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The contribution of moulds and yeasts to the fermentation of 'agbelima' cassava dough

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W.K. AMOA-AWUA, J.C. FRISVAD, S. SEFA-DEDEH AND M. JAKOBSEN. 1997. Agbelima, a fermented cassava meal widely consumed in Ghana, Togo and Benin, is produced by fermenting grated cassava with one of several types of traditional cassava dough inoculum. During fermentation a smooth textured sour dough is produced, the toxicity of cassava is reduced and there is a build up of volatile aroma compounds. Four types of inocula were included in the present investigation. In one type moulds were found to form a dominant part of the microbiota, the species present being *Penicillium sclerotiorum, P. citrinum, P. nodulum, Geotrichum candidum* and a basidiomycete. All these moulds were found to possess cellulase activity which was responsible for the hydrolysis of cassava tuber cellulose during fermentation leading to a breakdown of the coarse texture of cassava dough. The yeasts *Candida krusei, C. tropicalis* and *Zygosaccharomyces* spp. were present in high numbers in the four types of inocula including the mouldy inoculum. The yeasts *C. tropicalis* and some strains of *Zygosaccharomyces,* all of which possessed cellulase activity, were also found to contribute to the modification of cassava texture during fermentation. All yeasts and moulds exhibited linamarase activity and were therefore capable of breaking down the cyanogenic glucosides present in cassava.

INTRODUCTION

Traditional processing of cassava into various fermented indigenous products plays an important role in the food supply system of Ghana by contributing to the curtailment of the post-harvest loss of the highly perishable root crop. The root crop is processed by a variety of indigenous methods to convert the tubers into stable products with reduced toxicity and improved palatability. One of the most important of these products is agbelima, which is produced by fermenting cassava dough in the presence of an inoculum into a smooth textured sour cassava meal.

Previous work by Amoa-Awua and Jakobsen (1995) has shown that the breakdown of cassava texture during agbelima fermentation is due to the activity of microbial cellulase and in two types of traditional inocula examined *Bacillus* spp., mainly *Bacillus subtilis*, were identified as the sources of cellulase in the inocula. The souring of agbelima has been found by Amoa-Awua *et al*. (1996) to be due to a lactic acid fermentation by *Lactobacillus plantarum*, *Lact. brevis* and *Leuconostoc mesenteroides*.

In another type of cassava dough inoculum examined, the present authors did not isolate *Bacillus* species in any significant numbers in the microbiota. This type of inoculum, which is produced by superficially drying split cassava pieces which are then stuck under a thatch roof for 2–4 d to ferment, is often covered with dark mycelial growth pointing to a possible role of moulds in the fermentation of cassava dough when this type of inoculum is used for the fermentation.

The involvement of yeasts and moulds in cassava fermentation has been reported in the literature. Hahn (1989) described a solid-substrate air fermentation of cassava in East Africa in which peeled and sliced cassava tubers were surface-dried for 1–2 h, heaped together and covered with straw or leaves to incubate for 3–4 d to enable profuse mould growth. After removal of the fungal mycelium, the fermented pieces were sun-dried and milled into a flour. Essers (1995) made reference to the isolation of the fungi *Geotrichum candidum*,

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Mucor racemosus, *Neurospora sitophila*, *Rhizopus oryzae* and *R. stolonifer* as well as *Bacillus* spp. from farm heap-fermented cassava in Uganda. Aidoo (1986) isolated several species of moulds from dried fermented cassava chips, kokonte, with *Aspergillus* and *Penicillium* spp. as the dominant organisms.

Yeasts of the genus *Candida* have been frequently isolated from fermenting cassava (Okafor 1977; Ejiofor and Okafor 1981; Oyewole and Odunfa 1990). The influence of *Candida* spp. on the typical odour of fufu, a fermented cassava product, has been demonstrated by Oyewole and Odunfa (1990). Amoa-Awua *et al*. (1996) reported both yeasts and lactic acid bacteria to be responsible for the development of volatile aroma compounds produced in agbelima during fermentation.

This study was carried out to determine the mechanism through which the breakdown of cassava texture is achieved when the mouldy type of cassava dough inoculum is used to ferment cassava dough and to find out the possible role of moulds and yeasts in agbelima fermentation.

MATERIALS AND METHODS

Production of inoculum for cassava fermentation

Four different types of cassava dough inocula, A, B, C and D, and inoculated fermenting dough were produced by two traditional cassava processors near Accra (Ghana) for laboratory analysis. They were sampled in duplicate on four separate occasions over a 6-month period and were prepared as follows.

