Original article

Development of a tempe-related food using *kocho* and grass pea as substrate

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Abstract: Tempe was prepared by co-fermenting different proportions of *kocho* flour with grass pea using the traditional inoculum, *Usar*. The nutritive quality of tempe prepared from *kocho*-grass pea (20:80; 25:75; 30:70; 40:60; 50:50) was determined. The protein crude fibre and ash content increased significantly, while carbohydrates decreased slightly. The fat content of tempe made from all the *kocho*-grass pea combinations decreased by about one third. Soluble and reducing sugars increased by 2.6- and 2.9-, 4.8- and 9.5- and 7- and 10-fold, respectively, in 30:70, 40:60 and 50:50 *kocho*-grass pea combinations. Free amino acids and non-protein nitrogen similarly increased 8.5- and 24-, 23- and 7.5- and 6.5 and 20-fold, respectively, whereas minerals remained unaffected in 20:80, 25:75 and 30:70 *kocho*-grass pea combinations. Fermentation also significantly decreased the phytic acid and trypsin inhibitors but, increased tannin contents of the tempe. Co-fermentation of *kocho*-grass pea combinations into tempe greatly improved the nutrient of *kocho* in terms of protein, fat and ash contents. The tempe could be used for supplementary feeding. A fermentation scheme was therefore, developed for the production of an enriched product in which 40% to 80% grass pea was fermented with *kocho* flour for 48 hr. [*Ethiop. J. Health Dev.* 1997;11(1):67-73]

Introduction

In developing countries plant proteins are cheaper and more accessible than animal protein and could be used to alleviate protein energy malnutrition (PEM) and other dietetic problems, and improve the low nutritive quality cereals and starch staple foods (1, 2). Grain legumes are rich and cheap sources of dietary protein in developing countries. Grass pea (*Lathyrus sativus* L.) as one of the important legumes contributes more than 7.2% of the total legume production in Ethiopia. Its utilization, however, is undermined by the presence of trypsin inhibitions (3), phytate and tannins causing reduced bioavailability of minerals and prolonged cooking times (4, 5) as well as, β-NOxalyl-L-α, β-diaminopropionic acid (ODAP) causing lathyrism (6).

Enset (*Ensete ventricosum*) is one of the most important indigenous crops for Ethiopia. It grows in a wide range of environmental conditions. More than 10 million people in the south, south west and central regions of the country, directly or indirectly, depend on enset for their livelihood (7). *Kocho* is a semi-solid fermented product of enset. It generally contains only 1% or 2% protein and thus is completely unable to provide the consumer with sufficient protein. Being essentially a starch food, *kocho* is not only poor in protein but the protein has also been found to be deficient in the essential amino acids (8). *Kocho* has also another drawback that is, rapid post processing deterioration. The low protein content of *kocho* has been a major concern in its utilization and this has led to *kocho* products being regarded as nutritionally inferior foods. The possibility of increasing the protein content of *kocho* products could help to alleviate this limitation of its wider utilizations.

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Protein quality of starch food may be improved by amino acid complementation with legumes (1, 2) and fermentation (8). *Rhizopus* fermentation of cereal - legume mixture was reported to produce tempe with enhanced nutritive quality (9). Tempe is a popular Indonesian fermented food consisting of tender-cooked soy beans (or occasionally other legumes) bound together in to a white cake by a dense cottony mycelium of the mould *Rhizopus* spp. It owes much of its flavour, sliceable meat like texture, easy digestibility and excellent nutritional properties to the process of fermentation (10). In Indonesia, tempe has been sucessfully used in community-based therapy for the management of diarrhoea and rehabilitation of protein energy malnutrition (PEM) cases (11). The synthesis of vitamin B_{12} (12) during tempe fermentation might also help in anaemia managemet.

Binyam *et al* reported that the various steps and fermentation procedures used for tempe production from grass pea decreased antinutritional factors significantly including the neucotoxin principle ODAP (5). Tempe is cheap, can be incorprated in many foods, causes no malabsorption syndrome and can be used by all age groups (10).

