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Beneficial Changes and Drawbacks of a Traditional Fermentation Process on Chemical Composition and Antinutritional Factors of Yellow Maize (*Zea mays*)

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Abstract: The present study was designed to investigate the potential of nutritional improvement of yellow maize by a household fermentation. Yellow maize (*Zea mays*) was fermented according to a traditional fermentation process which included soaking the raw seeds in distilled water for four days at 30°C, followed by milling, washing, sieving, decanting and drying. Proximate composition, vitamins, minerals and some antinutritional factors such as phytates, trypsin and α-amylase inhibitors were measured and compared in raw and processed maize. Crude proteins, total fats, carbohydrates and fibers significantly decreased by 8.7, 11.2 and 8.8%, respectively ($\alpha \leq 0.05$). This was accompanied by a decrease of 38% for calcium, 69% for thiamin, 81.81% for riboflavin and 66% for Retinol Equivalent (RE). These nutrient losses were due to the various steps involved in the process, but thiamin, riboflavin and RE content were already low in raw maize. The pH drop to 3.87 and the reduction in antinutrient factors observed (61.5, 41.7 and 16.6%, for phytates, trypsin inhibitors and α-amylase inhibitors, respectively) might improve the safety and the nutritional value of fermented maize. However the decrease in macronutrients and the low content in vitamins observed in fermented or raw maize require complementation with other protein and vitamin food sources.

Key words: Yellow maize, nutritional value, antinutritional factors, fermentation

INTRODUCTION

Fermentation is one of the oldest, simple and most economical methods of producing and preserving foods[1]. It is well known that lactic acid fermentation, mostly applied at the household level in African countries, may provide a way to reduce volume of material to be transported, to enhance nutritive value (synthesis of certain amino acids, improvement of availability of B group vitamins, minerals and protein digestibility), to improve appearance and taste of some foods, to salvage material otherwise not usable for human consumption, to reduce energy required for cooking[2-7] and to destroy antinutritional compounds of food (phytate, condensed tannins, trypsin and α-amylase inhibitors)[1,4,8,9].

The most advantageous effects of lactic acid fermentation in developing countries is food safety and preservation, due to the rapid drop of pH close to 4, which inhibits the growth of enteropathic bacteria[10-12]. Traditional lactic acid fermentation is commonly used under non-standardized conditions to produce popular cereal products such as breakfast foods, snack items, beverages and porridges[4-7]. The nutritional value of the finished products vary greatly from culture to culture in various regions of the world, due to the type of raw material used for fermentation and the conditions of fermentation. Marfo *et al.*[4], Dillon[6], Ojofeitimi and Abiose[7] reported a decrease of more than 70, 29 and 26% in ash, crude protein and fat, respectively in fermented maize compared to raw maize, while Asiedu *et al.*[5] reported a decrease of 10 and 4% in ash and crude protein and an increase of 6% in fat in fermented maize. Some investigators have observed a significant reduction or total elimination of certain antinutrients (phytate and trypsin inhibitors) in cereals by fermentation[1]. The reduction of phytate in maize by fermentation is well documented[1,4,10], but studies on the effect of fermentation on trypsin inhibitors, α-amylase inhibitors, condensed tannins and the other antinutritional factors of maize are scarce. Since the preparation of many indigenous or traditional fermented foods and beverages from grains remains, even today, a household art it is therefore crucial

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to investigate the biochemical and nutritional changes during traditional fermentation, in order to highlight the effects of the technique used.

The aim of the present study was to investigate the nutritional advantages and drawbacks of a fermentation technique commonly used in Cameroon for the preparation of a variety of traditional meals on pH, proximate composition, some minerals, vitamins and antinutritional factors of yellow maize.

MATERIALS AND METHODS

Materials: Yellow maize (*Zea mays*) variety CMS 8704 was obtained from the Agricultural Research Institute for Development, Cameroon in May 2002 and the study was carried out in Joseph-Rhéaume's laboratory (Laval University, Sainte-Foy, Quebec from June to September 2002). Extraneous materials and broken seeds were removed by hand sorting. The raw seeds were divided into two portions, one portion being designated as control and stored in a cold room at 4°C. The remaining seeds were washed carefully with distilled water and the floats were discarded. The water was discarded and the seeds were allowed to undergo traditional fermentation.

