Natural fermentation of Enset *(Ensete ventricosum)* for the production of *Kocho*

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Abstract: Biochemical changes of fermenting enset were studied. After seven weeks fermentation, total protein, ash and total carbohydrates decreased by 15%, 16% and 34%, respectively. Significant ($p<0.05$) reduction in iron (15%), phosphorus (29%), calcium (51%), starch (23%), soluble sugars (93%), reducing-sugars (84%) and available carbohydrates (51%) were recorded. Free amino acids and non-protein nitrogen increased by 6- and 1.6-fold, respecively. The pH of the fermented mash fell from an initial value of 5.7 to 3.8 with a concomitant sharp rise in titratable acidity resulting in accumulation of organic acids. The predominant organic acids were lactic, iso-valeric and n-butyric acids followed by n-valeric and acetic acids. Other volatile fatty acids and ethanol were formed in lesser quantities. Fermentation of enset also resulted in significant reductions in tannins and trypsin inhibitors whereas oxalic acid remained unaffected. [*Ethiop. J. Health Dev.* 1997;11(1):75-81]

Introduction

In Ethiopia there are several traditionally fermented foods some of which are products of a lowtechnology process involving a solid state fermentation (SSF) system. The technique is usually cheap and simple to operate but can be time-consuming and labour-intensive. Biochemically, SSF is a complex system that has not been fully characterized and understood. The technology has been used in Ethiopia to produce such food items as kocho and bulla.

Kocho and bulla are naturally fermented food products of enset *(Ensete ventricosum)*. Enset is a perennial root crop which grows between altitudes of 1500-3000 meters above sea level (1). It provides an essential source of calories for as many as 10 million people in the South and Southwestern parts of Ethiopia (2, 3).

One of the processing methods of enset involves the fermentation of the corm and pseudo stem in an underground pit for several weeks during which period they soften and turn into a whitish, semisolid dough-like meal popularly called *Wassa* or *Kocho*. This dough-like meal (product) is prepared into various types of foods. Selinus *et al* (1) have listed food types prepared from *kocho*. *Kocho* is consumed widely by people of all social classes in South and Southwestern Ethiopia. Fermentation of enset is a microbial disintegration of the grated corm and pseudo-stem and is reported to impart flavour and textural qualities to the fermented food products, *kocho* and *bulla*. The odour change is much more pronounced in this fermentation than any other fermented food products locally encountered. Studies made to date on fermented enset products of *kocho* and *bulla* have tended to concentrate on carbohydrate and calcium composition (4) amino acid pattern (5) and identification of the microflora involved in enset fermentation processes (6, 7, 8). Virtually nothing is known about the biochemical changes due to fermentation and the content of antinutritional factors in fermented enset. This paper reports the biochemical changes brought about by natural lactic fermentation of enset into *kocho* including the amounts and types of organic acids produced during fermentation.

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Methods

Enset fermentation: Enset plants of variety Ado were purchased from a farmer in Sidama. All the fermentation processes were carried out in the enset farm at the backyard of the farmer. Enset was fermented using the traditional *wassa* fermentation procedure of Sidama as described by Selinus et al (1). This involved scrapping of the leaf bases with sharpedged bamboo split *(Sissicho*) to extract the long fibres. The liquid oozing out was collected in a separate pit lined with fresh enset leaves. Water was decanted after three days and the starch residue *(bulla*) wrapped with enset leaves and kept in the fermentation pit with "*Amulcho*" to ferment. Part of the enset corm pulverized with rough-edged serrated wooden piece (*Keho*) was mixed with the leaf scrapping locally called *Amulcho* or *Ambicho*, covered with enset leaves and stored in the open air for five to seven days. The remaining part of the corm is rubbed with decomposed enset leaves *(Shigido)*, recovered from the enset farm, covered with fresh leaves and left for four to five days in the open air. The retted corm is pulverized into fine pieces and thoroughly mixed with *Amulcho*. The mixture is then transferred to a fermentation pit lined with layers of fresh enset leaves, covered with same and left to ferment. The first sample, referred to as zero week (Ow) sample, was collected immediately after the mash was placed in the pit, thereafter samples were obtained at intervals of one week.

Chemical analyses: To measure the pH, a 10 g portion of the fermenting enset mash was homogenized with deionized water and the suspension measured using a pH meter (Orion, USA) equipped with a glass electrode.

The titratable acidity (TA) expressed as percent lactic acid was determined by titrating 25 g of the decanted homogenate samples used for pH determination against 0.1 N NaOH to pH 8.3.

