

Short communication

**SOYBEAN FORTIFICATION OF MAASA:
A GHANAIAN FERMENTED MILLET-BASED CAKE**

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ABSTRACT

Fortification of commonly consumed cereals with inexpensive plant protein sources such as soybeans has been exploited to improve the protein quality of staple foods through a mutual complementation of their limiting amino acids. In Ghana and other parts of West Africa, millet is used for the processing of many traditional fermented foods including maasa. The purpose of this study was to assess the effect of soybean fortification on the fermentation characteristics and proximate composition of fermented millet dough as well as consumer acceptability of maasa produced from different soy-millet blends. Maasa samples were prepared from a blend of steeped pearl millet grains and pre-soaked, blanched, hand dehulled soybean added at 0, 10, 20, 30 and 40% replacement levels. The millet-soybean blends were wet-milled, formulated into a dough, spontaneously fermented for 14 h and fried into cake known as maasa. During spontaneous fermentation, samples were analyzed for pH, total titratable acidity, microbial counts and proximate composition. Finally, maasa prepared from the fermented millet-soybean blends were assessed for consumer acceptability using a nine point hedonic scale. There was a general decrease in pH from 5.4-5.5 to 3.9-4.1 pH units and an increase in titratable acidity from 0.10-0.30 to 0.58-1.26 (%lactic acid) during the 14 h fermentation period. Lactic acid bacteria and yeast counts reached 9.7 and 8.0 logcfu/g respectively. Crude protein and fat contents increased with the addition of soybeans whereas carbohydrate content reduced. Consumer sensory evaluation showed that fortification with 20% soybean positively affected taste, colour, texture and the overall acceptability of maasa. Therefore, soybean can be used to fortify the Ghanaian millet-based maasa to improve nutrient quality and acceptability of maasa by replacing 20% of the millet with soybeans prior to milling and fermentation.

Keywords: Millet, soybeans, fortification, fermentation, maasa.

INTRODUCTION

Cereal grains serve as an important source of dietary proteins, carbohydrates, vitamins, minerals and fiber. Millet grains, one of such important cereal crops, remains a major source of calories and forms a vital component of the food security in the developing world (FAO, 1996). Throughout Africa, millet is processed into many different staples through fermentation. In Ghana, millet grains serve as the raw material for the processing of various fermented foods such as koko (Lei and Jakobsen, 2004), fura (Owusu-Kwarteng *et al.*, 2012), and maasa (Owusu-Kwarteng and Akabanda, 2013). Maasa is processed and consumed throughout the country, especially in communities of northern Ghana extraction and Muslim dominated areas. Maasa is popularly consumed as an adjunct to breakfast porridges or may be consumed alone as food (Owusu-Kwarteng and Akabanda, 2013).

Despite the widespread use and dependence on millet and other cereals in tropical Africa, they are considered to be

of lower nutritive value due to their low protein content and limitations in certain essential amino acids such as lysine, and also the presence of some antinutritional factors (Chavan and Kadam, 1989). This has therefore necessitated the need to investigate various methods of processing aimed at improving the nutritional quality and acceptability of traditional cereal-based foods. In this light, fortification of the cereals with soybeans (which is high in both proteins and lysine) is one method which has been exploited in many developing countries over the years. During such cereal-legume blends, protein quality of the staple is complimentary enhanced through the contribution of high protein and lysine by the legume and methionine by the cereal (Afoakwa *et al.*, 2002). Several cereal-legume blends for staples foods have been developed with varying degrees of success. Fortification of the Ghanaian fermented maize dough with soybeans has been investigated (Plahar *et al.*, 1997). It was found that the addition of boiled whole soybeans to soaked maize grains before milling and fermentation was the most appropriate and cost-effective technique for household, small-scale and medium scale operations

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(Plahar *et al.*, 1997).

The purpose of this investigation was to assess the influence of soybean fortification on the fermentation characteristics and proximate composition of spontaneously fermented millet dough. Maasa produced from different soy-millet blends at different fortification levels were also evaluated for consumer acceptability. This is geared towards improving nutritional value maasa and therefore the nutritional security of consumers.

MATERIALS AND METHODS

Cereal and legume grains

The local variety of pearl millet (*Pennisetum glaucum*) and *Salinatum* variety of soybeans were both purchased from a local retail outlet in Navrongo market, Ghana. They were cleaned and stored at ambient temperature ($29 \pm 1^\circ\text{C}$) until they were used.

