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# Effect of Natural Fermentation on the Chemical and Nutritional **Composition of Fermented Soymilk Nono**

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#### ABSTRACT

Changes in the chemical and nutritional composition of naturally fermented soy nono were studied at ambient temperature ( $27 \pm 2^{\circ}$ C) for 72 h. The differently fermented soy *nono* samples were collected at 6 h intervals and analysed for chemical, proximate and mineral composition using standard laboratory procedures. Biochemical changes in the fermenting soy nono showed a drop in pH from 6.90 to 4.09 while titratable acidity (lactic acid equivalent) increased from 0.42 to 1.82% after 72 h of natural fermentation. The moisture, carbohydrate and fat contents decreased from 93.45 to 92.70, 1.52 to 0.60 and 2.18 to 0.87 % respectively while total solids, ash and protein contents increased from 6.55 to 7.30, 0.23 to 0.74 and 2.62 to 5.09 % respectively. Results reveal that the calcium, iron and magnesium contents in fermenting soymilk increased from 52.86 to 71.43, 28.00 to 40.00 and 7.66 to 8.87 mg/l respectively within time intervals of 0 to 54 h and then decreased to 65.00, 28.00 and 7.83 mg/l respectively till the end of fermentation period while the zinc content increased from 4.42 to 6.75 mg/l throughout the fermentation period. It was evident that there was increase in protein, calcium, magnesium, zinc and iron contents during natural fermentation of soymilk.

Keywords: Fermentation, nono, soymilk, soybean.

#### Introduction

Soymilk is the rich creamy liquid extract of soybean (Tunde-Akintunde and Souley, 2009) and is the most available soy product. Soymilk is a popular nutritive alternative to cow's milk and is even cheaper (Soya-Agrodok, 2005). Soymilk has become a very interesting food due to its extraordinary nutritive value and health characteristics. It is a very rich source of highly valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibres, and isoflavones whose presence in everyday diet is very important (Boani, 2006). In some countries, soymilk is intended for the population who cannot digest milk due to lactose intolerance, allergy to

milk proteins, or vegetarian way of diet. Soymilk has limited consumer acceptability due to its undesirable beany flavour. However, its acceptability can be enhanced by the modification of its processing methods. Some of the modifications of coldwater extraction of soymilk include application of heat, soaking of soybean in ethanol or alkali and acid grinding. Several researchers have tried to ferment soymilk using either pure or mixed cultures of the following bacteria: Lactobacillus cellobiosis, L. plantarum, L. fermentum, L. delbrueckii, L. fermenti, L. pentosaceus and L. bulgaricus to improve its acceptability due to reduction in objectionable flavour and oligosaccharides such as starchyose and raffinose that cause flatulence (Mital and Steinkraus, 1975; Pinthong et al., 1980; Buono et al., 1990; Wang et al., 2002). Akabanda et al. (2010) reported that fermentation of soymilk provides a possibility for modifying or improving its flavour and texture

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so that it becomes more acceptable and can also lead to new types of soy products that resemble cultured dairy products.

Fermented milk products are sour-tasting milk which has been made by either fermenting the milk naturally or by the use of starter culture to produce the desirable milk products. Such fermented milk in Africa, Syria, India, America and Nepal include: Cheese, nono, buttermilk, yoghurt, irgo, kadam, laban, shenineh, dahi, shirkand, mahi, etc. (Ajayi, 2006). Nono is the Hausa name for naturally fermented thick milk product sold in Northern Nigeria. It is usually prepared from cow's milk but occasionally from goat's milk (Atanda and Ikenebomeh, 1991). To prepare nono, fresh milk is left to ferment in a covered calabash at room temperature. The fermentation of nono may be brought about by a number of different bacterial species from various sources that naturally contaminate the fresh milk (Eka and Ohaba, 1977). Microorganisms that have been reported to be implicated in the fermentation of nono include Lactobacillus acidophilus, Streptococcus cremoris, Micrococcus aureus and Streptococcus lactis (Akinyanju, 1989). Soymilk yoghurt fermented with starter cultures have been greatly researched recently because their nutritional attributes may be changed due to the metabolism of microorganisms. This study therefore evaluates changes that occur during the natural fermentation of soymilk for soy nono.

