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TECHNICAL NOTE

Production of improved cassava fufu, 'akpu', through controlled fermentation

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Controlled fermentation of cassava tuber sections using a starter culture consisting of *Citrobacter freundii*, *Geotrichum* spp., *Candida* spp. and *Saccharomyces* spp. yielded the most acceptable product 'akpu', with respect to odour and residual cyanide ($P < 0.05$; $P < 0.01$ in some cases). Although traditional fermentation resulted in a significantly higher protein and lower HCN ($P < 0.01$) content than in all controlled fermentations, the product is significantly more odorous and lower in dietary fibre ($P < 0.05$).

INTRODUCTION

Cassava fufu ('akpu') is made by fermenting freshly harvested cassava tubers in water in open containers for 3 days. 'Akpu' meal produced in this way (mixed culture fermentation) usually has an undesirable odour. This has largely restricted the consumption of the meal to rural areas. The aim of this work was to explore the possibilities of producing odourless 'akpu' through controlled fermentation, using microorganisms isolated from traditional (mixed culture) fermentation.

MATERIALS AND METHODS

Materials

Cassava

Freshly harvested cassava tubers (18 months old) were obtained from a farm in Benin City.

Microorganisms

The microorganisms used in this study were isolated and purified from traditional cassava fermentation in a previous study (Okolie, 1987). The organisms were selected on the basis of dominance. Bacterial and yeast strains were maintained on nutrient and potato dextrose agar slants, respectively.

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Agar, peptone and reagents

Nutrient agar, potato dextrose agar and peptone were products of Oxoid. All reagents used were of analytical grade and were obtained routinely from our laboratory.

Controlled-fermentation flasks

Conical flasks (500 ml) containing 250 ml of 0·1% peptone (in distilled water) were fitted with rubber bungs bearing two glass delivery tubes. The delivery tubes were connected to shorter glass tubes with rubber tubing. Before use, the flasks were sterilized by autoclaving at 121°C and 15 psi for 15 min in an Express model autoclave (Arnold & Sons Ltd, Basildon, UK) and allowed to cool to room temperature. (Peptone was used instead of water in order to provide a nutrient source to enable the microorganisms to adjust to change in a culture environment, thereby shortening the lag phase for cell multiplication.)

Methods

Controlled fermentation of cassava

Freshly harvested cassava tubers were thoroughly washed in tap water and surface sterilized in absolute alcohol for 5 min. After removal of the peel, the tubers were also surface sterilized in absolute alcohol for 5 min. Working in a sterile hood, transverse sections of the

tubers were cut into sterile trays, and a 20 g sample of these sections was rapidly weighed and transferred into each fermentation flask. Some of the flasks were than inoculated with single microorganisms, while others were inoculated with a combination of microorganisms. The size of inoculum in each flask was maintained at 3×10^6 cfu (colony forming unit), as determined by the pour plate technique (Cruickshank *et al.*, 1975). An uninoculated flask containing the same quantity of cassava served as control. A water pump was connected to each flask through one of the delivery tubes. When the taps were turned on, this caused sterile air to be drawn through each flask via the second delivery tube, which was plugged with cotton wool moistened with 0·1% (w/v) $HgCl_2$. Simultaneously, 20 g samples of cassava sections were soaked in 250 ml of water in an open beaker (traditional fermentation). The flasks and beaker were left for 3 days at room temperature (*c.* 29°C), and sensory evaluation and chemical analysis were carried out on the 'akpu' products. In addition, the flasks were checked for contamination by streaking aliquots of their contents on nutrient agar plates and incubating at *c.* 29°C for 48 h. Flasks in which the starter culture were not obtained in pure form were rejected and the fermentation repeated.

Sensory evaluation for odour

The various 'akpu' samples were scored for odour by a randomly selected panel of eight judges on a 10-point hedonic scale. This ranged from 1 (completely odourless) to 10 (extremely odorous).

Determination of residual cyanide

Residual cyanide was estimated according to the procedure outlined by Ikediobi *et al.* (1980). 'Akpu' (2 g) was ground thoroughly in a hand mortar with 30 ml of 0·2 M phosphate buffer (pH 6·8) for 15 min. After centrifuging at 2000 *g* for 5 min, 1 ml of the extract was incubated with 0·5 ml of partially purified cassava cortex linamarase and 0·5 ml of 0·2 M phosphate buffer, pH 6·8, in a quick-fit tube at room temperature (*c.* 29°C). After 10 min, 4 ml of alkaline picrate was added, and the contents were shaken. On warming at 95°C in a water bath for 5 min, an orange colour resulted, which was read at 495 nm in a Pye Unicam UV spectrophotometer against a reagent blank. Corresponding HCN concentrations were read off from a KCN standard curve prepared under the same conditions.

Determination of protein

This was carried out by the classical Kjeldahl digestion. The dried 'akpu' sample (1·5 g) was digested with 20 ml of 90% (w/v) sulphuric acid to clarity in a fume chamber. The diluted digest was added to 8 ml of distilled water and 2·0 ml of Nessler's reagent. It was left for 20 min at room temperature for colour development, and then read at 520 nm. Corresponding $(NH_4)_2SO_4$ concentrations were read off from an $(NH_4)_2SO_4$ standard curve, and multiplied by a factor of 6·25 to convert to protein.

