Quality Assurance and Safety of Crops & Foods, 2015; 7 (4): 441-447

Wageningen Academic Publishers

Nutritional quality and physico-chemical changes during breadfruit (*Artocarpus altilis*) natural fermentation

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Received: 11 October 2013 / Accepted: 24 April 2014

RESEARCH ARTICLE

Abstract

Breadfruit *(Artocarpus altilis)* being a nutritious, staple food with high economic importance is usually subjected to fermentation in order to preserve it, improve its flavour and digestibility, amongst other benefits. In order to provide information on the nutritional and physicochemical changes during the fermentation of breadfruit, this study was carried out. Breadfruits were subjected to solid and submerged state fermentation for 120 h (5 days) and analysed at intervals (24 h) for physicochemical and nutritional changes: pH, total titrable acidity, moisture content, mineral content, protein, total starch, total soluble sugar and total reducing sugar. The protein content increased during the first 72 h and decreased during the last 48 h of the solid state fermentation. There was a 51.67% decrease in the moisture content, 0.68% decrease in total soluble sugar, 45.90% decrease in the total reducing sugar and 41.35% decrease in the starch content of the breadfruit at 96 h of solid state fermentation. There was also an increase in the total titrable acidity content of the fermenting breadfruit during solid state fermentation and during submerged fermentation. For minerals, potassium and sodium increased at 72 and 48 h, respectively. The submerged state fermentation process caused a decrease in the concentration of potassium (3.69-1.35 mg/100 g) and iron (0.82-0.72 mg/100 g) after 120 h. The contents of minerals in mg/100 g were 0.07 for manganese, 0.76 for iron, 0.004 for copper and 0.04 for zinc at 48 h for submerged fermented breadfruit. In conclusion, solid state fermentation gave breadfruit more desirable chemical parameters than submerged state fermentation.

Keywords: breadfruit, fermentation, protein, minerals, total soluble sugar

1. Introduction

Breadfruit (*Artocarpus altilis*) is a multipurpose tree crop that is primarily used for its nutritious, starchy fruit (Ragone, 1997). Though breadfruit is grown in almost 90 countries, it is still considered as an underutilised crop (Deivanai and Subhash, 2010). The fruit has been described as an important staple food with high economic value (Soejipto and Lubis, 1981). Breadfruit tree belongs to the family Moraceae and the genus *Artocarpus* has about 50 species and it is reported that the genus is exotic to Nigeria. Breadfruit is a high yielding tropical food plants having from less than 100 to more than 600 fruits per tree with average yields of 150-250 fruits or 160-500 kg per year (Ragone, 2006).

Breadfruit is rich in carbohydrate, vitamins, fibre and protein (Dignan *et al.*, 2004). Ripe fruit, especially yellow-fleshed varieties, can be a good source of provitamin-A carotenoids (Ragone 2009). Breadfruit is a nutritious, high-energy food with moderate glycemic index, rich and a good source of potassium, magnesium and calcium, with small amounts of riboflavin, niacin, and iron. The fat content of breadfruit is low and can be used in low calorie diets (Ragone, 2009). Compared with other staple starch crops, it is a better source of protein than cassava and is comparable to sweet potato and banana (Graham and Negron de Bravo, 1981). The fruit is a very useful substitute to roots crops and this has been a traditional practice among people in the South-western part of Nigeria (Fasasi *et al.*, 2004). Due to its high amount of carbohydrate, it can easily replace such carbohydrate-rich fruits like banana, though its hydrolysable carbohydrates are thought to be higher (Parkison, 1984). The breadfruit pulps are made into various dishes. It can be pounded, fried, boiled or mashed to make porridge (Amusa *et al.*, 2002 and Ragone, 1997).

Fermentation is one of the oldest methods used to preserve this fruit (Ragone, 1997). It is one of the oldest methods of food preparation and preservation (Campbell-Platt, 1994). Fermentation leads to the enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins (Steinkraus, 1997).