## Materials and Methods Sample collection and preparation of soymilk

A bulk of healthy seeds of soybean (*Glycine max*) used for this study was purchased from Lanfenwa market in Abeokuta, Ogun State. These seeds were sorted manually to remove stones, damaged and immature seeds. The clean soybean seeds were soaked in tap water at room temperature (28°C) in a 2.0 l beaker for 18 h. The soaked water was drained from the soybean and beans thereafter were blanched at 98°C in boiling distilled water for 2 min and dehulled manually to remove their testa. They were placed in a blender and 1.0 l of boiled water at 87 – 90°C was added before blending for

3 min. The boiled water inactivates the enzyme, lipoxygenase during blending (Wilkens *et al.*, 1967). The slurry was filtered through two layers of 50 mesh cheese cloth. The resulting soymilk was boiled at  $100^{\circ}$ C for 5 min and thereafter held at  $5^{\circ}$ C.

## Fermentation of soymilk

Freshly prepared soymilk was dispensed into clean, dry conical flasks. The samples were loosely covered with aluminum foil to keep out dust and insects, while allowing air circulation. The samples were then left on the bench to ferment at room temperature at  $27 \pm 2^{\circ}$ C for 72 h.

#### Sample preparation

Fermented samples were collected at 6 h intervals until the end of fermentation period of 72 h for analyses.

#### Analytical procedures

#### Chemical analysis

The pH value was determined at 0 h immediately after production. It was done by taking 10 g from each of the fermenting soy *nono* with 100 ml of distilled water. The mixture was left at room temperature for 30 min. The pH of the supernatant was then measured with a pH meter. Titratable acidity was determined by dissolving 10 g of the sample in 100 ml of distilled water and titrating 10 ml aliquots with 0.1 N NaOH to phenolphthalein end point.

## Proximate composition

Samples of fermented and unfermented soy *nono* were analyzed for the following parameters, total solid, fat, protein, moisture and ash content using the methods of the Association of Official Analytical Chemists (2000). The carbohydrate content was determined by difference between 100 and total sum of the percentage of moisture, protein, fat and ash (AOAC, 1990).

## Nutritional composition

The mineral contents of the fermenting soymilk were determined by the procedure of AOAC (2000). Magnesium, calcium, iron and zinc were determined using the Atomic Absorption Spectrometer

(Thermo Scientific S Series Model GE 712354) after digestion with a perchloric-nitric acid mixture (AOAC, 2000). Prior to digestion, 0.50 g of soymilk samples were weighed into a 125 ml Erlenmeyer flask with the addition of perchloric acid (4 ml), concentrated HNO<sub>3</sub> (25.00 ml) and concentrated sulphuric acid (2.00 ml) under a fume hood. The contents were mixed and heated gently in a digester (Buchi Digestion unit K-424) at low to medium heat on a hot plate under perchloric acid fume hood and heating was continued until dense white fume appeared. Heating was continued strongly for half a minute and then allowed to cool, followed by the addition of distilled water (50.00 ml). The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was read on the Atomic absorption spectrometer.

# Statistical analysis

Mean values of duplicate determinations were reported with their standard deviations. Analyses of Variance (ANOVA) were achieved to calculate significant differences in the treatment means, and the mean separations were achieved by Duncan's Multiple Range Test (p 0.05).

# **Results and Discussion**

The effect of natural fermentation period on soymilk at 0 h was 6.90 and it was similar to pH value reported by Omojasola (2000), Favaro et al. (2001), Oluwabamiwa and Kolapo (2007) and Namrata and Gurmukh (2007), but was lower than pH of 7.2 reported by Osundahunsi et al. (2007). The pH value decreased to 4.09 at the end of fermentation period after 72 h (Figure 1). This was very close to the value of 4.81 reported by Oluwabamiwa and Kolapo (2007) when soymilk was fermented by commercial starter culture. Though the pH of all yoghurt made by soymilk decreased with fermentation time, however, the differences observed in the pH decrease in the fermenting yoghurt pre-mix might be a reflection of the ability of the yoghurt bacteria to grow in the pre-mix and ferment the carbohydrates they contained. Results from the present study revealed that fermentation by commercial starter culture brought about greater reduction in pH value. This was closely followed by starters from cow's milk while starters from soymilk brought about the least pH reduction.

Titratable acidity (TTA) is the amount of acid present from titration with an alkali. Titratable acidity of the fermented soy *nono* ranged from 0.42 to 1.82% while the highest value was recorded (1.82) at 72 h of fermentation. The lactic acid contents obtained in this study compared favourably with the values (0.17 - 1.16) reported in the earlier studies (Oluwabamiwo *et al.*, 2007; Jimoh and Kolapo, 2007). Acidity developed in the different fermented soy products produced depended on the culture for the fermentation. Present results further corroborate the earlier report of Tuitemwong and Tuitemwong (2003) who observed that LAB from different sources was different in their efficiencies to ferment soymilk.

