

NUTRITIONAL POTENTIAL OF MAIZE (*ZEA MAYS* L.) AND MILLET (*PENNISETUM GLAUCUM*) CEREALS FERMENTED AND GERMINATED**Amandou Ouattara¹, Gbocho Serge Elvis Ekissi^{*2}, Nestor Kouakou Kouassi¹, Didier Aristide Ikpe Kouamé² and Denis Yao N'dri¹**¹Food Biochemistry and Tropical Products Technology Laboratory, Department of Food Science and Technology, Nangui Abrogoua University (Abidjan, Côte d'Ivoire).²Biocatalysis and Bioprocessing Laboratory, Department of Food Science and Technology, Nangui Abrogoua University (Abidjan, Côte d'Ivoire).***Corresponding Author: Gbocho Serge Elvis Ekissi**

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ABSTRACT

Present study was conducted to investigate the effects of germination and fermentation on nutrient composition of two cereals (maize, millet) flours. Maize and millet grains were soaked in both tap and boiled waters respectively for 24h and 6h and germinated / fermented for 4 days. Native maize and millet flours were used as control. The samples were analyzed for biochemical properties using standard methods. Nutrient compositions of germinated and fermented cereals flours were dominantly increased when compared with the raw sample. Protein content of flours varied between $9.33 \pm 0.05\%$ (RwMaF) and $15.83 \pm 0.02\%$ (GMaF) for maize and $7.32 \pm 0.07\%$ (RwMiF) to $12.15 \pm 0.41\%$ (FMiF) for millet. Germination and fermentation lead to a decrease in reducing and total sugars. Ash content of germinated maize flour increase while that of fermented maize millet flours decreased. Fibers content of flours varied between $3.37 \pm 0.00\%$ (RwMaF) and $5.45 \pm 0.92\%$ (FMaF) for maize and $2.25 \pm 0.01\%$ (RwMiF) to $4.43 \pm 0.26\%$ (FMiF) for millet. Vitamin C content of maize and millet flours increased sharply after fermentation and germination. Germination greatly increased the tannin content from 219.52 ± 4.37 to $315.21 \pm 4.23\%$ (maize), 189.24 ± 1.78 to $287.56 \pm 5.40\%$ (millet), total polyphenols from 127.15 ± 2.15 to $143.17 \pm 5.72\%$ (maize), 190.17 ± 6.21 to $275.15 \pm 8.74\%$ (millet). Fermentation lowered contents of total polyphenols, tannins and flavonoids in maize and millet flours. These two processing technologies (germination and fermentation) could be used to improve the nutritional properties of complementary foods for infants and young children.

KEYWORDS: Fermentation, Germination, Maize, Millet, Flours, Composition.**INTRODUCTION**

Nutritional quality of food is a key element in maintaining human overall physical well-being.^[1] Cereals form a significant portion of the food supply for humans and other animals, as they are a major source of macro and micronutrients.^[2,3] Generally a cereal-based porridge is the main complementary food in most developing countries.^[4] Maize, millet and sorghum are the principal cereals crop product in Côte d'Ivoire. Maize (*Zea mays* L.) is originating to South America and belongs to the Poaceae family (Gramineae).^[5] In Côte d'Ivoire, maize is the most cultivated cereal after rice.^[6] It's a cereal which is more consumed in north of Côte d'Ivoire in porridge form and cooked flour paste ("toh"), "congodé" and fried pancakes commonly known as "claclo".^[7,5] It's also used for children and infants foods (Brou *et al.*, 2013), starch, flours, ethanol and cooking syrup production.^[8]

Millet (*Pennisetum glaucum*) is a food grain that is mainly grown for its kernels.^[9,10] Their nutritional value is higher than that of some commonly consumed cereals such as rice, wheat and maize.^[11,10] Millet is an energetic and nutritious food particularly recommended for children and elderly or convalescent people.^[12] It is used in porridges preparation, dough commonly called "tô" in Africa, biscuits, pasta, beer and other preparations.^[13,10] To improve the nutritional quality and organoleptic acceptability of cereal grains, commonly used processing techniques include soaking in water, high temperature boiling in water, roasting, sprouting and fermentation.^[14,15,16]