Investigations have been carried out on methods of improving the protein content of cassava (*Manihot esculanta* Cratz) products by fermentation with protein enriching organisms (13) and legumes (14). Virtually no such study has been carried out on *Kocho*. The aim of the report here was to develop grass pea *Kocho* tempe and estimate its nutrient contents and antinutritional factors.

Methods

Sample preparations: Rice-grown mixed culture *Rhizopus oligosporus* and *Rhizopus oryzae* obtained from the Institute of Sciences, Bandug, Indonesia was used for the inoculation of the substrate.

The local grown grass pea (*Lathyrus sativus* L) was used for this study. The grass pea was dehulled by the traditional grinding mill. *Kocho* purchased from a market in Sidama, Ethiopia, was freezedried and ground in a Cyclotec (Tecator, Sweden) sample mill and sieved.

Tempe was prepared according to the procedure developed by Mugula (15) to manufacture sorgum-common bean tempe. Grass pea and *Kocho* were mixed at different weight ratios of *kocho to* grass pea.

A 0.2% inoculum by dry weight of raw materials was used to obtain an acceptable tempe with respect to better mould growth texture and ease of slicing. Incubation was made at room temprature ($^{28}\pm2^{o}$C) in 250 g capacity perforated polyethylene bags. Tempe was sliced into small pieces and freeze-dried for 12 hrs and ground in a Cyclotec sample mill into powder and used for analysis.

Analysis: Proximate composition of samples was analyzed according to AOAC (16) methods. The moisture was estimated by drying to constant weight in the oven at 105^{o} C. The micro-Kjeldahl method was used for analysis of nitrogen and estimation of protein (6.25 x N%). Crude fat was extracted with diethyl ether. Ash and crude fiber were determined according to AOAC (16).
Carbohydrate was estimated by difference.

Calcium and iron concentrations were determined according to AOAC (16) methods whereas, phosphorus was determined as described by Fiske and Subbarrow (17). Phytic acid was extracted with 1.5% HCl in 10% Na_2So_4 and determined by the method of Haug and Lantsch (18). Tannins were estimated by the modified Vanillin-HCl method of Maxon and Rooney (19) and expressed as catechin equivalent. Trypsin inhibitor activity was determined colorimetrically and expressed as TIU/g dry weigt (20). pH of samples was monitored periodically during fermentation using pH meter.

To estimate the soluble carbohydrates, a 10 g sample was suspended in 80 ml deionised water, heated at 70^{o}C in an agitating water bath for one hour and made up to 100 ml. The protein in 20 ml aliquot of homogenate was precipitated with 20 ml saturated lead acetate solution. Soluble carbohydrate supernatants obtained after centrifugation were quantified using the phenol-sulphuric acid method of Dubois *et al* (21) and with glucose standard. Total soluble sugars in the tempe were extracted with 80% ethanol, then determined using the phenol-sulphuric acid method of Miller (22). Starch was extracted with perchloric acid from the residue and measured according to McCready *et al* (23)

The non-protein nitrogen (NPN) was estimated by the method of Cocon and Soltess (24) and the free amino acids were estimated by the ninhydrin method of Rosen (25).

Statistical analyses: All experiments were conducted three times and analyzed by analysis of variance. Differences were considered statistically significiant at $p<0.05$.