Experimental procedures
Raw samples: Raw seeds were milled in a Tecator 1093 cyclotech sample mill (Germany) to pass through a 1 mm sieve. One part of the flour was immediately analyzed for thiamin, riboflavin and β-carotene. The remaining was packed, sealed in polyethylene bags and stored in a Fisher Scientific desiccator in a cold room at 4°C until further analysis.

Fermentation: Based on the traditional technique used in Cameroon, yellow maize seeds were washed with distilled water and separated into three equal portions. Each portion was dispersed in distilled water (1:4, w/v) and allowed to undergo natural lactic acid fermentation for four days in a E7H Controlled Environment Conviron chamber (Manitoba, Canada) set for 80% Relative Humidity (RH) and 30°C, to reflect atmospheric conditions prevalent in Cameroon (Fig. 1). Recording of pH was made in the steeping water at the beginning and at the end of fermentation. At the end of the four days fermentation, water was discarded; fermented seeds were washed with running distilled water for 10 min and milled in a 720 Braun domestic grinder (Germany). The fermented paste was mixed with distilled water (1:4, w/v). Washing followed by sieving with a domestic metallic inox sieve was performed three times in order to remove the hulls or testa. After 24 h decanting, water on the top of the container

was easily drained. About 300 g of the milled, washed, sieved and decanted dough were immediately analyzed for thiamin, riboflavin and β-carotene, the remaining were freeze dried (Virtis, Gardiner, New York), milled, handled and stored as raw samples.

Analytical methods
Proximate and mineral composition: The moisture and ash contents were determined by standard AOAC method 925.10[13]. The pH was measured using a Fisher scientific 925 accumet pH meter (Pittsburgh, PA, USA). Nitrogen was determined by AOAC Kjeldahl method 979.09[13] using a nitrogen autoanalyzer (Foss Electric, Denmark) and crude protein content was calculated (N × 6.25). Lipid content was determined by the AOAC Goldfish method No. 945.16[13] and total carbohydrate and fiber was calculated by difference.

Fe, Zn and Ca contents were simultaneously determined by flame atomic absorption spectrophotometry (Perkin-Elmer Analysis 100, USA) following wet digestion of sample with HNO_3 and $HClO_4$[14].

Energy value: Gross Energy (GE) was determined with an automatic adiabatic bomb calorimeter (Parr, model 1720, Il, USA).

Thiamin, riboflavin and β-carotene: The measurement of thiamin and riboflavin was simultaneously made by an HPLC system equipped with a Millipore pump (Waters 501, Milford, MA), a reversed phase column (LiCrospher ® 100 RP-18, 5 μm endcapped) and a LiChrocart ® Cartridge 250-4 both from Hewlett Packard (Waldbronn, Germany). Short body μBondapak C18 Sep-Pak Classic cartridges (Waters, Milford, MA) and Minisart® (Sartorius, Goettingen, Germany) were used for sample condensation and purification, respectively. Sample preparation and extraction procedure were carried out according to Fellman *et al.*[15].

β-carotene was measured by the same HPLC system using the methods described previously[16,17]. β-carotene was expressed as Retinol Equivalent (RE) which was calculated by the following conversion: 1 RE = 3.33 International Unit (IU); 1IU = 0.60 μg β-carotene[18], then 1RE = 3.33×0.6 μg β-carotene ≈ 2 μg β-carotene.

Phytates: Phytates were extracted with 2.4% HCl (1:20, w/v). The mixture was centrifuged at 400 g (Sorvalle®-rotor GSA, Newton, USA) for 10 min and the supernatants were collected and diluted at 4%. Bio-Rad (medium size) columns were filled with AG1-X8 200-400 mesh resin to effect phytate purification. Wade solution was used as

described by Latta and Eskin[19] to assess total phytate content.

Trypsin inhibitor activity: Trypsin inhibitor activity was measured with the method developed by Kakade *et al.*[20] with modifications and limitations[21,22]. The method used α-N-benzoyl-DL-arginine-p-nitroanilide hydrochloride (Sigma, B 4875) or BAPNA as a substrate for trypsin. Trypsin inhibitor from bovine pancreas was used to release P-nitroanilide. The absorbency was measured at 410 nm against a reagent blank and trypsin inhibitory activity (TIA) expressed as trypsin inhibitory units (TIU)/mg DM was calculated. One trypsin unit was defined as 0.01 increase in absorbency unit.