Determination of dietary fibre

This was carried out by the method of Schweizer & Wursch (1979).

Statistical analysis

The results obtained for each parameter in the various fermentations were analysed, using one-way analysis of variance according to the procedure outlined by Bailey (1981).

RESULTS AND DISCUSSION

The results obtained in the sensory evaluation and chemical analysis of the various 'akpu' samples are summarized in Table 1. No results are presented for control cassava because this did not ferment. The absence of fermentation in control flasks would seem to indicate that cassava tubers do not carry endogenous microflora, and that the controlled fermentations were carried out under sterile conditions. A starter culture of *Citrobacter freundii*, *Geotrichum* spp., *Saccharomyces* spp. and *Candida* spp. yielded the most acceptable product with respect to odour and residual cyanide ($P < 0.05$; $P < 0.01$ in some cases). On the other hand, 'akpu' from traditional fermentation was the least acceptable with respect to odour ($P < 0.05$), although it has the least level of residual cyanide. Generally, participation of yeasts resulted in significantly lower HCN in the final product than in purely bacterial fermentations. This is probably due to the fact that yeasts are generally more acid tolerant than bacteria (Akinrele, 1964). Therefore, a combination of bacteria and yeast would result in more efficient lowering of HCN since cassava fermentation results in a decrease in pH (Ngaba & Lee, 1979).

'Akpu' from traditional fermentation has significantly higher protein ($P < 0.01$) than the product of any of the controlled fermentations. Although traditional fermentation resulted in the highest protein enrichment (26·9 mg/g dry wt) and lowest HCN (62 mg/kg dry wt), it was not acceptable on grounds of odour. An acceptable product should, first and foremost, be virtually odourless. It should also be low in residual cyanide. Again, controlled fermentation with *C. freundii* and yeasts satisfies these requirements. Due to the low protein content of cassava, enrichment with single cell protein through controlled microbial growth has been advocated (Azoulay *et al.* 1980). Since traditional fermentation involves a wider range of microorganisms, it is not surprising that it resulted in higher protein enrichment than in controlled fermentations.

Table 1. Sensory evaluation and chemical analysis of 'akpu' from controlled and traditional fermentations

Starter microflora	Odour score[a]	Residual cyanide[b] (mg/kg dry wt)	Protein[c] (mg/g dry wt)	Dietary fibre[d] (% dry wt)
Leuconostoc spp.	6·82 ± 0·31	162 ± 7	6·20 ± 0·45	9·87 ± 0·62
Citrobacter freundii	2·50 ± 0·45	159 ± 6	7·18 ± 0·62	9·02 ± 0·61
Alcaligenes spp.	8·57 ± 0·22	237 ± 17	7·45 ± 0·44	10·4 ± 0·26
Lactobacillus spp.	5·00 ± 0·46	139 ± 5	7·97 ± 0·47	7·17 ± 0·42
C. freundii + *Lactobacillus* spp.	3·38 ± 0·24	110 ± 6	9·25 ± 0·38	7·00 ± 0·71
C. freundii + yeasts[e]	1·47 ± 0·19	87 ± 4	17·0 ± 1·06	4·47 ± 0·56
Alcaligenes spp. + *C. freundii*	5·75 ± 0·32	130 ± 6	8·32 ± 0·51	8·92 ± 0·05
Alcaligenes spp. + yeasts	4·75 ± 0·33	72 ± 3	14·8 ± 0·49	5·62 ± 0·42
Citrobacter + *Lactobacillius* + *Alcaligenes* + *Leuconostoc*	5·87 ± 0·31	136 ± 7	8·90 ± 0·44	4·60 ± 0·29
Citrobacter + *Alcaligenes* + yeasts	4·88 ± 0·13	71 ± 4	16·9 ± 0·81	4·20 ± 0·36
Traditional fermentation	8·00 ± 0·35	62 ± 6	26·9 ± 0·44	2·85 ± 0·51

Values are mean ± SEM of four measurements.
[a] Least significant difference (LSD) = 1·15 (95%); 1·75 (99%).
[b] LSD = 18 (95%); 27 (99%).
[c] LSD = 1·12 (95%); 1·69 (99%).
[d] LSD = 1·75 (95%); 2·65 (99%).
[e] *Saccharomyces* spp., *Geotrichum* spp. and *Candida* spp.

Traditional and controlled fermentations involving yeasts resulted in significantly lower dietary fibre than fermentation involving bacterial strains only ($P < 0·05$; $P < 0·01$ in some cases).

This may be due to the fact that yeasts tolerate lower pH better. As a result, degradation of polysaccharides would proceed for a longer period in yeast fermentations, resulting in lower levels of dietary fibre. Advantages of dietary fibre in the diet include shortening of transit time (Holmgren & Mynors, 1972), and improvement of bowel habit (Burkitt, 1971). In this regard, the results suggest that bacterial fermentations are better than those involving yeasts.

However, since most people shun 'akpu' meal due mainly to sensory (odour) rather than chemical considerations, the most crucial aspect of these results concerns production of odourless 'akpu' meal. This can be achieved by controlled fermentation of cassava, using *C. freundii* in combination with the yeasts *Saccharomyces* spp., *Geotrichum* spp. and *Candida* spp. This finding is considered vital, especially now that importation of foreign food items is being discouraged through government legislation in order to conserve scarce foreign exchange.

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