Results

The best tempe was obtained by using grass pea to *Kocho* ratio of 20:80, 30:70, 25:75, 40:60 and 50:50 and 0.2% inoculum. The proximate compostion of *Kocho*-grass pea tempe is shown in Table 1. Mould, fermentation for up to 48 hr apparently increased significantly ($p<0.05$) the crude protein, fat and ash contents of tempe while it slightly decreased the carbohydrate. Highest increase in protein content was observed in combinations 20:80 (31%) and 25:75 (42%). Fat content in all fermented combinations decreased by about a third compared to the unfermented combinations. The ash content in all fermented mixtures increased significantly ($P<0.05$) due to fermentation. The total carbohydrate content decreased slightly (4.5-13%) in the fermented mixtures. The crude fibre increased by 31%, 34% and 35% in the fermented 20:80, 30:70 and 50:50 *kocho*-grass pea combinations, respectively. Moisture content of the tempe samples decreased with the increasing proportion of *kocho* in the mixtures. However, fermentation by the tempe mould did not change the content of minerals.

Table 1: **Proximate composition of fermented and unfermented *kocho*-grass pea combinations (% dry weight basis)**

kocho-grass pea ratio	Process	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrate
20:80	Unfermented		22.5±1.5^{a}	1.98±0.12^{a}	4.79±0.51^{a}	0.84±0.03^{a}	69.5±2.5 a
	Fermented	55.3±2.1^{a}	29.3±1.2^{b}	1.32±0.01^{b}	6.27±0.37 b	1.19±0.07 b	61.6±3.1 b
25:75	Unfermented	-	21.5±1.1^{c}	1.90±0.15^{a}	5.01±0.27^{c}	0.88±0.01^{a}	70.61±1.5 a
	Fermented	49.8±2.5^{b}	26.7±2.1^{d}	1.24±0.03^{c}	5.30±0.32 c	1.20±0.00 b	65.6±1.8 c
30:70	Unfermented	-	20.7±1.3^{e}	1.89±0.09^{a}	4.29±0.21^{c}	0.91±0.03^{a}	72.2±5.1 d
	Fermented	50.9±2.2^{c}	24.1±1.5^{f}	1.14±0.01^{d}	5.75±0.41 e	1.33±0.11 b	67.0±1.3 e
40:60	Unfermented	-	19.1±0.9^{g}	1.76±0.13^{e}	.49±0.17^{d}	0.98±0.04^{a}	73.4±3.1 f
	Fermented	48.2±3.2^{d}	22.4±1.1^{h}	1.11±0.09^{d}	4.69±0.19 f	1.40±0.09 b	70.4±1.5 a
50:50	Unfermented	-	17.4±0.5^{i}	1.65±0.14^{f}	3.29±0.12^{g}	0.95±0.05^{c}	76.5±3.2 g
	Fermented	46.6±2.3^{e}	20.9±0.9^{e}	1.14±0.10^{e}	.44±0.11^{d}	1.62±0.10 d	71.9±1.0 h

Values are means of five determinations ±S.D. Values in a column with different letters are significantly different ($p<0.05$).

The effect of fermentation on carbohydrate components is shown in Table 2. As a result of fermentation, all soluble carbohydrate in all the mixtures increased significatly ($p<0.05$). This was followed by significiant decrease in starch content in all the tempe. Highest loss in starch due to fermentation was observed in combinations 40:60 (21.4%) and 50:50 increased 2.6-, 4.8- and 7-fold, respectively. Reducing sugars increased similarly, by about 3- and 10- fold for these combinations. Significant ($p<0.05$) reductions were also observed in available carbohydrate after 48 hr fermentation. Fermentation also resulted in increased pH from an average value of 6.3 to 7.3 in all fermented tempe samples.

As fermentation increased, the quantity of non-protein nitrogen and free amino acids increased significantly ($p<0.05$). None protein nitrogen increased 24-, 23-, 21-, 17-, and 12- fold, respectively, in all the five *kocho*-grass pea combinations. Similarly, free aminoacids increased 2.4- two 8.5- fold in all the combinations.