Alcohols were determined by gas liquid chromatography on a Varian 3700 VISTA (Varian Associates, USA) instrument equipped with a flame ionization detector using a glass column (2mx2mm, i.d.) packed with Propack QS and temperature progrmmed from 120^{o}C to 220^{o}C at 20 min $^{-1}$ with nitrogen as carrier gas at a flow rate of 40 ml min $^{-1}$. Pentan-2-ol was used as an internal standard and quantisation was performed with the aid of a Varian 4270 computing integrator. Volatile fatty acids were determined by gas liquid chromatographic analyses of the aqueous extracts of fermenting enset according to Rogossa and Love (9) and Patrick and Timothy (10). A column (2m x 3.2mm i.d.) of 15% FFAP on Chromosorb WAW - mesh 80/100 held at 180^{o}C and isothermal operation was used. Injector port, and detector temperatures were 210^{o}C and 220^{o}C, respectively. 2-Methyl valeric acid was employed as internal standard and quantisation was performed as before. Lactic acid was estimated by the method of Alcok (11).

Table 1: **Effect of fermentation on proximate composition of *kocho* (dry weight basis)**

Period	Moisture	Total protein	Fat	Crude fiber	Ash	NPN	Free amino acids
wk	%	%	%	%	%	%	mg/g
0	84±3	4.07±0.02	0.43±0.01	3.43±0.03	0.75±0.01	0.01±0.01	8.1±1.2
1	66±4	3.97±0.01	0.43±0.00	4.07±0.02	0.76±0.03	0.01±0.00	9.0±1.0
2	63±2	3.87±0.03	0.42±0.00	4.13±0.03	0.78±0.04	0.03±0.00	10.7±0.8
3	60±5	3.75±0.01	0.41±0.01	4.21±0.05	0.75±0.03	0.05±0.02	12.4±0.9
4	60±3	3.63±0.00	0.53±0.04	4.33±0.02	0.67±0.00	0.06±0.01	16.9±1.0
5	60±7	3.53±0.04	0.43±0.05	4.37±0.03	0.65±0.02	0.06±0.00	18.8±1.2
6	60±2	3.47±0.03	0.43±0.02	4.50±0.05	0.64±0.01	0.07±0.01	20.0±0.8
7	60±4	3.47±0.01	0.43±0.05	5.00±0.04	0.63±0.04	0.07±0.01	20.7±0.7

Values are means±S.D. of three determinations.

For analysis of proximate compostion, the fermenting enset mashes were freeze dried and ground in a Cyclotec (Tecator, Sweden) sample mill and kept in glass jars at 4^{o}C until used for analysis. The samples were analyzed for proximate compositions by AOAC (12) methods. Crude protein was calculated using the factor 6.25. Carbohydrate content was determined by difference.

Total sugars other than starch were extracted in ethanol by reflux method and were estimated colorimetrically according to Dubois *et al* (13). Starch from sugar free pellet was estimated by the method of McCready *et al* (14). Reducing-sugars were estimated by the method of Miller (15) and non-reducing- sugars calculated by the difference between total sugars and reducing sugars.

The method of Vanillin-HCl described by Maxon and Rooney (16) was employed for the examination of tannin content using catechin as a standard and expressed as catechin equivalents (mg/100 g, dry wt).

The trypsin inhibitor activity was determined by the method of Kakade *et al* (17) using N-benzoylDL-arginine-p-nitroaniline (BAPNA) as the trypsin substrate and expressed as TIU/g (dry wt). Oxalic acid in the fermenting enset was determined by precipitation with calcium salt and potassium permanganate titration (12).

Total iron was determined using bathophenanthrolein as described in AOAC (12), phosphorus by the Fiske-Subbaraw method (18) and calcium according to the AOAC (12).

The ninhydrin method of Rosen (19) was used to estimate free amino acids with a standard of leucine. Non-protein nitrogen (NPN) was determined by the procedure of Concon and Soltess (20). *Statistical analysis*: Tests were replicated three times. Data were subjected to analysis of variance (ANOVA). Differences were considered statistically significant at $p<0.05$.

Table 2: **Effect of fermentation on carbohydrate components of kocho (% dry weight)**

Period	Starch	Total Sugar	Reducing sugar	Non-Reducing Sugar	Available carbohydrate
0	68.8±2.5	4.3±0.1	1.9±0.3	2.4±0.4	72.1±0.4
1	68.5±1.4	4.0±0.4	1.5±0.1	2.5±0.3	72.1±0.5
2	65.9±3.7	3.5±0.3	1.2±0.2	2.3±0.0	72.0±0.7
3	57.3±5.1	6.2±0.7	2.6±0.4	3.6±0.2	61.5±0.6
4	55.3±0.7	4.5±0.8	0.8±0.0	3.7±0.4	57.8±0.8
5	53.3±1.2	1.2±0.1	0.5±0.0	0.7±0.1	54.1±0.5
6	53.1±0.5	1.1±0.2	0.3±0.1	0.8±0.2	54.0±0.9
7	53.1±1.2	0.3±0.0	0.3±0.0	--	54.0±0.7