α-amylase inhibitor activity: α-amylase inhibitor activity was evaluated according to the methods described previously[23] with some modifications[24-26]. A 1 g sample was extracted with 10 mL deionized water for 16 h at 4°C, centrifuged at 5000 g (Sorvalle SS-34 8000 rpm, Newton, USA) for 20 min and the supernatants were tested for α-amylase inhibitor activity (AIA). The absorbency was recorded at 540 nm. One unit of enzyme activity was defined as that which liberates, from soluble starch, one μmole of reducing groups (calculated as maltose) per 3 min at 37°C and pH 7.0.

Statistical analysis: Treatments were conducted in three repetitions. Macronutrients, micronutrients, phytates and tannins analyses were performed in triplicate for each treatment repetition, with two readings for vitamins. Enzyme inhibitor activity (α-amylase and trypsin) was measured in triplicate with four readings. Results were given as treatment Mean±SD. Analyses of Variance (ANOVA) of the results were performed at 95% confidence interval ($\alpha \leq 0.05$) using the t test. This analysis was done by using SAS system Copyright © 1989-1996 by SAS Institute Inc., Cary, NC, USA.

RESULTS AND DISCUSSION

The pH: A 37% decrease in pH ($\alpha \leq 0.05$) was observed after four days of fermentation (Table 1) thus indicating the production of lactic acid. This was observed in a previous study in which a 42% decrease in pH (from 6.1 to 3.35) and an 88% increase in titratable acidity after 72 h of maize dough fermentation were recorded[12]. Nago *et al.*[11] recorded a pH of 3.8 and 3.5 in ogi (fermented maize product) supernatant and sediments, respectively after one week fermentation at 35°C and a pH of 3.8 and 3.9 after two weeks of fermentation. The pH drop was probably the result of microbial

Table 1: pH and proximate composition of yellow maize as affected by four days traditional fermentation

Elements	Yellow maize	
	Raw	Fermented
pH	6.16±0.11*	3.87±0.06 (37.18↓)
Moisture (%)	5.73±0.15*	1.36±0.66 (76.27↓)
Ash (%)	1.45±0.18*	0.66±0.42 (54.48↓)
Gross energy (Kcal/100 g DM)	446.63±5.48	442.54±13.7 (0.9)
Crude protein (%)	10.34±0.16*	9.44±0.12 (8.7↓)
Fat (%)	5.36±0.17*	4.76±0.07 (11.2↓)
Carbohydrate and fibers (%)	77.13±0.27	83.95±0.8* (8.84↓)

Results are the means of 3 determinations±SD, *The mean differences were significant at 95% confidence interval ($\alpha \leq 0.05$) through the t test, Figures in parentheses indicate the percent increase↓/decrease↓ of the corresponding element over raw maize where significant differences were demonstrated.

activities on the soaked seeds converting some of the carbohydrates into organic acids such as lactic, citric and acetic acids.

Energy value: Energy value of the fermented yellow maize was 0.9% lower than the raw product (443 versus 447 Kcal/dry matter), but the decrease was not significantly different (Table 1). A previous study[5] reported an energy value of 409 Kcal/100 g Dry Matter (DM) in fermented white maize dough compared to 404 Kcal/100 g DM in unprocessed white maize. Spontaneous fermentation of corn is mostly lactic acid fermentation, because chemical analysis in a previous study showed that 94.5% of the 1.1% total acidity was lactic and that the resulting dominant bacteria was lactic acid bacteria[27]. Lactic acid bacteria are microaerophilic, which means that the metabolic processes they carry out require little or no oxygen and they do not decompose foods into their basic constituents such as carbon dioxide, water, oxygen, simple nitrate and sulfates[28]. Other volatile carboxylic acids such as acetic, propionic and butyric acid have been identified during the fermentation of maize meal and could lead to the involvement of varieties of aerobic microorganisms (*Saccharomyces cerevisae, Candida tropicalis* and *Candida krusei*) associated with amylolytic activity[2]. Lactic and other acids are produced with only minor changes to the food components and there should be little loss of energy and nutrients.