Table 2: **Carbohydrate components of fermented and unfermented *kocho*-grass pea combinations (% dry weight basis)**

kocho-grass pea ratio	Process	Soluble Carbohydrate	starch	Available Carbohydrate	Total Sugars	Reducing Sugars	Nonreducing	pH Sugars

20-80	Unfermented	1.80±0.3^{a}	41.7±0.2^{a}	44.2±1.2^{a}	0.68±0.10^{a}	0.17±0.01^{a}	0.50±0.02^{a}	6.57±0.03^{a}
	Fermented	-	36.8±0.3^{b}	37.5±1.5^{b}	0.82±0.09^{b}	0.29±0.03^{b}	0.53±0.01^{a}	7.23±0.02^{b}
25-75	Unfermented	2.1±0.2^{b}	44.1±0.7^{a}	46.5±2.1^{a}	0.68±0.02^{a}	0.19±0.00^{a}	0.48±0.04^{b}	6.50±0.03^{a}
	Fermented	-	37.6±0.3^{b}	38.6±1.3^{b}	1.24±0.11^{c}	0.31±0.01^{b}	0.93±0.05^{c}	.20±0.01^{b}
30-70	Unfermented	2.50±0.4^{c}	44.9±0.4^{a}	48.8±2.4^{d}	0.67±0.04^{a}	0.22±0.02^{c}	0.46±0.01^{b}	6.40±0.01^{a}
	Fermented	-	38.7±0.1^{b}	40.3±1.7^{e}	.77±0.12^{d}	0.63±0.04^{d}	1.14±0.11^{d}	7.03±0.02^{b}
40-60	Unfermented	2.97±0.2^{d}	51.4±0.2^{c}	53.4±3.3^{f}	0.66±0.03^{a}	0.26±0.02^{e}	0.41±0.02^{e}	5.80±0.01^{c}
	Fermented	-	40.4±0.4^{h}	44.0±2.1^{a}	.85±0.21^{e}	2.48±0.10^{f}	1.37±0.13^{f}	7.17±0.03^{b}
50:50	Unfermented	4.93±0.5^{e}	56.3±0.7^{e}	58.0±2.4^{g}	0.65±0.01^{a}	0.30±0.03^{g}	0.36±0.01^{g}	5.60±0.02^{c}
	Fermented		41.8±0.5^{a}	46.4±1.8^{c}	4.54±0.23	3.03±0.12^{h}	1.51±0.13^{h}	7.23±0.02^{b}

Values are means of five determinations ±S.D. Values in a column with different letters are significantly different ($p<0.05$)

Soluble protein also increased significantly ($p<0.05$) in the tempe samples. Fermentation by the tempe mould also resulted in dry matter losses. The dry matter losses decreased as content of *Kocho* in the combinations increased (Table 4). *Kocho*-grass pea combinations with higher grass pea content have higher trypsin inhibitors. However, fermentation by the tempe mould has decreased significantly ($p<0.05$) this antinutrient in tempe samples. Trypsin inhibitors in 50:50, 40:60 and 30:70 *kocho*-grass pea tempe decreased by 73%, 58% and 56%, respectively.

Fermentation in the fermented *kocho*-grass pea combinations also significantly decreased the phytic acid contents in the 20:80 (80%), 25:75 (79%) and 30:70 (77%) *kocho*-grass pea combinations. However, in fermented 50:50 *kocho*-grass pea combination less (56%) reduction in phytic acid content was observed. Tannin content of the unfermented *kocho*-grass pea combinations appeared to be low, but decreased as the content of grass pea in the unfermented combinations decreased. Fermentation, however, significantly increased tannins in *kocho*-grass pea combinations ($p<0.05$) (Table 4). Such increases in tannin content were evident in the 20:80 (42%), 25:75 (40%) and 30:70 (37%) *kocho*-grass pea combinations.

Discussion

After 17 hr, the *kocho*-grass pea combinations were tied together in rather firm cake by the hyphae. The smell was rather distinctive and may be described as between a yeast-like and a fruit-like aroma. The hyphae seemed even more dense and reached a maximum after 30 hr. After 48 hr, spore formation began and release of free amonia was noted. Fermentation for 30 to 48 hr, therefore, yielded the most acceptable products.