Values are means±S.D. of three determinations

Results

The fermentation of enset is one of the most important steps in kocho preparation. *Amulcho* has no smell or characteristic flavourital when transferred to the fermentation pit. When the pH of the mash reached 4.4 with 0.96% titratable acidity (as lactic acid), the desired sour flavour and characteristic aroma was attained. The proximate composition of fermenting enset are shown in Table 1. Fermenting enset as it was initially prepared (week 0) had a high moisture content (84%) and a near neutral pH (6.5). However, at week seven, when the fermentation reached the desired fermentation stage, the moisture content significantly ($p<0.05$) decreased to about 60%. The fermentation process also resulted in a significant ($p<0.05$) losses of protein, ash, and total carbohydrate the losses being 15, 16, and 34%, respectively, after seven weeks of fermentation. Fat content remained unaffected whereas crude fibre increased by 30%. The loss in ash content of the fermenting enset similarly resulted in significant ($p<0.05$) losses of iron (51%), phosphorus (29%) and calcium (51%), respectively.

As fermentation progressed, the free amino acids and non-protein nitrogen increased to values which were six times and 1.6 times, respectively, the sample at week zero. A significant ($p<0.05$) and positive correlation was observed regarding the relative levels of free amino acids and the amounts of non protein nitrogen in *kocho*.

The changes in carbohydrate components are shown in Table 2. The starch content significantly ($p<0.05$) decreased from a starting value of 69% to 53% at the end of the fermentation. During fermentation, sugars were not completely depleted. Two distinct patterns of sugars consumption can be observed. In the first case, there was an increase in sugars in the first three weeks of fermentation. In the second case, the accumulation of sugars is not observed. After seven weeks of fermentation period, the concentrations of total and reducing sugars declined to about 0.3%. The

changes in starch and sugars resulted in a significant loss of available carbohydrate (51%) ($p<0.05$). Fermentation also resulted in a sharp increase in titratable acidity from the first week to the 6th week of fermentation and it

Table 3: **Production of organic acids during enset fermentation (mg/100g dry weight basis)**

Period wk	Acetic acid	Propionic acid	n-Butyric acid	i-valeric acid	n-valeric acid	-caproic acid	i Lactic acid	Ethanol	TA %	pH
0	8.5±1.5	3.8±0.2	5.0±0.3	8.7±0.1	-	-	51.3±2.1	5.3±0.3	0.27±0.02	5.7±0.1
1	1.0±1.7	4.5±0.3	8.1±0.2	23.1±0.4	-	-	126.4±3.1	5.4±0.1	0.30±0.01	5.1±0.1
2	3.8±1.9	5.3±0.2	10.5±0.5	25.4±0.8	2.5±0.8	-	67.8±2.7	5.7±0.3	0.52±0.83	4.9±0.2
3	6.0±2.0	6.6±0.3	27.5±1.2	60.0±2.1	8.9±0.3	-	90.2±3.7	6.3±0.2	0.96±0.04	4.4±0.2
4	9.1±2.1	8.9±0.4	34.4±1.3	63.7±3.2	15.5±0.4	-	210.0±3.1	12.1±0.4	0.98±0.06	4.2±0.1
5	3.5±2.6	9.5±0.6	35.3±1.2	65.5±3.0	22.0±0.4	1.7±0.2	486.1±4.3	15.0±0.2	1.28±0.01	4.0±0.1
6	1.4±2.1	10.3±0.7	35.6±1.2	66.9±2.1	24.4±0.5	2.6±0.1	504.0±3.5	15.3±0.5	1.50±0.20	3.9±0.2
7	1.9±2.0	12.5±0.6	35.7±2.0	68.9±1.8	26.3±0.6	3.0±0.4	520.4±3.0	21.0±0.4	1.54±0.40	3.8±0.1

Values are means of five determinations ± S.D.

gradually levelled off at the 7th week (Table 3). At the same time, pH also decreased in a similar manner broadly paralleling total acid accumulation in the fermentation mixture. A significant negative correlation was observed between pH and titratable acidity ($p<0.05$).

The major organic acids produced were acetic, lactic, n-butyric, propionic, iso-valeric, and isocaproic acids. Ethanol was the only alcohol detected during fermentation but the compound attained a maximum concentration of only 21mg/100 g (dry weight basis) after seven weeks of fermentation rendering it unlikely that yeasts are involved in anaerobic degradation of substrate. Lactic and isovaleric acids were the predominant organic acids and accounted for more than 84% of the total organic acids followed by n-butyric and acetic acids. The concentrations of n-valeric, iso-hexanoic and propionic acids appeared to be low.