Germination of cereals is a better alternative because it is simple, inexpensive and improves the availability of certain nutrients.^[17] It improves the quality of nutrients and bioactive compounds in cereals, thus increasing the content of proteins, amino acids, sugars and vitamins.^[18] It has been widely used for its ability to decrease the

levels of anti-nutritional factors in plant seeds, while improving the concentration and bioavailability of their nutrients.^[19] Fermentation is a metabolic process that allows certain microorganisms to obtain energy through the digestion of simple fermentable sugars, mainly glucose and fructose. It improves the nutritional value of foods through the biosynthesis and bioavailability of vitamins,^[20] essential amino acids and improves the quality of proteins and the digestibility of fibers. It serves as a means of providing a major source of food for large rural populations and contributes significantly to food security.^[21]

The aim of this study was to evaluate effects of germination and fermentation on the composition of millet and maize in order to obtain new foods with improved nutritional properties.

MATERIAL AND METHODS

Materials

Grains of millet (*Pennisetum glaucum*) and maize (*Zea mays*) used for this study were bought from local market in Abobo (Côte d'Ivoire). Grains of millet and maize

were used to study the effect of germination and fermentation.

Methods

Processing of maize and millet flours

Grains of millet and maize were thoroughly cleaned and divided into 3 equal parts for study.

The first part (1 kg) was not subjected to any treatment. It served as control.

Second portion (1 kg) was soaked in tap water 1:3 (p/v) for 24 hours. The soaked seeds were kept on a cotton cloth and allowed to germinate in the dark at room temperature for 4 days. They were kept wet by frequent spraying of water. Then, the germinated seeds were dried in an oven at 45°C for 2 days and degerminated by hand. The dried seeds were milled, sieved, packed and stored at 4°C as before (Fig 1). Third portion (1 kg) of grains were soaked in boiled water in a ratio of 1:4 (p/v) for 6 hours and allowed to ferment by the natural microorganisms present on the seed for 4 days. The fermented seeds were washed, dried in an oven at 45°C for 2 days. Then, the dried seeds were milled and sieved through 0.25 mm wire mesh before packing in a sealed airtight plastic container and storage at 4°C prior to analysis (Fig 1).

