmonella and lower values in Yeasts and Molds within the acceptable sanitary safety limits for FCMPSO, FCMPCV, FCMPPV, FCMLSO, FCMLCV, and FCMLPV fortified flours could be due to the good hygienic practices applied during collection and preparation of Moringa leaves; processing techniques (sorting, washing, cooking, precooking, drying), roasting, hulling, grinding and also the low moisture content, pH (slightly acid), and the antimicrobial and antifungal effects of *Moringa oleifera* effects and quality processing material in inox. Drying, precooking and torrecfaction reduced the germs, the microbial flora, and the ant-nutritional properties and inactivate the antitrypsin properties in foods [34, 35]. The phenolic chemical compounds in *Moringa oleifera* were also reported to have antimicrobial and antifungal effects [36, 37]. Moreover, the foliar extracts of Moringa were revealed to present antimicrobial activities, inhibit the growth of *Staphylococcus aureus* isolated from foods and would be used against bioceutic agent to replace the antibiotics [34, 37]. Whereas, the presence of *Escherichia coli* and *Staphylococcus aureus* in only FCMPCE, FCMPPE flours could be due to the effect of water during cooking and precooking, the residual moisture content, the water activity, the nutrients dissolution, the drying time or the contamination during storage. Meanwhile, De Saint Sauveur and Broin [10], reported that drying at ambient temperature cannot totally guarantee Moringa leaves from Molds and can't generally allow to attain the maximal moisture value of 10% recommended. Otherwise, only FCT had the highest microbial loads in Yeasts and Molds of 10.63×10^4 CFU/g, superior to the acceptable limits and could be explained by the absence of *Moringa oleifera* in its formulation. There might not be suitable for human consumption and storage. Higher values in Yeasts and Molds compromise sustainable storage of products, alter the organoleptic qualities and lead to the accumulation of toxins [19, 20, 38]. The FCT flour could therefore be easily contaminated and spoiled during storage.

The complementary flours fortified with *MoPKM1* and *MoL* had variable levels of phytates, oxalates and polyphenols which means that Moringa varieties contained anti-nutrients. Moringa leaves contained anti-nutritional properties such as phytates, oxalates and oligosaccharides [9-11]. The fortification with *MoPKM1,* shadow drying, water cooking and precooking of fresh Moringa leaves had significantly decreased the values of phytates from 188 for FCT to 172; 176; 182 and 187 µg/100 g respectively for FCMPCE, FCMPSO, FCMPCV and FCMPPE. The processing techniques of Moringa leaves reduced the values of phytates [39], whereas the quasi-total reduction of phytates allowed to improve the bioavailability of iron of the food [40]. Therefore, the phytates levels for flours fortified with *MoPKM1* were feeble and could when consumed improve the bioavailability of iron, prevent or fight anemia and malnutrition. Nevertheless, the fortification with *MoL* had more increased the values of phytates from 188 for FCT to 196, 199, 213, 217, 217 µg/100 g for FCMLSO, FCMLPE, FCMLCE, FCMLCV and FCMLPV respectively. Otherwise, the oxalates values of 11,6; 12,3; 13,1 and 14,7 µg/100 g for FCMPPE, FCMPCV, FCMPSO and FCMPCE fortified with *MoPKM1* were inferior to 21,2; 23,4; 24,4 and 26,8 µg/100 g for FCMLPV, FCMLCE, FCMLCV fortified with *MoL* and FCT respectively. The variety *MoL* contained more phytates and oxalates. The significant difference between *MoPKM1* and *MoL* in their content for phytates and oxalates could be due to their genotypes and the effects of processing techniques (washing, roasting, cooking, precooking and drying). The lower level of oxalates in the fortified flours could therefore have nutritional and health benefits such as the bioavailability of calcium as oxalic acid antagonist for the utilization of calcium will be negligible. The quantities of phytates and oxalates are not significantly important in Moringa leaves [41]. The phytates are the chelators of minerals and decrease the bioavailability of iron in the body. According to Zimmermann and Hurrell [42], the anti-nutritional properties such as phytic acid limit the bioavailability of micronutrients. So, the enhancement of iron content from vegetable food origin through fortification increase bioavailability of iron [42]. On the other hands, the values in polyphenols of 131; 129 and 126 µg/100 g for FCMLCV, FCMLPV, FCMLCE fortified with *MoL* were higher than 91; 98; 100; 112 µg/100 g for FCMPSO, FCMPPE, FCMPCV, FCMPPV fortified with *MoPKM1* respectively. The high value in polyphenols for fortified flours with *MoL* could be due to the specific genotype of *MoL*. Fortunately, the anti-nutrients phytates, oxalates and polyphenols of the fortified complementary flours were smaller than values of 1000 mg/100 g reported by Elke and Zannini [43] and, of 0.064 to 0.556 mg/kg; 0.317 to 0.571 mg/kg revealed by Mengeneh and Ariahu [44] respectively earlier reported in complementary flours. They could therefore be consumed safely and provide nutritional health benefits. According to MA/MS, Ministry of Agriculture, and Ministry of Health [34], the consumption of legumes rich in nutrients and phytochemical compounds such as Moringa leaves, improve the immunity response.