In Micronesia, Western Pacific and in several other countries, fermented breadfruit is produced using pit fermentation technologies. This fermentation process involves placing peeled, cored breadfruit in a pit lined with breadfruit leaves (Ragone, 1997). Despite the economic and social importance of breadfruit fermentation in Western Pacific Islands and several other countries in Africa including Nigeria, only limited information is available on the nutritional content and physico-chemical properties of the solid state and submerged fermented breadfruit considering the numerous advantages of fermentation processes. This research was therefore aimed at evaluating the changes in the nutritional and physicochemical characteristics of breadfruit during solid and submerged state natural fermentation.

2. Materials and methods

Matured breadfruits purchased from a local market in Abeokuta, Ogun State, Nigeria and a batch of the breadfruit was subjected to solid state fermentation. The whole breadfruit was washed, peeled, cored and cut up to an average length of 3-5 cm. The breadfruit tissue was grated and wrapped in a sack to undergo natural fermentation for 120 h (Figure 1) at room temperature (27±2 °C). Samples from the naturally fermenting breadfruit were aseptically collected at 24 h intervals into sterile bottles and subjected to physico-chemical and nutritional analyses, another batch of breadfruit was subjected to submerged state fermentation: the whole breadfruit was washed, peeled, cored and cut up to an average length of 3-5 cm.

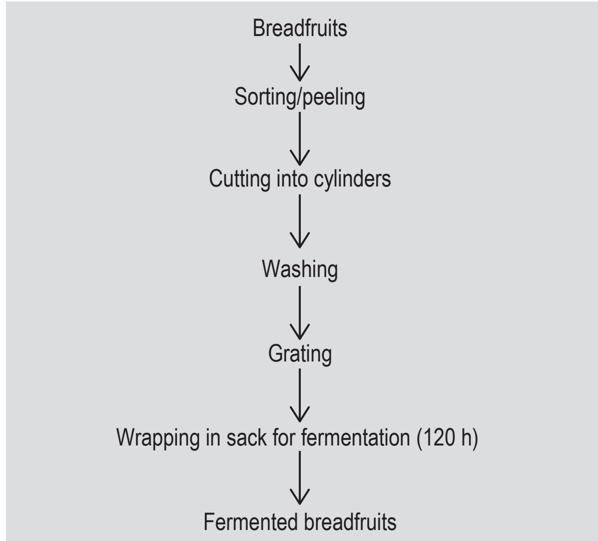

Figure 1. Flow chart for preparation of submerged fermentation of breadfruit.

Breadfruits (2.60 kg) were soaked in sterile water (4,000 ml) and allowed to undergo natural fermentation for 120 h at room temperature (27±2 °C). Samples from the fermenting breadfruit were aseptically collected at 24 h intervals into sterile bottles and subjected to physico-chemical and nutritional analyses. All experiments were conducted in triplicates.

The pH values of the samples were measured, and the total titrable acidity values were determined at 24 h intervals of fermentation according to the AOAC (2000) method. The temperature change during the fermentation was taken using a thermometer inserted into the fermenting water. The thermometer was sterilised by swabbing with 95% ethanol prior to insertion.

The moisture content, protein content and mineral content was determined AOAC (2000). Magnesium, calcium, potassium, copper, iron and zinc were determined using the atomic absorption spectrometer (iCE 3300 Series; Thermo Scientific, Franklin, MA, USA).

A modified method of Southgate (1976) was employed in extracting the sugars, the total sugars in the clarified extract was then determined by the method of Dubois *et al.* (1956). The absorbance of the yellow orange colour that developed during this process was measured at 490 nm using a spectrophotometer (Cecil 2483; Cecil Instruments Ltd., Cambridge, UK). Distilled water subjected to the same treatment was used as blank. The amount of sugar was determined by reference to a standard curve based on known concentration of glucose. The standard curve was prepared with glucose following the procedure employed by Ayoola (1985).

The starch of the breadfruit was extracted and hydrolysed. The sugars which were produced as a result of the hydrolysis of the starch content by the perchloric acid were determined using phenol sulphuric acid method of Dubois *et al.* (1956). The amount of starch was estimated by multiplying the weight of sugars read from the glucose curve by 0.9.