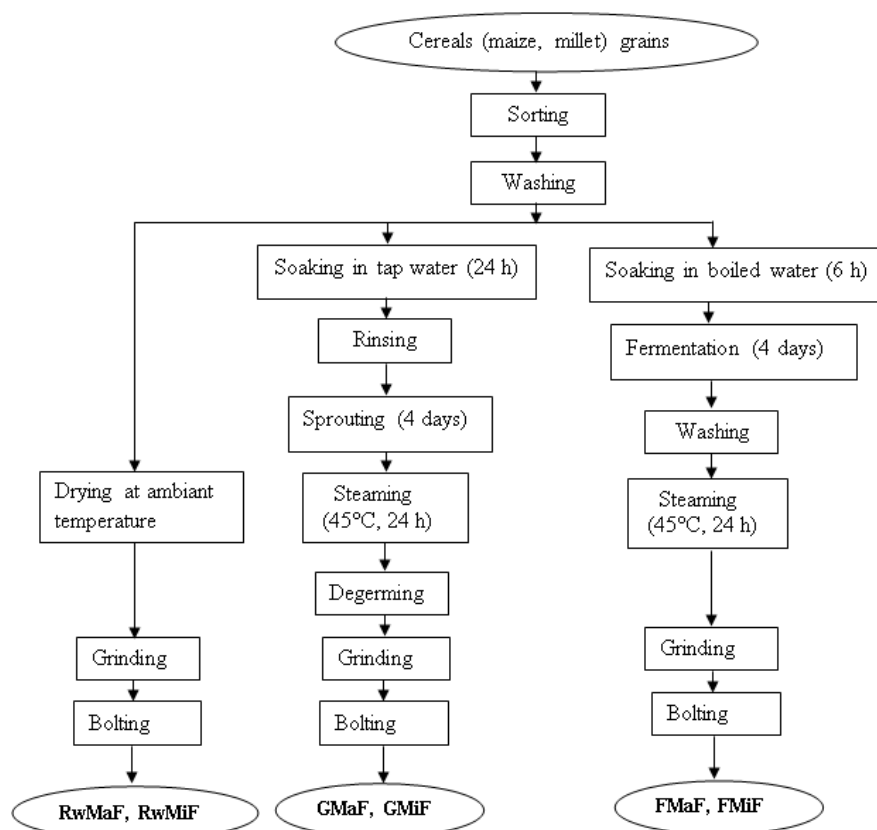


Fig. 1: Flow chart illustrating preparation of germinate and fermented cereals (maize, millet) flours.

Proximate analysis

The proximate composition of samples was determined.^[22] Moisture was determined by drying in an oven at 105°C during 24 h to constant weight. Crude protein contents were calculated from nitrogen contents

(Nx6.25) obtained using the Kjeldahl method. Crude fat contents were determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent. Ash contents were determined by incinerating flour (2g) in a furnace at 550°C for 6 h, then weighing the residue after

cooling to room temperature in a desiccator. For crude fibers, 2g of cereals (maize, millet) flour sample were digested with 50 mL of sulphuric acid (0.25N) and 50 mL sodium hydroxide (0.3N) solution. Insoluble residue obtained was washed with hot water and dried in an oven at 100°C until constant weight. Dried residue was then incinerated (550°C), and weighed for the determination of crude fibers content. Carbohydrate contents were determined by difference as follows: % carbohydrate = 100 % - (% moisture + % crude protein + % crude fat + % ash). Total sugar was determined by Chow & Landhäusser^[23] and reducing sugar was analyzed according to Garriga et al.(2017) using 3,5 dinitrosalicylic acids (DNS). Samples pH was determined using a pH meter as protocol described by Nielsen (2003). Titratable acidity was made according.^[25] Energy value were obtained by the summation of multiplied mean values for protein, fat and carbohydrate by their respective Atwater factors, 4, 9 and 4. Energy value (%) = (4 x % Carbohydrates) + (9 x % Fat) + (4 x % Proteins).

The total carbohydrate content was calculated by using the equation: 100 - (% moisture + % proteins + % lipids + % ash)^[26]. The energy value of samples was calculated^[27] using the conversion factor: 9 kcal.g⁻¹ of lipids, 4 kcal.g⁻¹ of carbohydrate and 4 kcal.g⁻¹ of proteins. Vitamin C content was measured by titrimetric assay with 2,6-dichloroindophenol.^[28]

Polyphenols determination

Phenolic compounds were extracted.^[29] Sample of cereal flour (1 g) was soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 min. The pellet was collected in 10 mL of methanol 70% (w/v) and centrifuged again. A third extraction was carried out under the same conditions. The three (3) supernatants were pooled in a 50 mL vial and the volume was adjusted with distilled water to the mark. This mixture constituted the total phenolic extract.

Total polyphenols determination

Total polyphenol content was determined using the Folin-Ciocalteu reagent-based colorimetric assay as described.^[29] An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of Na₂CO₃ (20%, w/v). The reaction mixture was incubated for 30 min at ambient temperature and absorbance was recorded at 745 nm by using a spectrophotometer. The polyphenol content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

Tannins determination

Tannins contents of cereal flours samples were quantified by the spectrophotometric method.^[30] About 1 mL of the methanolic extract was mixed with 5 mL of vanillin reagent and the mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was read at 500 nm by using a

spectrophotometer. Tannin content of samples was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

Flavonoids determination

Total flavonoids were evaluated using the spectrophotometric method.^[31] Briefly, 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL AlCl₃ (10%, w/v), 0.5 mL potassium acetate (1 M) and 2 mL distilled water. The absorbance was measured using a spectrophotometer at 415 nm after 30 min. Total flavonoids were calculated using a calibration curve of quercetin (0.1 mg/mL) as standard.