Results of the present study showed that there was a fall in the pH and increase in Total Titratable Acid (TTA) content of fermented soy *nono*. This trend in decrease in pH and increase in TTA was also reported by several researchers (Gesinde *et al.*, 2008; Almeida *et al.*, 2007) which could be attributed to the accumulation of some organic acid and acetic acid resulting from the activities of some fermentative organisms such as lactic acid bacteria and yeasts in the fermenting foods.

The effect of fermentation on the proximate composition of fermenting soymilk is presented in Table 1. It was observed that there was decrease in moisture, carbohydrate and fat contents while an increase was observed in total solids, ash and protein contents of fermenting soymilk. During the natural fermentation of soy *nono* for 72 h, the moisture content decreased from 93.45 % at 0 h to 92.50 % at 54 h and then increased gradually to 92.70 % at 72 h of fermentation. The initial moisture content falls within the range and is similar to reports of Namrata and Gurmukh (2007), Tunde-Akintunde and Souley (2009) and

Orhevba (2011). The moisture content decreased to 92.50% at 54 h of fermentation and then later peaked to 92.70 at the end of fermentation period (72 h). The decrease observed in moisture content as fermentation time progressed could be due to increased dry matter content as a result of microbial cell proliferation. Decrease in moisture content was also reported by Omojasola (2000) in soymilk fermented for 30 h. As fermentation time increased, moisture content decreased while the total solid content in fermenting soymilk increased. Morris *et al.* (2004) reported that decrease in moisture generally increased the concentration of nutrients. The carbohydrate content recorded in this study decreased significantly (p < 0.05) from 1.52% at 0 h to 0.60% at 72 h of fermentation. The decrease observed in the carbohydrate content of fermenting soymilk as fermentation period increased could be explained by the activities of the fermenting microorganisms which utilized and transformed them into energy for growth and other cellular activities. Osundahunsi *et al.* (2007) also reported a decrease in carbohydrate content of soy-yoghurt fermented with starter cultures.

Table 1: Changes in proximate composition of soymilk nono during natural fermentation

| Time (hr)   | Total solid         | Moisture             | Ash content                 | Fat content                 | Protein content   | Carbohydrates        |
|-------------|---------------------|----------------------|-----------------------------|-----------------------------|-------------------|----------------------|
|             |                     | content              |                             |                             |                   |                      |
| 0           | $6.55 \pm 0.07^{a}$ | $93.45 \pm 0.07^{a}$ | $0.23 + 0.14^{a}$           | $2.18 \pm 0.01^{f}$         | $2.62 + 0.14^{a}$ | $1.52 \pm 0.14^{d}$  |
| 6           | $6.55 \pm 0.21^{a}$ | $93.45 + 0.35^{a}$   | $0.25 + 0.02^{ab}$          | $2.13 \pm 0.01^{f}$         | $2.75 + 0.07^{a}$ | $1.37 + 0.01^{d}$    |
| 12          | $6.70 \pm 0.42^{a}$ | $93.30 + 0.28^{a}$   | $0.26 + 0.01^{abc}$         | $2.08 \pm 0.00^{\text{f}}$  | $3.12 + 0.02^{a}$ | $1.23 + 0.14^{d}$    |
| 18          | $6.75 \pm 0.41^{a}$ | $93.25 + 0.14^{a}$   | $0.56 + 0.14^{abcd}$        | $1.43 + 0.14^{de}$          | $3.75 + 1.41^{a}$ | $.01 + 0.00^{bc}$    |
| 24          | $6.90 + 1.41^{a}$   | $93.05 + 0.07^{a}$   | $0.57 + 0.2^{8abcd}$        | $1.43 + 0.28^{de}$          | $4.00 + 0.14^{a}$ | $0.90 + 0.14^{abc}$  |
| 30          | $7.10 + 1.41^{a}$   | $92.90 + 1.41^{a}$   | $0.58 + 0.28^{\text{abcd}}$ | $1.48 + 0.00^{ce}$          | $4.15 + 1.41^{a}$ | $0.89 + 0.00^{ab}$   |
| 36          | $7.20 + 1.41^{a}$   | $92.80 + 1.41^{a}$   | $0.60 + 0.14^{abcd}$        | $1.25 + 0.70^{\text{bcde}}$ | $4.43 + 1.42^{a}$ | $0.87 \pm 0.14^{ab}$ |
| 42          | $7.25 + 1.41^{a}$   | $92.75 + 1.41^{a}$   | $0.65 + 0.07^{bcd}$         | $1.20 + 0.14^{bcd}$         | $4.53 + 0.00^{a}$ | $0.87 + 0.00^{ab}$   |
| 48          | $7.27 + 1.41^{a}$   | $92.73 + 1.41^{a}$   | $0.67 + 0.14^{d}$           | $1.09 + 0.00^{abc}$         | $4.72 + 0.70^{a}$ | $0.79 + 0.14^{ab}$   |
| 54          | $7.50 + 1.41^{a}$   | $92.50 + 0.07^{a}$   | $0.70 + 0.14^{d}$           | $1.11 + 0.00^{ab}$          | $4.96 + 1.41^{a}$ | $0.73 + 0.28^{ab}$   |
| 60          | $7.35 + 0.21^{a}$   | $92.60 + 0.00^{a}$   | $0.71 + 0.14^{d}$           | $1.08 + 0.00^{ab}$          | $4.96 + 1.41^{a}$ | $0.65 + 0.14^{a}$    |
| 66          | $7.35 + 1.41^{a}$   | $92.65 + 1.41^{a}$   | $0.71 + 0.28^{d}$           | $1.01 + 0.00^{ab}$          | $5.00 + 1.41^{a}$ | $0.63 + 0.14^{a}$    |
| 72          | $7.30 + 1.41^{a}$   | $92.70 + 1.41^{a}$   | $0.74 + 0.14^{d}$           | $0.87 + 0.14^{a}$           | $5.09 + 2.82^{a}$ | $0.60 + 0.14^{a}$    |
| $\pm$ S.E.M | 0.17                | 0.16                 | 0.04                        | 0.09                        | 0.24              | 0.06                 |