Preparation of traditional unfortified and soy-fortified maasa

The processing of unfortified and soy-fortified maasa is

shown in figure 1. The processing of traditional unfortified maasa involved steeping 2 kg of cleaned millet grains in 3L de-mineralized water for 12 h. After steeping, the millet grains were washed and excess water drained off. After draining, the millet grains were milled using a plate attrition mill (model: No 2A – Amuda grinding mill) to obtain a wet milled dough. The dough samples were then divided into two portions of two-thirds ($\frac{2}{3}$) and one-third ($\frac{1}{3}$). The $\frac{1}{3}$ portion was used to prepare a 30% (w/v) slurry, cooked into a pre-gelatinized meal and mixed with the $\frac{2}{3}$ portion to obtain a thick paste. The mixture was then allowed to spontaneously ferment for 14 h. The fermented paste was fried (in portions of about 100 g) in oil for approximately 5 min to obtain maasa. For soybean fortification, portions of millet grains were replaced with separately weighed soybeans at 0, 10, 20, 30 and 40 % replacement levels. The soybeans were pre-soaked in de-mineralized water for 2 hours and boiled for 20 min to inactivate trypsin inhibitor activity and reduce be any flavor (Plahar *et al.*, 1997). The boiled beans were hand dehulled by rubbing in cold water. The dehulled soybeans were then mixed with the decanted soaked millet grains prior to milling and fermentation (Fig. 1).

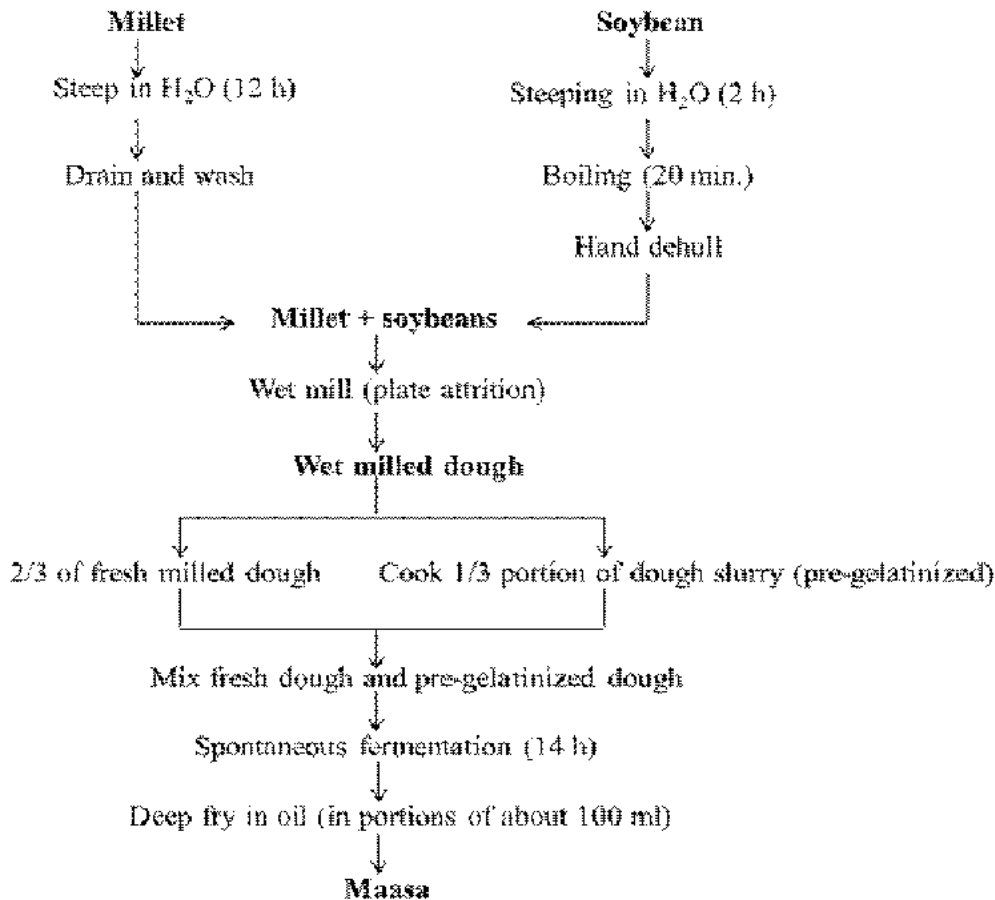


Fig. 1. Processing of soybean fortified maasa.