The sugar extract was produced by homogenizing five grams of the fermenting breadfruit tissues with 25 ml distilled water. The homogenate was mixed and filtered through a glass wool. Further solid materials were removed by centrifuging (10,000 rpm) for 20 min. The clear supernatant which was decanted served as the sugar extract. The reducing sugar contents were determined by reference to a standard curve based on known concentration of maltose. Triplicate analyses were carried out in all cases.

Titratable acidity was determined using the standard method described by Amoa-Awua *et al.* (1996). The amount of acid (as lactic acid) in the sample was determined by using the formula in Equation 1 (Kimaryo *et al.*, 2000).

$$\text{Lactic acid (mg/ml)} = \frac{V \times N \times 0.009}{W} \quad (1)$$

Where V is the volume of the base used, N is the normality of alkali, and W is the sample weight.

Statistical analysis

One way analysis of variance (ANOVA) was performed to test for the significance of differences between sample means. Duncan's multiple range test was applied to separate the significantly different means of all the parameters (Duncan, 1955). SPSS version 16.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis.

3. Results and discussion

pH and titratable acidity

The pH of the submerged breadfruit decreased from 6.57 during the first day of fermentation to 4.77 at 96 h of fermentation and the continued acidification of the broth brought the pH to 4.65 at the end of the fermentation period. The low pH of fermented breadfruit is therefore a key factor for safety and adequate preservation of the final product (Steinkraus, 1994). There was also an increase in the total titrable acidity content of the fermenting breadfruit during solid state fermentation and during submerged fermentation. This trend of decrease in pH and increase in total titrable acidity have been reported by several researchers (Oyewole, 2003) and could be attributed to the activities and accumulation of some fermentative organisms such as lactic acid bacteria and yeasts during the fermentation process (Almeida *et al.*, 2007). Analyses reveal that the pH value of the solid state fermented breadfruit was lower than that of the submerged fermented and likewise, the total titrable acidity was higher during solid state fermentation than during submerged fermentation (Figure 2 and 3). This may be attributed to increase in the surface area of the fermenting breadfruit which enabled the growth of lactic acid bacteria and also the microbial load of the steeping water during submerged fermentation.

Moisture content

The moisture content of the fermenting breadfruit decreased significantly from 66.30 to 49.77% within the 120 h of fermentation for the solid state fermenting breadfruits (Table 1) and increased significantly to 83.30% for submerged fermented during the same period (Table 2). The moisture increase observed during submerged fermentation was probably caused by the combined effects of dry matter consumption and water production during aerobic and anaerobic catabolism by microorganisms as earlier described (Hounhouigan *et al.*, 1993) and as a result of the steeping of breadfruit in water during the fermentation.

Total starch content

Changes in the starch contents of breadfruit during the solid state and submerged fermentation periods are shown in Table 1 and 2. The starch contents decreased from 67.44 at the beginning to 36.89% at the end of fermentation (120 h) for the solid state fermenting breadfruit. There was a decrease in the starch content of the breadfruit during the solid state fermentation process than during

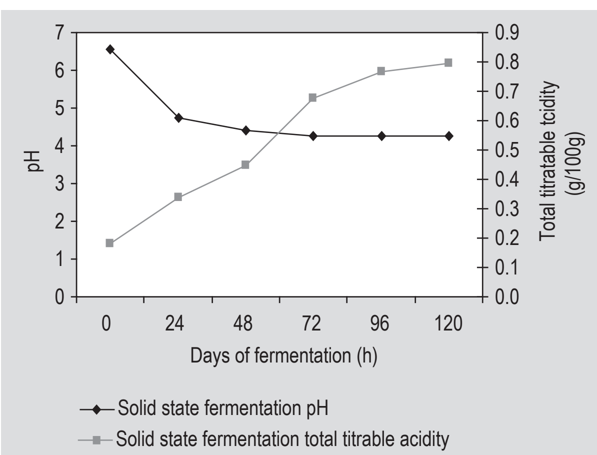

Figure 2. Changes in pH and total titratable acidity of fermenting breadfruit during solid state fermentation.