Table 4 shows that the content of tannins and trypsin inhibitor activity in fermenting enset was decreased significantly ($p<0.05$). The reduction was about 51% for tannins and 53% for trypsin inhibitor activity at the end of the fermentation period. However, the content of oxalic acid remained unaffected during fermentation of enset.

Discussion

Fermentation of enset into *kocho* is one of the most important processing steps in enset utilization. During the enset fermentation, significant losses in protein, ash and carbohydrates were observed. Most of the losses may be attributed to the leaching of these nutrients through the permeable fermentation pit. Abraham *et al* (5) and Taye (4) also observed losses in protein and minerals during the fermentation of enset.

An increase in the concentrations of nonprotein nitrogen and free amino acids was also apparent as a result of fermentation, a finding which duplicates observations during the *ogiri* fermentation (21). Such significant increases may be accounted for by the activity of proteolytic fermenting enset. During the enset fermentation process, a progressive decline in carbohydrate content was observed. This may be due to the activity of the microflora which most likely derived its energy from carbohydrate metabolism. The level of soluble and reducing sugars increased by about 1.4fold during the first three weeks of fermentation and then decreased significantly at the end of the

fermentation period. The increase in sugars content in the first case is probably due to increased activity of native or microbial amylases which hydrolysed starch into sugars (22). The decrease in sugar levels thereafter is probably due to the metabolism of these sugars to glycolytic end products such as lactic acid and other volatile organic acids. Similar trends were observed during the fermentation of cassava (23, 24).

Table 4: **Effect of fermentation on minerals and antinutritional factors of kocho (dry weight basis)**

Period	Ca mg/100g	Iron mg/100g	P mg/100g	Tannin mg/100g	TIA TIU/g	Oxalic acid mg/100g
0	288.0±23	3.4±0.1	154.3±11	676±41	8356±11	230±32
1	283.7±23	2.8±0.3	149.0±10	644±34	8137±120	228±23
2	271.7±17	2.5±0.4	140.7±12	440±23	7954±177	225±24
3	210.0±13	2.4±0.0	133.3±9	430±31	6520±123	224±22
4	186.3±21	2.4±0.0	131.7±11	422±30	5184±130	220±21
5	157.3±20	2.2±0.3	131.3±7	390±32	4288±137	224±19
6	149.0±11	1.7±0.2	129.0±10	331±18	4079±112	235±32
7	142.3±10	1.6±0.2	128.0±21	333±21	3924±110	220±34

Values are means ± S.D. of three determinations.

Enset fermentation into *kocho*, like cassava, is primarily a lactic fermentation (25). This is indicated by the sharp drop in pH and rapid rise in titratable acidity. The fall of pH into the acid range could be attributed to an increased amylolytic activity of enzymes causing liberation of acids and possibly other acidic end products of carbohydrates fermentation (26, 27). Amund *et al* (22) similarly reported that the available sugars were transformed into acid during the natural fermentation of cassava.

Fermented enset, *kocho*, has a characteristic penetrating butyrous smell. Although these minor acids contribute only a small amount to the total organic acids, they make significant contribution to the flavour of the *kocho* because of their characteristic odours.

Various parallels may also be drawn between lactic acid fermentation of cassava and enset. In cassava fermentation, lactic and acetic acids comprise most of the total organic acids while other minor volatile fatty acids (propionic, iso-and n-butyric acids) contribute less (25). However, the presence of iso-valeric and n-butyric acids in fermenting enset which may impart the peculiar aroma to *kocho*, in appreciable amounts is a difference.

The bases and corms of enset show enzymic browning reactions when cut and exposed to the air. This can occur with the corm in the fresh state, after storage or when physiologically damaged which can be associated with the reactions of phenolic substances. The diminishing effect on tannins during enset fermentation may be due to the activity of polyphenoloxidase enzyme in fermenting enset. Dhankher and Chauhan (28) also reported decreases in the polyphenols of *rabadi* with an increase in fermentation time.

Trypsin inhibitor activity was significantly ($p<0.05$) reduced due to fermentation of enset. On the other hand studies on the stability of trypsin inhibitors to cooking or baking of *kocho* is required which may throw light on the problem. The presence of appreciable levels of oxalic acid, trypsin inhibitors and tannins in enset that contributes the major component of the diet of the South and South Western people of Ethiopia is likely to limit its utilization. However, natural fermentation of enset markedly reduced the content of trypsin inhibitors and tannins thus enhances the digestibility of kocho protein and bioavailability of minerals. This study has provided useful data. However, there is a need for greater understanding on changes brought about by natural fermentation of enset as the trend in this fermentation is to devise ways to shorten *kocho* production time.

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