Determination of dough acidity

Titrateable acidity and pH of unfortified and soy-fortified millet were determined in 10% (w/v) slurry of dough samples. Particles in the slurry were kept in suspension by stirring for 15 min and filtered. Ten milliliter aliquots of the filtrate were then titrated 0.1 N NaOH standard solution to determine the total titrateable acidity, while pH was measured using a pH meter (CrisonBasic, model 20) calibrated with standard buffers. Acidity was expressed as lactic acid based on the conversion of 1 ml of 0.1 N NaOH being equivalent to 9.008×10^{-3} g lactic acid (Annan *et al.*, 2005).

Microbiological analysis

Duplicate 10 g samples of fermenting dough were homogenized with 90ml sterile peptone physiological saline solution (5g bacto-peptone, 8.5g NaCl, 1000ml distilled water, pH 7.0 ± 0.2). The homogenate was serially diluted in the 10^{-9} concentration and 0.1ml aliquots of the dilutions directly inoculated by surface plating on different isolation media. Lactic acid bacteria were isolated on MRS agar (Merck, Darmstadt, Germany). Plates were incubated anaerobically (BBL gas pack, Anaerocult A, Merck) at 30°C for 48h. Aerobic mesophilic bacteria were enumerated on Plate count agar (PCA) (Oxoid Ltd, Basingstoke, Hampshire, England), pH 7.0 and incubated at a temperature of 32°C for 48 h. Sabour and Dextrose Agar (Merck), supplemented with 250mg/100ml chloramphenicol (selective supplement, Oxoid) with pH adjusted to 3.5 with tartaric acid was used for the isolation and enumeration of yeasts. Inoculated plates were incubated at 25°C for 5 days. Following incubation, the number of colony forming units (cfu) per gram of sample was determined using a digital colony counter and recorded.

Proximate analysis of unfortified and soybean-fortified fermented millet dough

Proximate composition on dry matter basis was determined according to the AOAC (2000) methods. Crude protein content was determined using the Kjeldahl method (Method 960.52) ($N \times 5.83$). Crude lipid content was determined using the Soxhlet extraction method (Method 920.39C). Ash content was determined by heating the dried sample in a furnace at 550°C (Method 923.03). Carbohydrate content was calculated by difference.

Consumer sensory evaluation of maasa samples

The final products (maasa) prepared from millet with different levels of soybean fortification were served to a 35 member panel of judges (drawn from the Faculty of Applied Sciences of the University for Development Studies) who are familiar with maasa. The panel evaluated the products for sensory qualities (taste, colour, odour, texture and overall acceptability) using a nine-point hedonic scale (1 and 9 representing extremely dislike and

extremely like respectively). The judges were made to wash their mouths with clean water before and after evaluating each product.

Statistical analysis

All analyses were carried out in duplicates in two independent fermentation trials. Data obtained were subjected to one-way analysis of variance (ANOVA) and means were separated by Tukey's family error rate multiple comparison test ($P < 0.05$).

RESULTS AND DISCUSSION

Dough acidity and microbial changes

Changes in acidity and microbial counts of unfortified and soybean-fortified millet dough is presented in table 1. Differences in pH between unfortified and soybean-fortified millet dough samples were not significant ($p < 0.05$). However, there were significantly higher levels of total acids expressed as percent lactic acid observed in soybean-fortified dough than in the unfortified dough samples. Similar observations of high titrateable acidity in soybean fortified cereals have been observed for fermented millet and maize (Owusu-Kwarteng *et al.*, 2010; Annan *et al.*, 2005; Plaharet *et al.*, 1997). This observation has been attributed to a buffering effect from the higher content of soluble proteins and amino acids contributed by the beans in such soy-cereal blends (Annan *et al.*, 2005; Nche *et al.*, 1994; Plahar *et al.*, 1997, 1983; Zamora and Fields, 1979). Thus, free fatty acids from soybeans may contribute significantly to soybean-fortified fermented millet dough as observed in table 1, where higher levels of total titrateable acids were recorded in soybean-fortified millet than unfortified millet dough samples, even at time zero (0h), when fermentation had not started. In general, similar trends of acidification with fermentation time have been associated with several African fermented cereal-based foods (Owusu-Kwarteng *et al.*, 2012; Sawadogo-Lingani *et al.*, 2007; Vieira-Dalodé *et al.*, 2007; Lei and Jakobsen, 2004; Muyanja *et al.*, 2002; Hounhouigan *et al.*, 1994).