Values with different superscript along the column differ significantly by Duncan's multiple range test at 5% level of significance

\* Results are in %

\*\* S.E.M: Standard error of mean

! by difference

| Time (hr) | Energy Kcal/100g   | Calcium Mg/l              | Iron Mg/1                   | Magnesium Mg/l      | Zinc Mg/l           |
|-----------|--------------------|---------------------------|-----------------------------|---------------------|---------------------|
| 0         | $36.18 + 1.41^{a}$ | 52.86 +1.41ª              | 28.00 + 1.41a               | $7.66 + 1.41^{a}$   | $4.42 + 1.41^{a}$   |
| 6         | $38.85 + 2.82^{a}$ | $52.86 + 2.82^{a}$        | $32.00 + 1.41^{ab}$         | $7.91 + 2.82^{a}$   | $4.65 + 141^{a}$    |
| 12        | $36.21 + 1.41^{a}$ | 58.57 +1.41 <sup>ab</sup> | $32.00 + 1.41^{bc}$         | $8.00 + 1.41^{a}$   | $5.35 + 1.41^{a}$   |
| 18        | $31.91 + 1.41^{a}$ | $58.57 + 4.24^{ab}$       | $32.00 + 2.82^{bcd}$        | $8.13 + 1.41^{a}$   | $5.58 + 0.00^{a}$   |
| 24        | $32.47 + 2.82^{a}$ | $60.00 + 2.82^{ab}$       | $34.00 + 2.82^{ab}$         | $8.16 + 2.82^{a}$   | $5.58 + 0.00^{a}$   |
| 30        | $33.48 + 1.42^{a}$ | $60.14 + 0.00^{a}$        | $36.00 + 1.41^{ab}$         | $8.24 + 1.41^{a}$   | $6.05 + 0.00^{a}$   |
| 36        | $32.90 + 1.41^{a}$ | $67.14 + 1.41^{bc}$       | $36.00 + 1.41^{cd}$         | $8.25 + 0.00^{a}$   | $6.12 + 1.41^{a}$   |
| 42        | $32.40 + 1.41^{a}$ | $67.77 + 1.41^{bc}$       | $37.00 + 1.41^{cd}$         | $8.36 + 1.41^{a}$   | $6.28 + 1.41^{a}$   |
| 48        | $31.85 + 1.41^{a}$ | $68.43 + 1.41^{bc}$       | $37.00 + 1.41^{cd}$         | $8.47 + 1.41^{a}$   | $6.28 + 2.82^{a}$   |
| 54        | $32.75 + 1.41^{a}$ | 71.43 + 1.41c             | $40.00 + 1.41^{d}$          | $8.87 + 2.82^{a}$   | $6.28 \pm 0.00^{a}$ |
| 60        | $32.16 + 1.41^{a}$ | $65.71 + 1.41^{bc}$       | $38.00 + 0.00^{\text{bcd}}$ | $8.00 + 1.41^{a}$   | $6.74 + 1.41^{a}$   |
| 66        | $31.61 + 1.41^{a}$ | $65.00 + 1.41^{bc}$       | $32.00 + 1.41^{ab}$         | $7.87 + 1.42^{a}$   | $6.75 + 0.00^{a}$   |
| 72        | $30.59 + 1.41^{a}$ | $65.00 + 0.00^{bc}$       | $28.00 + 1.41^{a}$          | $7.83 \pm 0.00^{a}$ | $6.75 + 1.41^{a}$   |
| ± S.E.M   | 0.75               | 1.45                      | 0.77                        | 0.26                | 0.24                |