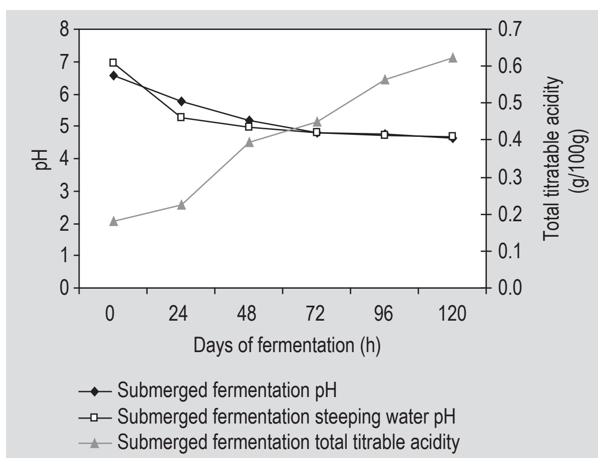

Figure 3. Changes in pH and total titratable acidity of fermenting breadfruit during submerged fermentation.

Table 1. Nutritional changes in solid state fermentation of breadfruit.[1,2]

PH (h)	% MC	TSS (g/10 g)	TS (g/100 g)	TRS (μg/g)	% Pt	K (mg/100 g)	Na (mg/l00 g)	Mn (mg/l00 g)	Fe (mg/l00 g)	Cu (mg/l00 g)	Zn (mg/l00 g)
0	66.30^{f}	0.89^{d}	67.44^{f}	74.90^{e}	2.24^{c}	3.79^{d}	1.52^{c}	0.07^{a}	0.82^{b}	0.04^{a}	0.06^{a}
24	63.40^{e}	0.91^{e}	59.88^{e}	79.21^{f}	2.21^{c}	3.83^{de}	1.52^{c}	0.08^{a}	0.75^{a}	0.04^{a}	0.04^{a}
48	60.97^{d}	0.93^{e}	54.00^{d}	60.00^{d}	2.27^{d}	3.24^{b}	1.58^{d}	0.09^{a}	0.82^{b}	0.04^{a}	0.02^{a}
72	56.80^{c}	0.80^{c}	50.79^{c}	56.40^{c}	2.29^{d}	3.80^{d}	1.55^{c}	0.09^{a}	0.78^{b}	0.05^{a}	0.02^{a}
96	51.67^{b}	0.68^{b}	41.35^{b}	45.90^{b}	1.85^{b}	3.34^{c}	1.48^{b}	0.08^{a}	0.81^{ab}	0.04^{a}	0.01^{a}
120	49.77^{a}	0.53^{a}	36.89^{a}	40.10^{a}	0.92^{a}	3.11^{a}	1.34^{a}	0.06^{a}	0.72^{a}	0.03^{a}	0.01^{a}

[1] PH = period in h; % MC = moisture content; TSS = total soluble sugar; TS = total starch; TRS = total reducing sugar; Pt = protein.
[2] Values with different subscripts along the column differ significantly by Duncan's multiple range tests at 5% level of significance.

Table 2. Nutritional changes in submerged fermentation of breadfruit.[1,2]

PH (h)	% MC	TSS (g/10 g)	TS (g/100 g)	TRS (μg/g)	% Pt	K (mg/100 g)	Na (mg/l00 g)	Mn (mg/l00 g)	Fe (mg/l00 g)	Cu (mg/l00 g)	Zn (mg/l00 g)
0	66.04^{a}	0.88^{c}	67.51^{f}	73.40^{e}	2.22^{d}	3.69^{f}	1.54^{a}	0.07^{a}	0.82^{b}	0.04^{a}	0.06^{a}
24	71.50^{b}	0.92^{f}	63.72^{e}	77.10^{f}	2.19^{d}	2.39^{e}	1.62^{b}	0.09^{a}	0.79^{b}	0.06^{a}	0.04^{a}
48	74.69^{c}	0.57^{d}	60.02^{d}	70.94^{d}	2.26^{d}	2.00^{d}	1.64^{b}	0.07^{a}	0.76^{a}	0.04^{a}	0.04^{a}
72	75.69^{c}	0.35^{c}	56.30^{c}	63.00^{c}	1.81^{c}	1.57^{c}	1.65^{bc}	0.06^{a}	0.76^{a}	0.04^{a}	0.01^{a}
96	82.55^{e}	0.26^{b}	51.28^{b}	60.33^{b}	1.72^{b}	1.42^{b}	1.65^{bc}	0.07^{a}	0.76^{a}	0.04^{a}	0.01^{a}
120	83.30^{f}	0.21^{a}	48.70^{a}	54.22^{a}	0.61^{a}	1.35^{a}	1.60^{b}	0.05^{a}	0.72^{a}	0.03^{a}	0.01^{a}