Yeasts counts during millet dough fermentation increased from 4.9-5.4 to 7.6-8.0 log cfu/g after 14 h fermentation period. Counts were similar for both unfortified and soybean-fortified millet dough samples. Lactic acid bacteria counts however, significantly increased with the addition of soybeans (Table 1). An accelerated growth of lactic acid bacteria in fermenting cereals in the presence of amino acids has been reported (Gobetti *et al.*, 1994; Spicher and Schroeder, 1978). Thus, the increased protein content of fermented millet as a result of soybean fortification may have contributed additional amino acids and thereby accelerating the growth of lactic acid bacteria. Additionally, interactions between yeasts and lactic acid bacteria during the production of fermented foods are suggested to involve a 'symbiotic' association due to a mutual growth stimulation based on their amino

Table 1. Acidity and microbial counts during spontaneous fermentation of unfortified and soybean fortified millet dough.*

| Acidity and microbial count | Fermentation time (h) | Level of soybean addition (%), by replacement | | | | |
|-----------------------------|-----------------------|---|------------------------|------------------------|------------------------|------------------------|
| | | 0 | 10 | 20 | 30 | 40 |
| pH | 0 | 5.56±0.05 ^a | 5.50±0.04 ^a | 5.61±0.09 ^a | 5.44±0.13 ^a | 5.58±0.12 ^a |
| | 14 | 4.08±0.07 ^a | 4.01±0.04 ^a | 3.91±0.15 ^a | 3.88±0.11 ^a | 4.03±0.17 ^a |
| TTA (% lactic acid) | 0 | 0.10±0.02 ^a | 0.17±0.04 ^b | 0.21±0.04 ^c | 0.24±0.03 ^d | 0.30±0.04 ^c |
| | 14 | 0.58±0.06 ^a | 0.64±0.05 ^b | 0.85±0.09 ^c | 0.98±0.11 ^d | 1.26±0.08 ^c |
| LAB (logcfu/g) | 0 | 6.9±1.2 ^a | 7.0±1.9 ^a | 6.7±1.4 ^a | 6.9±0.9 ^a | 6.8±1.3 ^a |
| | 14 | 8.0±0.7 ^a | 8.9±1.3 ^b | 9.2±1.9 ^{bd} | 9.4±1.4 ^d | 9.7±1.0 ^c |
| yeast (logcfu/g) | 0 | 5.2±2.3 ^a | 5.0±1.7 ^a | 4.9±1.0 ^a | 5.2±2.1 ^a | 5.4±1.3 ^a |
| | 14 | 7.8±1.5 ^a | 7.6±0.9 ^a | 8.0±1.7 ^a | 7.6±1.3 ^a | 7.7±1.6 ^a |

*Values are means of duplicate determinations from two independent fermentation trials. Means with the same superscript in a row are not significantly different ($P < 0.05$)

Table 2. Proximate composition of unfortified and soybean fortified spontaneously fermented millet dough (dry matter basis)*

| Soybean addition (%), by replacement | Protein | Fat | Ash | Fiber | Carbohydrates |
|--------------------------------------|----------|---------|---------|---------|---------------|
| 0 | 11.2±0.4 | 4.9±0.7 | 1.6±0.2 | 4.2±0.5 | 77.8±0.7 |
| 10 | 14.6±0.6 | 5.2±1.2 | 2.1±0.1 | 3.6±0.3 | 74.5±0.6 |
| 20 | 18.4±0.5 | 5.7±0.4 | 2.4±0.3 | 3.1±0.3 | 70.3±0.8 |
| 30 | 22.0±1.1 | 6.1±0.4 | 2.6±0.2 | 2.5±0.6 | 66.8±0.8 |
| 40 | 25.5±0.9 | 6.2±0.2 | 2.9±0.2 | 2.1±0.4 | 63.4±0.5 |