Proximate composition: There was a significant decrease in ash (54%), crude protein (9%), fat (11%) and a significant increase in carbohydrate and fiber (9%) in fermented maize, compared to raw maize (Table 1). A comparison of previous studies (Table 4) on ash, protein, fat and carbohydrate and fiber reveals variable results. Losses in ash, crude protein and fat were therefore probably not the result of the metabolic processes carried out by lactic acid bacteria which could offer many

nutritional advantages[29,30], but would rather be due mostly to the variety of maize, the fermentation conditions and the steps such as multiple washings and wet-sieving involved in the process (Fig. 1). For instance, Asiedu *et al.*[5] registered less losses in ash and crude protein which could be attributed to the use of a white variety of maize in the form of dough, the influence of solids: water ratio and the fermentation technique (Table 4), but the proportion of water in relation to solids in fermenting medium significantly influence the level of acidity and dry matter yields. With less water, higher levels of acids are present, inhibiting the flora[1]. However the general trend was comparable to previous studies, Asiedu *et al.*[5] and Dillon[6] reported an increase in fat after maize fermentation. The increase in total carbohydrates and fiber calculated by difference could be due to the water content of the finished products before chemical analysis: 5.73 and 1.36% for raw and fermented dry maize flour, respectively corresponding to 77.13 and 83.95% DM, respectively and may not reflect the real values in the foods. Carbohydrates, particularly starch and soluble sugars are principal substrates for fermenting microorganisms, therefore degradation and a subsequent decrease in starch content are expected to occur.

On the other hand, the expected decrease in fiber content during fermentation could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by microbial enzymes[1]. A previous study has reported a significant decrease of 8.5 and 16% in starch and total sugars, respectively after 18 h fermentation at 28-29°C and an increase of 141.6% in crude fiber[1].

Iron and zinc contents were not significantly affected by the fermentation of yellow maize, as opposed to calcium where the decrease was 38% ($\alpha \le 0.05$) (Table 2). Adeyeye *et al.*[31] reported an 8% decrease in calcium in whole maize vs. fermented maize (49.2 mg/100 g on a dry weight basis (dwb) vs. 45.1 mg/100 g dwb. Asiedu *et al.*[5] who used lactic acid fermentation without washing, decanting and wet sieving reported a 6.6% increase in calcium, but fermentation did not affect iron and zinc contents. Calcium is mostly located in the seed coat of cereals and legumes[32]. The decrease in calcium observed might be due to mechanical elimination of maize seed coats and embryo during repeated washing, wet-sieving and decanting, followed by discarding excess of water.

Thiamin, riboflavin and β-carotene: There was a significant decrease ($\alpha \le 0.05$) in thiamin, riboflavin and β-carotene contents: 69, 82 and 66%, respectively

Table 2: Mineral and vitamin content of yellow maize as affected by four days traditional fermentation

Elements	Yellow maize	
	Raw	Fermented
Calcium (mg/100 g DM)	43.48±0.7*	26.86±1.18 (38.2↓)
Iron (mg/100 g DM)	2.15±0.21	2.09±0.34 (2.79)
Zinc (mg/100 g DM)	1.57±0.12	1.45±0.11 (7.64)
Thiamin (mg/100 g DM)	0.49±0.06*	0.15±0.02 (69.39↓)
Riboflavin (mg/100 g DM)	0.11±0.01*	0.02±0.003(81.81↓)
β-carotene (RE/100 g DM)	61.16±1.26*	20.80±2.3 (66↓)