The shadow drying under ventilation, water cooking and precooking and fortification with *MoPKM1* and *MoL* had decreased phytates, oxalates while total polyphenol levels significantly increased with MoPKM1 fortification and steam cooking and precooking ($p < 0.05$) for FCMPSO, FCMLSO, FCMPPV, FCMLPV, FCMPCV and FCMLCV. This reduction in anti-nutritional properties could be explained by the effects of thermal processing techniques, the dissolution of anti-nutrients, the air velocity from ventilation during drying and the controlled time and temperature durations. According to De Saint Sauveur and Broin [10], to ensure a good nutritional and microbiological quality of *Moringa oleifera* powder, the residual humidity must not exceed 7.5%;

the duration of the drying must be short and the drying temperature must not be too high (50 – 55°C maximum). The water cooking and precooking had more decreased phytates, oxalates and polyphenols content for fortified flours FCMLCE, FCMPPE and FCMLPE. The presence of anti-nutrients in foods hindered the efficient utilization, absorption or digestion of some micronutrients and thus, reduce their bioavailability [45]. But, the high values of polyphenols as antioxidants in fortified flours could be beneficial in maintaining good health and preventing cancer. Natural antioxidants play significant roles in the prevention and treatment of cancer from inflammatory diseases, cardiovascular, neurodegenerative and hyperlipidemias diseases [46-51]. Fortunately, the duration of storage of foods containing lipids could be improved because of the presence of antioxidants such as ascorbic acid, flavonoids, phenolic and carotenoid compounds [50, 52, 53].

4. Conclusion

The fortification with 26% *MoL* and *MoPKM1* steam cooked and precooked and shadow dried under ventilation had produced antimicrobial and antifungal effects. The fortified flours FCMPSO, FCMLSO, FCMPCV, FCMLCV, FCMPPV, and FCMLPV were exempts of *Escherichia coli, Staphylococcus aureus* and Salmonella (0 CFU/g). The microbial loads in Yeasts and Molds for FCT were also significantly decreased for FCMPSO, FCMLPV and FCMLPE. The variety *MoL* had higher phytates and oxalates content and as such fortified complementary flours had higher values than those with *MoPKM1*. Otherwise, the fortification with *MoPKM1* decreased significantly phytates and oxalates content. The variety *MoPKM1* contained more polyphenols content, while steam cooking and precooking preserved polyphenols better than shadow drying, water cooking and precooking and as such fortified complementary flours FCMLCV, FCMLPV, FCMPPV, and FCMPCV had the highest total polyphenol values. The lower values in phytates, oxalates for FCMPPE, FCMPCV, FCMPSO and FCMPCE and higher total polyphenols for FCMLCV, FCMLPV, FCMPPV, and FCMPCV have nutritional and health benefits such as bioavailability and absorption of iron and calcium; prevention and treatment of cancer and anemia.

Therefore, the variety *MoPKM1*, steam precooking, shadow drying under ventilation, fortification and flours formula FCMPPV, FCMLPV, FCMPSO and FCMLSO are free from pathogens *Escherichia coli, Staphylococcus aureus* and Salmonella, have lowest values in phytates, oxalates and total polyphenols and conformed to recommended sanitary safety standards. Scaling-up results could enhance food and nutrition security, competitiveness of complementary flours, prevention and fight against malnutrition and anemia, and poverty in developing countries. *Moringa oleifera* Periyakulam1 is safer and nutritious for human nutrition and could be used as vector for quality fortification of complementary flours.

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