*Values are means of duplicate determinations from two independent fermentation trials.

acids and carbohydrate metabolisms (Martinez-Anaya *et al.*, 1990; Wood and Hodges, 1985). Thus, in a co-metabolism between yeasts and lactic acid bacteria, the bacteria provide the rapid acidic environment, which selects for the growth of yeasts, whereas the yeasts provide essential metabolites such as pyruvates, vitamins and amino acids to the bacteria (Gadaga *et al.*, 2001; Steinkraus, 1996; Gobetti *et al.*, 1994; Leroi and Pidoux, 1993) and thereby accelerating the growth of the lactic acid bacteria. The faster growth of microorganisms and the accelerated acid production could be important in reducing fermentation time as well as positively affect safety and shelf stability of the product.

Proximate analysis

Crude proteins, fats and ash contents increased with the addition of soybeans whilst carbohydrates decreased (Table 2). The addition of 20% soybeans by replacement resulted in about 60.8% increase in protein content whilst the addition 30% soybeans by replacement resulted in a 100 % increase in the protein content of soybean-millet blends for maasa production.

Several studies have previously reported increased protein content in soybean fortified cereals and tubers, which make a very significant contribution towards the

alleviation of protein-energy malnutrition (Kolapo and Sanni, 2005; Plahar *et al.*, 1997, 1983; Sanni and Sobamiwa, 1994; Nout, 1993). Although soybean is known to contain some anti-nutritional factors which inhibit the availability of the desirable elements such as protein, these anti-nutritional factors can be destroyed through boiling and other processing methods (Loo, 1978; Enwere, 1998; Osho and Dashiell, 1998). Therefore, the incorporation of soybeans in the processing of maasa a cereal-based food of the rural poor and underprivileged communities in Ghana, will greatly contribute towards efforts aimed at alleviating protein-energy malnutrition, as animal protein is beyond the reach of many of these people.

Consumer sensory analysis of maasa samples

Consumer sensory evaluation of maasa produced from different soybean- millet blends is shown in Table 3. The addition of boiled whole soybeans to millet for maasa preparation did not significantly taste, texture and overall acceptability at 10 and 20% fortification levels but were significantly and negatively affected beyond 20% soybean fortification level.

The odor was negatively affected whereas color improved with the addition of soybeans. Legumes such as soybeans

Table 3. Consumer sensory evaluation of unfortified and soy-fortified millet based maasa.

| Soybean addition(%) by replacement | Taste | Odour | Colour | Texture | Overall acceptability |
|---------------------------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| 0 | 5.8±1.6 ^a | 7.1±1.5 ^a | 3.8±2.2 ^a | 5.1±1.2 ^a | 5.8±0.8 ^a |
| 10 | 5.5±1.4 ^a | 6.0±1.2 ^b | 4.5±1.6 ^b | 5.3±1.9 ^a | 6.1±1.3 ^a |
| 20 | 5.7±1.6 ^a | 6.3±0.9 ^b | 5.2±2.0 ^c | 5.0±1.7 ^a | 5.9±1.0 ^a |
| 30 | 3.3±1.1 ^b | 3.5±1.7 ^c | 5.1±1.5 ^c | 4.3±1.2 ^c | 4.1±1.5 ^b |
| 40 | 2.8±1.8 ^b | 2.9±1.2 ^c | 5.5±1.1 ^c | 3.5±2.0 ^d | 3.3±0.7 ^c |

Means with same superscript in a column are not significantly ($p < 0.05$).

used in foods to enhance nutritional and functional qualities have often been found to alter the organoleptic qualities of the food. However, the addition of boiled whole soybeans at 20% fortification level prior to milling and fermentation was found to be appropriate for maasa processing and accepted by consumers.

CONCLUSION

Soybean fortification accelerates the production of total acids and the growth of lactic acid bacteria during the fermentation of millet dough to produce maasa. This is significant in safety and stability since accelerated acid production contributes to the inhibition of pathogens and spoilage microorganisms. Again, protein quality of soy-millet blends improved with the addition of soybeans, and maasa produced by fortification of millet with 20% soybeans replacement level, prior to milling and fermentation was found to be most acceptable to consumers.

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