Results are the means of at least 3 determinations±SD, *The mean differences were significant at 95% confidence interval ($\alpha \le 0.05$) through the t test, Figures in parentheses indicate the percent increase↑/decrease↓ of the corresponding element over raw maize where significant differences were demonstrated. RE = Retinol Equivalent
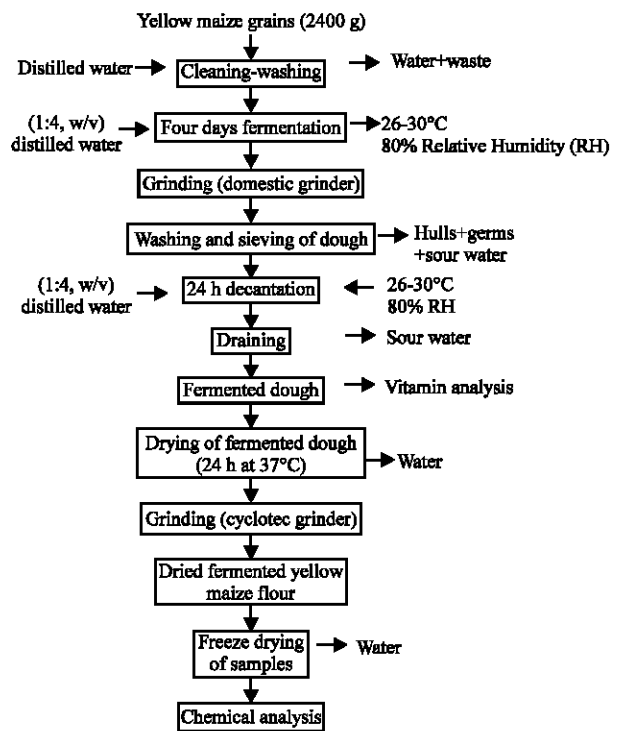

Fig. 1: Flow diagram of fermented yellow maize production

compared to raw maize (Table 2). Dillon[6] also used multiple sieving and washing. He reported a decrease of 35 and 60%, respectively for thiamin and riboflavin. Losses were greater compared to Dillon[6], who did not provide information regarding the variety of maize used. The reduction of β-carotene contained in the starchy endosperm of the yellow maize grains was the result of mechanical loss by leaching of grain particles through repeated washing, sieving and decanting steps associated with the fermentation process and to the resulting loss of total fat, since the embryo has the highest concentration of lipids and hence lipid-soluble vitamins in cereal grains[33].

Antinutritional factors

Phytates: Phytate content (9.87 mg g^{-1} DM) in raw yellow maize (Table 3) was found to be greater than 7.1 mg g^{-1} DM in Malawian white maize flour[10] and 6.98 mg g^{-1} DM in pounded white maize flour[34]. On the other hand, phytate content in fermented yellow maize (3.8 mg g^{-1} DM) compared well with 3.06 mg g^{-1} DM reported by Hotz and Gibson[10] after 12 h soaking of maize flour at 25°C and with 3.42 mg g^{-1} DM after 1 h soaking of pounded maize flour at 26-30°C. The decrease in phytate content observed in fermented vs. raw yellow maize was 62%, compared to 57% (12 h soaked white maize flour) and 51% (1 h soaked and pounded white maize flour)[10]. During spontaneous fermentation, the fermentative microorganisms contain phytase and phosphatase, which hydrolyze phytate into inositol and orthophosphate. The degradation occurs during the first 12 to 14 h of soaking[29].

It has been suggested that the loss of phytate during fermentation might be due to phytase activity naturally present in the cereals and activity of the fermentative microorganisms in the dough[4]. Phytic acid content of some cereals can also be removed via passive diffusion of water-soluble phytate, phytate being present primarily as the water-soluble sodium or potassium salts[35]. Fermentation may be a method for improving the availability of iron, zinc and calcium, since less phytate can bind those minerals and thus prevent hydrolysis of the resulting insoluble complexes by digestive enzymes. However the optimum pH for activity of cereal phytase ranges from 5.1 to 5.3[4]. The drop of pH to 4 occurring after 48 h fermentation may denature the enzyme phytase. The capacity of fermentation to lower phytate levels is therefore optimum up to 48 h[4]. In the present work, the pH was 3.87 after 96 h fermentation and 24 h decanting. After 96 h fermentation, phytase was probably denaturated, inhibited or phytate was inaccessible. The drop of pH under 5 contributed to the slow breakdown of phytate after 48 h, which may explain why all the phytate was not removed[4].