Statistical Analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) was done. If necessary, Duncan test was done to determine significant differences at 5% probability between means. Statistical differences with a probability value less than 0.05 (P <0.05) are considered significant.

RESULTS AND DISCUSSION

Proximate composition of raw, germinated, fermented maize and millet flours were presented in Table 1. Germination and fermentation significantly (p<0.05) increased dry matter (88.07±0.08 to 93.50±0.15% and 86.20±0.04 to 94.52±0.03%) and protein (9.33±0.05 to 15.83±0.02% and 7.32±0.07 to 12.15±0.41%) content for maize and millet flours, respectively. The increase in nutrient composition of germinated and fermented maize and millet flours could be attributed to biochemical activities of sprouting grains and also due to the increase in microbial mass during fermentation, causing extensive hydrolysis of the protein molecule to amino acid and other simple peptides.^[21,32]

Increase in protein may confer nutritional advantage on the germinated and fermented flours. Otherwise, sprouted maize flour has the highest protein content while fermented millet flour has the lowest. The germinated maize sample was significantly higher in protein content when compared with the fermented and raw samples. Therefore, germination is the best technique to significantly increase the protein content of maize flour.

The result also showed significant decrease (p<0.05) in fat contents with germination, from 5.62±0.01 in control (untreated grain) to 3.08±0.02% and from 2.80±0.02 (control) to 1.75±0.03 respectively for maize and millet flours. As for fermentation, a significant increase in fat content was noted 7.33±0.21% (maize) and 6.95±0.08% (millet). This decrease might be due to the increased activities of the lipolytic enzymes during germination, which hydrolyse fats to fatty acids and glycerol.^[33]

The simpler products can be used for synthesis of carbohydrate and protein or as a source of energy for developing embryo.^[34] This also explains the increase in protein observed in this study. The increase in fat content during fermentation could be attributed to extensive break down of large fat molecule to simpler fatty acid units.^[32]

Reducing sugar of samples decreased from $0.15 \pm 0.03\%$ (raw maize flour) to $0.02 \pm 0.00\%$ (germinated maize flour) and $0.11 \pm 0.00\%$ (fermented maize flour). For millet, there was also a decrease in values from $0.17 \pm 0.00\%$ to $0.11 \pm 0.00\%$ and $0.03 \pm 0.00\%$ respectively for the control, germinated and fermented millet flours. Total sugar content of both germinated ($1.48 \pm 0.00\%$) and fermented ($0.64 \pm 0.04\%$) maize flour was lower than the raw sample ($2.45 \pm 0.00\%$). The same observation applies to sprouted and fermented millet flours. Germination and fermentation are therefore techniques for reducing total sugars. Decrease in total sugar may be due to increasing of metabolic activity accompanying germination and to consumption of the later sugars by bacteria during fermentation.^[35]

When maize grains are germinated, there is an increase in ash content (1.35 ± 0.05 to $2.15 \pm 0.08\%$) while fermentation causes a reduction (1.35 ± 0.05 to $0.85 \pm 0.03\%$). This difference could be justified by the increase in the dry matter rate during germination because, ash content is correlated with that of dry matter and varies proportionally.^[36] The increase in ash content could also come from the tap water used to soak, rinse and water the grains during germination.^[37] As for millet, germination and fermentation cause a significant reduction in ash content (1.25 ± 0.04 to $0.94 \pm 0.02\%$). This decrease could be the result of hydrothermal treatment of grains prior to fermentation. It could also be due to the use of certain essential salts by the microorganisms for their metabolic activities.^[38]

Fiber contents of maize ($5.45 \pm 0.92\%$) and millet ($4.43 \pm 0.26\%$) fermented flours were higher than the maize ($3.37 \pm 0.00\%$) and millet ($2.25 \pm 0.01\%$) raw flours. Consumption of fermented millet flours would have positive health effects (preventing constipation, colon cancer and cardiovascular disease) as suggested.^[39] In contrast, crude fiber content decreased significantly ($P < 0.05$) during maize germination. It would be due that part of the seed fiber may be solubilized enzymatically during seed germination. In fact, as germination progressed, partial utilization of cell wall carbohydrate can occur and consequently, the content of structural carbohydrates such as lignin and cellulose can be affected negatively with the germination time.^[40,41]