Table 2: Nutritional changes in soymilk nono during natural fermentation

Values with different superscript along the column differ significantly by Duncan's multiple range test at 5% level of significance

\*\* S.E.M: Standard error of mean

! by difference

Fat content as reported in this study varied from 2.18 - 0.87%. Fat content of soymilk was found to decrease as fermentation time increased and this might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components (triacylglycerol) into fatty acid and glycerol. The fatty acids were reported by Astuti *et al.* (2000) to be used as sources of energy by some microorganisms such as moulds resulting in lower fat content in fermenting soymilk at the end of fermentation.

This result shows an increase in protein content of soymilk (2.62% - 5.09%) as it was being fermented for the time under consideration. The improvement reported in protein content of fermenting soymilk as compared to soymilk might be due to some anabolic processes leading to polymer build-up or due to microbial cell proliferation.

Ash content of fermenting soymilk ranged from 0.23 - 0.74%. The ash content increased as fermentation period increased. The higher ash content of fermenting soymilk compared with the unfermented soymilk could be due to reduction of certain chemical components such as carbohydrate, moisture and fat as reported earlier in this study.

Soymilk is rich in minerals such as calcium, iron, magnesium and zinc. The value of calcium, iron, magnesium and zinc contents in the fermenting soymilk ranged from 52.86 to 65.00, 28.00 to 28.00, 7.66 to 7.83 and 4.42 to 6.75 mg/l respectively. The calcium, iron and magnesium contents in soymilk increased with increase in natural fermentation period up to 54 h and then decreased to 72 h (Table 2). The zinc contents in soymilk increased sharply from the beginning until they reached a constant value at 42 h (6.28 mg/l) and then increased till

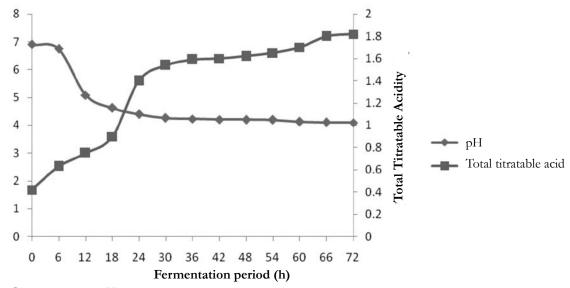


Fig. 1: Changes in the pH and total titratable acidity of fermenting soy nono during natural fermentation

the end of natural fermentation period. Gabriel et al. (2011) reported increase in some mineral contents of Jack beans as affected by the use of Mould starter cultures for fermentation. The increase in the mineral contents of fermenting soymilk compared to unfermented soymilk was an indication that these minerals were released from chelated complex compound through the activities of microorganisms responsible for the fermentation (Gabriel, 2002). The decrease in the magnesium, iron and calcium contents of fermenting soymilk towards the end of fermentation time could be an indication that certain organisms utilize them for their growth and metabolism (Hassan et al., 2005). Fermentation improves the nutrient composition of fermented soy nono as there was an increase in protein, ash and mineral contents of soymilk at the end of fermentation (72 h). This product is superior to cow's milk, being inexpensive, lower in cholesterol and rich in substantial amounts of protein attributes that are important to poor nursing mothers and therefore suggest possible use of fermented soy nono as a remedy to solve the menace of protein-energy malnutrition in developing countries.

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