Fermentation of *kocho*-grass pea combinations by the tempe mould has resulted in various chemical changes. The increase of protein in the present study reflects the decrease of other constituents particularly fat and carbohydrates utilized by the mould. Wang *et al*.(9) attributed the increase in the protein content to the decrease of constituents utilized by moulds for growth and production of heat during fermentation. The high fiber contents of tempe from *kocho*-grass pea combinations could also be due to enzyme resistant starches. Matsuo (26) also reported an increase in fiber content of *Okara* tempe manufactured using *R.oligosporus*.

During prolonged fermentation (0 to 48 hr), starch decreased significiantly ($p<0.05$) in all combinations of the *Kocho*-grass pea tempe

Table 3: **Nintrogenous constituents, soluble protein and dry matter loss of fermented and unfermented *koch*-grass pea combinations (dry weight basis)**

kocho grass pea ratio	Process	NPN%	Free amino acids mg/g	Soluble protein %	Soluble solids %	Dry matter loss%
20:80	Unfermented	0.10±0.01^{a}	11.2±1.5^{a}	1.88±0.02^{a}	7.14±0.10^{a}	

	Fermented	2.40±0.11^{b}	93.3±1.4 ^{b}	19.63±0.63 ^{b}	26.03±4.1 b	12.0±2.1 a
25:75	Unfermented	0.10±0.01^{a}	11.6±1.1 ^{a}	1.83±0.05 ^{a}	6.91±0.11 a	
	Fermented	2.26±0.13^{b}	87.2±3.5 ^{c}	17.43±0.41 ^{c}	25.90±3.2 b	10.7±1.7 b
30:70	Unfermented	0.09±0.01^{a}	12.3±0.9 ^{d}	1.77±0.04 ^{d}	6.66±0.12 c	
	Fermented	1.91±0.02^{c}	79.3±5.3 ^{e}	14.50±0.39 ^{e}	23.40±1.3 d	8.8±2.3 c
40:60	Unfermented	0.09±0.02^{a}	13.6±0.7 ^{f}	1.66±0.03 ^{f}	6.18±0.10 e	
	Fermented	1.55±0.04^{d}	56.4±5.3 ^{g}	8.17±0.12 ^{g}	21.77±1.2 f	5.7±1.1 d
50:50	Unfermented	0.08±0.01^{a}	14.8±0.8 ^{h}	1.55±0.01 ^{h}	5.70±0.12 g	
20:80	Fermented	1.40±0.02^{d}	34.8±5.6 ^{f}	6.83±0.20 ^{f}	20.00±1.5 h	3.6±0.9 e

Values are means of five determinations ±S.D. Values in a column with different letters are significantly different ($p<0.05$).

with concomitant significiant ($p<0.05$) increases in total and reducing sugars. These changes may be attributed to the amylolytic activity of the mould which increased the content of sugars by starch hydrolysis and its fermentation to organic acids (27). Berghofer (28) also reported significiant decrease in starch during field bean tempe preparation.

The tempe mould also possesses a strong lipolytic activity hydrolysing fermentation of *kocho*grass pea combinations. The lipolytic activity of tempe mould was also reported by Wagenkeencht *et al.* (29). The pH showed a progressive increase to more than 7.0, which could be largely attributed to the active proteolysis and deamination of amino acids by the mould. The increase in pH with fermentation time is not unusal, since a similar trend occurs in tempe fermentation (29).

The proteolytic activity of tempe mould could also account for the significant ($p<0.05$) increases in free amino acids and non-protein nitrogen content of fermented *kocho*-grass pea combinations. Matsuo (26) reported an increase in soluble nitrogen/total nitrogen ratio, free fatty acids/crude fat ratio, and free sugar carbohydrate ratio on frementation of *okara* into tempe.

Because of enzymatic degradation of macromolecules into substances of lower molecular weight, there is solublisation effect in the *kocho*-grass pea tempe samples. Due to the digestion of the martix between the kocho and grass pea cells, a significiant reduction in total dry matter and increases in total solube solids, soluble protein and solube carbohydrate were observed. Similar modifications took place in common beans (30), chickpeas and horse beans (31) when made into tempe.