Reduction of carbohydrate content might be as a result the microorganisms utilizing some of the sugars needed for their growth and metabolism by secreting saccharolytic enzymes which broke down the complex carbohydrates into smaller units like sugars and alcohols

One of the key events reported to occur during germination is the activation of amylolytic enzymes.^[35] This enzymes breaks down complex carbohydrates to simpler and more absorbable sugars (glucose, short-chain glucose polymers and oligosaccharides) which are utilized by the growing seedlings during the early stages of germination.^[34] This explains the drop in carbohydrate content during germination. With regard to fermentation,^[42] explained that during this process, carbohydrate was used as source of energy for fermentables microorganisms growth. So, germinated/fermented maize and millet flours will become soft, more palatable as well as more digestible because of hydrolysis of complex polysaccharides.

Control maize flour provides more energy than millet meal. Besides, the energy value of fermented maize sample (434.94 ± 1.34 Kcal) and fermented millet sample (413.07 ± 0.52 Kcal) were higher than germinated and raw samples. This observation could be a result of the biochemical activities of the growing grains that utilized parts of the protein, fat and carbohydrate content of the grains.^[19] It is also attributed to the decrease of fat contents on samples. Indeed, fat on its own contains about twice the food energy values of protein and carbohydrate.^[43]

pH obtained in this study are low compared to control values. The decrease is even greater when the grains cereals are fermented. Lower pH observed during fermentation could be a result of acids production by the fermenting microorganisms. Indeed, they hydrolyzed available carbohydrate to lactic acid before embarking on proteolysis of available protein.

Titrate acidity increased at the same time especially it was a measure of total organic acid amount present in a sample. This result was important because acidification was the key mechanism during fermentation.^[42] It should be noted at the level that acid production has been reported to be responsible for product stability and flavour development.

Vitamin C contents varied significantly ($P < 0.05$) from $7.14 \pm 2.10\%$ (RwMaF) and $8.78 \pm 0.44\%$ (RwMiF) to $10.14 \pm 1.59\%$ mg/100g DM (GMaF) and $11.95 \pm 1.53\%$ (GMiF) during germination respectively for maize and millet flours (Table II). This would be due to the fact that ascorbic acid has been directly implicated in the modulation of plant growth, including the early stage of embryos.^[41] Increase in vitamin C content during germination was related to the increase in activity of key enzymes in ascorbic acid biosynthesis pathway such as L-galactono- γ -lactone dehydrogenase (GLDH, EC 1.3.2.3) as reported by.^[44] Moreover, according to^[45] during germination, respiration process is triggered by ascorbic acid. Several studies have reported that germination leads to increase in antioxidant activity of which ascorbic acid is the main contributor.^[46] Vitamin C content also increased during fermentation. This result

is consistent with that of Adetuyi and Ibrahim (2014) on the fermentation of okra (*Abelmoschus esculentus*) grains.

Significant increase ($P < 0.05$) were observed between phenolic content of cereals (maize, millet) flours during germination (Table II). This would be due to the resumption of the metabolic activities of grains which led to a large consumption of oxygen and resulting in the formation of free radicals called reactive oxygen species (ROS).^[48] Thus, phenolic content act as antioxidants to

protect cells against the stress induced by oxidation of these free radicals.^[41] Their synthesis is enzymatically from phenylalanine ammonia lyase,^[49] This would explain the increase in levels during germination of cereals grains. During fermentation, a significant drop in phenolic content was noted. This decrease could be explained by diffusion of these compounds in the environment or their oxidation by polyphenol oxidases.^[47] The increase of phenolic content improved antioxidant potential flour derived from germinated maize and millet grains.

Table I: Proximate composition of germinated and fermented cereals (maize, millet) flours.