[1] PH = period in h; % MC = moisture content; TSS = total soluble sugar; TS = total starch; TRS = total reducing sugar; Pt = protein.
[2] Values with different subscripts along the column differ significantly by Duncan's multiple range tests at 5% level of significance.

submerged fermentation. This suggests that the amount of starch utilised or converted to sugars and organic acids during solid state fermentation was comparatively low. The decrease in the starch contents may be due to breaking down of starch to sugar by some microorganisms which contains amylase enzyme. This could further enhance the exploration of its potential in food formulation. Compared to a fermented cassava product ('fufu'), fermented breadfruit still had higher starch content. In the study of Oke and Bolarinwa (2012), there were no significant differences between the starch content of fermented and unfermented cocoyam flours.

Total soluble sugar content

Table 1 also shows the change in the total soluble sugar contents for the solid state fermented breadfruit. The total soluble sugar increased from 0.89 to 0.93 g/100 g at 48 h fermentation but decreased gradually to 0.53 g/100 g at the end of fermentation. Similar trend was also noted during the submerged fermentation of breadfruit in Table 2, the starch content decreased from 67.51 at the beginning to 48.70 at the end of fermentation. The total soluble sugar increased from 0.88 to 0.92 g/100 g at the end of 24 h fermentation but decreased to 0.21 g/100 g at the end of fermentation. The transient increase in total soluble sugar despite their consumption by an increasing population of microorganisms was probably as a result of amylolytic activities which could have led to the production of larger amounts of sugars than the microorganisms required for the metabolism of those sugars. Amylolytic activities of lactic acid bacteria have been reported in fermenting cereal grains (Umeta and Faulks, 1988). The amylolytic activity of the breadfruit no doubt contributes to this phenomenon, thus explaining the higher sugar content of the naturally fermented breadfruit. Amylolytic activities can further explain the dramatic higher increase in total soluble sugar in solid state fermentation as compared with the submerged fermentation, the latter providing a better substrate for amylases that will hydrolyse the gelatinised starch of the breadfruit much more quickly than the native starch of the submerged fermentation. The sugar content fell as soon as amylolytic activity was insufficient to provide fermentable sugars for microorganism metabolism, i.e. after 24 h fermentation for both solid state fermentation and submerged fermentation.

Total reducing sugar

The reducing sugar contents increased as shown in Table 1 and 2 during the natural fermentation processes. There was an increase in the total reducing sugar from 74.9 g/100 g at the beginning of fermentation to 79.21 at 24 h of fermentation followed by a gradual decrease to 40.10 at 120 h of fermentation. There was also an initial increase in the total reducing sugar from 73.40 at the beginning of submerged fermentation to 77.10 at 24 h of the fermentation and a gradual decrease to 54.22 at 120 h. Decrease in the total reducing sugars content during both solid state fermentation and submerged fermentation contributed to the production of lactic acids during the lactic acid fermentation. Even if Panda *et al.* (2008) demonstrated that *Lactobacillus plantarum* could produce α-amylase and reducing sugars from hydrolysed starch, the decrease in the concentration of the reducing sugars could be explained by the activities of total fermentative micro flora which metabolised and converted them into energy and organic acids. These results are similar to those obtained by Kouassi (2006) who observed a drastic reduction of total sugars content and a fall of reducing sugars concentration after 12 h of fermentation of cassava. Although the decrease in content of reducing sugars was more significant in the solid state fermentation compared to the submerged fermented breadfruit. This showed that reducing sugars were significantly degraded faster in the breadfruit subjected to solid state fermentation than in the submerged fermentation. In parallel with sugar degradation, the accumulation of fermented metabolites such as lactic acids was observed. The availability of high content of total reducing sugar as substrate for the growth of microorganisms and the presence of aerobic conditions could have favoured the development of diverse groups of microorganisms during the solid state fermentation of breadfruit.