Trypsin and α-amylase inhibitors: Trypsin and α-amylase inhibitors (Table 3) dropped by 42 and 17%, respectively after fermentation. Trypsin inhibitor is a small protein, which inhibits the digestive enzyme trypsin. Hoffman et al.[8] reported the inactivation and proteolysis of trypsin inhibitor protein by *in vitro* microbial fermentation of soybean in rumen fluid. In a previous study, significant decrease of 28% in trypsin inhibitor activity after four days fermentation at 32°C of a corn meal was attributed to microbial degradation of trypsin inhibitors[1]. Kanekar et al.[36] reported a 5-6% decrease in trypsin inhibitor activity after 18 h of fermentation by lactic acid bacteria in soybean seeds. On the other hand, Sharma and Kapoor[9] reported a reduction of 80% in amylase inhibitor units after 48 h of natural fermentation at 30°C of finely ground pearl millet. It should be pointed out that the previous studies[1,8,9] were made on soybean and pearl millet, but the findings in the present study showed the reducing effect of lactic acid fermentation on trypsin and α-amylase inhibitors.

Overall reductions of 54.5, 8.7 and 11.2% occurred in ash, crude protein and fat, respectively, after four days of fermentation. In the same period, 38.2, 69.4, 81.8 and 66% reduction in calcium, thiamin, riboflavin and β-carotene, respectively occurred. A drop of pH close to 4 and a significant reduction of antinutritional factors, which could be beneficial in further utilization and preservation of the finished products, accompanied these nutrient losses. The most important reductions were observed in

Table 3: Antinutrient content of yellow maize as affected by four days traditional fermentation

Elements	Yellow maize	
	Raw	Fermented
Phytates (mg g^{-1} DM)	9.87±0.28*	3.8±0.16 (61.5↓)
Trypsin inhibitors (TIU/mg DM)	3.65±0.28*	2.13±0.19 (41.7↓)
α-amylase inhibitors (AIU/g DM)	917.00±6.6*	765.30±40.1 (16.6↓)

Results are the means of at least 3 determinations±SD, *The mean differences were significant at 95% confidence interval (α≤0.05) through the t test, Figures in parentheses indicate the percent increase↑/decrease↓ of the corresponding element over raw maize where significant differences were demonstrated.

Table 4: Studies on traditional fermentation of maize: percent increase/decrease of ash, protein, fat and carbohydrate and fiber of previous studies, compared to the present study (calculated on a DM basis)

Ref. No.	Maize variety	Fermentation conditions	Elements			
			Ash	Crude protein	Fat	Carbohydrate and fiber
[4]	Yellow maize meal	3:1, w/v at ambient temperature, 48-72 h	70↓	82↓	68↓	39↓
[5]	White maize dough	3:1 w/v 30°C, 48 h (after overnight soaking of grains)	10↓	4↓	6↓	_
[6]	Unknown	Step 1: grains (24-72 h), washing and sieving; Step 2: dough (24-72 h), both steps at 30 to 32°C	82↓	29↓	26↓	_
[7]	Yellow maize	5 days, sieving and washing, temperature not specified	73↓	30↓	61↓	_
Present study	Yellow maize	4 days controlled environment chamber, 1:4 w/v, 26-30°C, 80% relative humidity, 3 washing and sieving	54↓	9↓	11↓	9↑

↓ percent decrease/↑ percent increase of the corresponding elements over raw maize

micronutrients such as calcium and vitamins and in phytates and trypsin inhibitors contents ranging from 38.2 to 81.81% while losses in protein and fat were less than 12%. It could therefore be concluded at a first sight that, at 30°C, the fermentation technique used in Cameroon to process yellow maize needed to be improved in order to avoid the drastic losses of calcium and vitamins recorded. However changing the fermentation technique may not be the best solution for several reasons: 1) the difficulties related to the introduction and acceptance of new technologies at the domestic level, 2) thiamin, riboflavin, retinol equivalent and minerals such as iron and zinc were already low in raw maize despite the losses recorded, 3) the traditional technique allowed the reduction of antinutritional factors, 4) cereals are low in crude protein content[1] and the losses observed were only 8.7% (Table 1).

It is therefore recommended to find inexpensive ways of replacing the lost nutrients or improving the nutritional content of fermented yellow maize, which would be easily implemented at the home level. Supplementation or complementation of the fermented maize with other food sources containing the deficient nutrients, in order to make the final product suitable for human consumption could be a possible approach.

ACKNOWLEDGEMENT

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