Parameters (%)	Maize			Millet		
	RwMaF	GMaF	FMaF	RwMiF	GMiF	FMiF
Dry matter	88.07±0.08 ^c	91.93±0.19 ^c	93.50±0.15 ^a	86.20±0.04 ^a	90.94±0.42 ^c	94.52±0.03 ^c
Protein	9.33±0.05 ^b	15.83±0.02 ^b	14.45±0.19 ^c	7.32±0.07 ^c	11.50±0.03 ^a	12.15±0.41 ^c
Fat	5.62±0.01 ^a	3.08±0.02 ^a	7.33±0.21 ^c	2.80±0.02 ^e	1.75±0.03 ^b	6.95±0.08 ^a
Reducing sugar	0.15±0.03 ^b	0.02±0.00 ^a	0.11±0.00 ^b	0.17±0.00 ^a	0.11±0.00 ^d	0.03±0.00 ^a
Total sugar	2.45±0.00 ^a	1.48±0.00 ^c	0.64±0.04 ^a	0.65±0.00 ^c	0.03±0.00 ^b	0.18±0.00 ^c
Ash	1.35±0.05 ^a	2.15±0.08 ^a	0.85±0.03 ^e	1.25±0.04 ^b	0.97±0.04 ^a	0.94±0.02 ^a
Fibers	3.37±0.00 ^a	1.95±0.02 ^e	5.45±0.92 ^a	2.25±0.01 ^c	3.01±0.26 ^a	4.43±0.26 ^a
Carbohydrates	83.71±0.03 ^c	84.07±0.27 ^b	80.15±0.76 ^a	86.15±0.57 ^b	76.17±0.45 ^b	77.54±0.41 ^b
Energy	403.57±0.10 ^b	395.35±0.98 ^a	434.94±1.34 ^c	397.05±0.15 ^c	355.15±0.02 ^c	413.07±0.52 ^b
pH	6.03±0.01 ^d	5.43±0.02 ^a	4.76±0.01 ^a	5.28±0.01 ^c	4.47±0.00 ^b	4.26±0.01 ^a
Titrateable acidity	11.73±0.46 ^a	15.33±1.15 ^{ab}	8.00±0.00 ^a	16.80±1.39 ^c	23.47±0.92 ^d	18.93±1.85 ^d

RwMaF: Raw Maize Flour; **GMaF:** Germinated Maize Flour; **FMaF:** Fermented Maize Flour; **RwMiF:** Raw Millet Flour; **GMiF:** Germinated Millet Flour; **FMiF:** Fermented Millet Flour

Table II: Vitamin C and polyphenol contents of germinated and fermented cereals (maize, millet) flours.

Parameters (%)	Maize			Millet		
	RwMaF	GMaF	FMaF	RwMiF	GMiF	FMiF
Vitamin C	7.14±2.10 ^b	10.14±1.59 ^a	9.29±0.87 ^a	8.78±0.44 ^b	11.95±1.53 ^a	10.62±0.88 ^a
Total polyphenol	127.15±2.15 ^a	143.18±5.72 ^c	113.75±0.55 ^a	190.17±6.21 ^b	275.15±8.74 ^c	165.31±6.25 ^c
Tannins	219.52±4.37 ^a	315.21±4.23 ^b	177.47±8.66 ^b	189.24±1.78 ^b	287.56±5.40 ^c	169.15±3.17 ^c
Flavonoid	10.45±0.17 ^a	12.92±0.29 ^b	8.71±0.50 ^b	19.56±0.41 ^d	28.77±0.27 ^c	12.07±1.57 ^b

RwMaF: Raw Maize Flour; **GMaF:** Germinated Maize Flour; **FMaF:** Fermented Maize Flour; **RwMiF:** Raw Millet Flour; **GMiF:** Germinated Millet Flour; **FMiF:** Fermented Millet Flour

CONCLUSION

Germination and fermentation of cereals (maize, millet) are traditional and simple methods of adding value to cereals. Protein content increases and gives a nutritional advantage to sprouted and fermented flours (corn and millet). Fermentation increases fibers content of flours and its consumption would have beneficial effects on health (prevention of constipation, colon cancer and cardiovascular diseases). The increase in phenolic compounds and vitamin C improves antioxidant potential of flours obtained from the germination of maize and millet kernels.

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