Protein content

Table 1 shows the changes in the mineral and protein contents of breadfruit during the solid state fermentation process. The protein content increased from 2.24 to 2.29 at 72 h of fermentation and decreased to 0.92 at 120 h of fermentation. Fermented breadfruit contained higher amounts of protein than the unfermented breadfruit and this conforms to the findings of Appiah *et al.*, 2011 who found out that the protein content of fermented breadfruit flour (4.43%) was higher than the unfermented breadfruit flour. The result of the study also showed that the protein content increased with fermentation period and later decreased and this conforms to the report of Irtwange and Achimba (2009) who reported an increase in the protein content of gari within 72 h fermentation period and its decrease after 72 h fermentation period. An increase in the protein content was reported for fermented tiger nut flour (Adejuyitan *et al.*, 2009), jackfruit seed flour (Mukprasirt and Sajjaanantakul, 2004), conophor nut flour (Odoemelan, 2003) and sesame seed (Olagunju and Ifesan, 2013). The higher protein contented of fermented breadfruit obtained in this work could be due to the action of extracellular enzymes produced by the fermenting microorganisms. It can also be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles (Elyas *et al.*, 2002). In most human diets, protein is more limiting than other nutrients, therefore, application of a fermentation process that appears to increase the protein content even at the expense of other nutrients may be advantageous nutritionally (Abdelhaleem *et al.*, 2008).

Mineral content

Breadfruit is rich in potassium, manganese, iron, copper and zinc. Potassium was the predominant mineral in the solid, submerged state 0 h fermented *A. atilis* and this observation conforms to the study of Appiah *et al.* (2011). Potassium has been reported to be an important mineral that help in maintaining electrolyte balance in humans (NTBG, 2009) and its presence in *A. altilis* indicates *A. altilis* is a good source of potassium. Potassium decreased throughout the fermentation process from 3.69 mg/100 g at 0 h to 1.35 mg/100 g at the end of the fermentation. Table 1 shows that the solid state fermentation increased the sodium content from 1.52 to 1.58 mg/100 g at 48 h and decreased sodium to 1.34 mg/100 g at 120 h. Zinc and potassium increased within the 24 h of fermentation by 0.04 and 3.83% respectively while zinc concentration decreased to 0.01 mg/100 g at 96 h and the manganese also decreased to 0.06 mg/100 g at 120 h (Table 2). Submerged fermentation caused an increased in the sodium content from 1.54 to 1.65 mg/100 g at 72 h and decreased to 1.60 mg/100 g at the end of fermentation period, while the 72 h solid state fermented breadfruit had the lowest sodium content. Morgan (1999) indicated that reducing intake of sodium ameliorates the development of hypertension. The moderate sodium content, therefore, suggests that 72 h submerged and solid state fermented *A. altilis* can be used to make low-sodium diet and could be consumed without apprehension of consuming excess sodium. Zinc is an important trace element in man and animals and it is responsible for many biological functions such as growth, fertility, vision, cell division, etc., the zinc content increased to 0.04 mg/100 g at 48 h of fermentation and decreased to 0.10 mg/100 g at 72 h. Iron is an important constituent of haemoglobin found in blood, the iron concentration decreased throughout the fermentation processes from 0.82 mg/100 g at 0 h to 0.72 mg/100 g at 120 h of the process. This result conforms to the findings of Appiah *et al.* (2011) who reported the iron content of unfermented breadfruit flour was higher than the fermented flour. The iron content of fermented breadfruits was above the recommended daily allowance for iron (8-18 mg/day) by National Academy of Science (2004).

Conclusions

Protein, total reducing sugar and total solids of the fermented breadfruits began to reduce after a 24 h period. Solid state fermentation of breadfruit gave fermented breadfruit of lower moisture content, lower total reducing sugar, higher total solids and higher protein content than submerged fermentation. Also, it was observed that there were no significant changes in zinc, copper and manganese content with the different types of fermentation.

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