Fermentation for 48 hr significantly ($p<0.05$) decreased the antinutritional factors, trypsin inhabitors and tannins, in the tempe samples. The significiant loss in trypsin inhibitor contents in fermented kocho-grass pea combinations may be attributed to the capability of the tempe mould to hydrolyse the trypsin inhibitors of the combinations. Wang *et al*. (32) also reported that *R. oligosporus* is capable of hydrolysing the trypsin inhibitors of soy beans. Tannin content of unfermented kocho-grass pea combinations appeared to be low. However, fermentation for 48 hr caused a significant ($p<0.05$) increase in the content of this antinutrient. The increase in tannin content of the fermented combinations may be due to the activity of tempe mould enzymes to relaease tannins from tannin-protein complexes during fermentation. Mugula (15) also reported a 42% increase in tannins during the fermentation of sorghum-common bean mixtures into tempe. From the nutritional point of view, tannins depress growth rate, lower protein digestibility, amino acid availability and increase faecal nitrogen (33).

Fermentation also caused significant ($p<0.05$) reduction of phytic acid in tempe

Table 4: **Minerals and antinutritional factors in unfermented and fermented *kocho*-grass pea combinations (dry weight basis)**

kocho grass pea ratio	Process	Fe mg/100g	Ca mg/100g	P mg/100g	Tannins Mg/100g	Phytic acid mg/100g	Trypsin inhibitors IU/g
20-80	Unfermented	2.6±0.3^{a}	131±3 ^{a}	351±3 ^{a}	124±3 a	880±51 a	8088±113 a
	Fermented	2.6±0.2^{a}	130±2 ^{a}	357±3 a	176±6 b	173±19 b	4285±131 b
25:75	Unfermented	2.6±0.5	31±5 a	346±5 ^{a}	120±3 ^{c}	825±38 c	7967±223 a

	Fermented	2.6±0.4[a]	126±2 [a]	341±5 [a]	168±4 [d]	174±17 [b]	3957±103 [c]
30:70	Unfermented	2.7±0.6[a]	131±3 [a]	342±4 [a]	115±2 [e]	770±42 [d]	7697±317 [d]
	Fermented	2.6±0.1[a]	134±4 [a]	349±0 [a]	158±5 [f]	80±9 [b]	3404±97 [e]
40:60	Unfermented	2.7±0.7[a]	131±5 [a]	353±6 [a]	105±3 [g]	660±34 [e]	7158±231 [f]
	Fermented	2.6±0.3[a]	133±3 [a]	345±1 [a]	138±9 [h]	392±21 [f]	2993±177 [g]
50:50	Unfermented	2.6±0.4[a]	131±2 [a]	334±7 [a]	96±6 [f]	550±23 [g]	6619±212 [h]
	Fermented	2.6±0.6[a]	133±6 [a]	347±4 [a]	125±3 [g]	569±27 [g]	1791±87 [f]

Values are means of five determinations ±S.D. Values in a column with different letters are significantly different ($p<0.05$)

from *kocho*-grass pea combinations which may be due to endogenous and mould phytase. Production of phytase by the fungus is well documented (11). The decrease in phytate content would increase the bioavailability of minerals particularly zinc and iron which might be important in the management of anaemia.

The present study has shown that high potential exists for the use of tempe fermentation in improving the nutritional quality of starch foods. The co-fermentation of *kocho* with grass pea in the present study has dual advantages in that protein content was increased while the antinutritional factors content of the *kocho*-grass pea combination decreased, resulting in more nutritive and safer food product. The use of such improved product is suggested at a household level as a way to improve the nutritive value of enset foods such as *kocho* and *bulla*.

Acknowledgement

This study was financially supported by the Ethiopian Nutrition Institute.

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