Inoculum A : blanched. Small chunks of peeled cassava tubers, weighing 2 kg, were blanched by boiling for 5 min and wrapped in a piece of cloth which had previously been used to prepare inoculum. They were placed in a basket and left in a warm place for 2 d to ferment into inoculum.

Inoculum B : roasted. Peeled cassava tubers, weighing 2 kg, were cut into small chunks, roasted on a subdued open fire for 10 min and wrapped in a piece of cloth which had previously been used to prepare inoculum. They were placed in a small basket and left in a warm place for 2 d to ferment into inoculum.

Inoculum C : warmed. Small chunks of peeled cassava tubers, weighing 2 kg, were exposed in the open air for 30 min (ambient temperature 30°C) and wrapped in a piece of cloth which had previously been used to prepare inoculum. They were placed in a basket and left in a warm place for 2 d to ferment into inoculum.

Inoculum D : thatch. Small chunks of peeled cassava, weighing 2 kg, were surface-dried in the open air for 6 h (ambient temperature 30°C) and then placed directly under the thatch roof of a hut for 2 d to ferment into inoculum.

Fermentation of agbelima

Fermented cassava meals were produced with the four different types of inocula by adding 0·8 kg of an inoculum to 30 kg of peeled, washed cassava tubers; this was then grated in a cassava grater with milled rasp teeth (Cassava Processing Demonstration Unit, Pokuase, Ghana). The grated cassava mash was collected into aluminium bowls and carefully packed into new polyethylene sacks. The sacks were covered with plastic sheets, placed in baskets and weights placed on top to slowly dewater the mash during fermentation. The bagged doughs were left in the open for 2 d to ferment into the cassava meal, agbelima.

Sampling of inocula and fermenting dough

Samples of inocula or dough at 0, 24 and 48 h of fermentation, weighing between 500 and 1000 g, were aseptically collected into stomacher bags (Seward Medical, London, UK) and taken immediately to the laboratory for analysis. Fermenting dough samples were taken from within the dough after the surface layers had been removed aseptically.

Microbiological analyses

For all samples of inocula or dough, 10 g were added to 90 ml sterile diluent containing 0·1% peptone, 0·8% NaCl, with pH adjusted to 7·2, and homogenized in a stomacher (Lab Blender, Model 4001; Seward Medical) for 30 s at normal speed. From appropriate 10-fold dilutions, yeasts and moulds were enumerated on Malt Agar (MA : 5398; Merck, Darmstadt, Germany) containing 100 mg chloramphenicol (Chloramphenicol Selective Supplement; Oxoid, Unipath Ltd, Basingstoke, Hants, UK) and 50 mg chlortetracycline (C-4881; Sigma, St Louis, MO, USA) per litre and incubated at 25°C for 9 d.

All yeast colonies from a segment ($>15\%$ of the area of a standard Petri dish) of the highest dilution or suitable plate were subcultured in malt-yeast-peptone-glucose broth (MYPG) containing ($g\,l^{-1}$) : yeast extract (0127-17-9; Difco), 3·0; malt extract (0186-17-7; Difco, Difco Laboratories, Detroit, USA), 3·0; bacteriological peptone (L37; Oxoid), 5·0; glucose (1·08342; Merck), 10·0 and streaked onto MYPG agar until pure cultures were obtained. The same procedure was used for moulds except that colonies were subcultivated onto MA.

Identification of yeasts

Yeast isolates were examined by colony and cell morphology and identified to species level by testing for the fermentation and assimilation of carbon compounds in API 20C AUX and ID 32 galleries (bioMerieux sa, Marcy l'Etoile, France) and identified to species level with reference to Kreger-van Rij (1984). The protein and chromosome profiles were determined to support these tests and to investigate subspecies variations within isolates.

SDS-PAGE of yeast isolates. Whole cell yeast proteins were extracted and prepared for electrophoretic analysis as described by Guillamon *et al.* (1993). SDS-PAGE was run in a Multiphor II electrophoresis unit (Pharmacia, Pharmacia LKB Biotechnology, Uppsala, Sweden) with Power Supply EPS 3500 XL (Pharmacia) and a Pharmacia LKB MultiTemp II Cooling Unit (Pharmacia) using either 12 or 15% homogeneous or 8–18% gradient precast gels (ExcelGel; Pharmacia). Electrophoresis was run at 600 V, 50 mA and 20 W, with a cooling temperature of 15°C, and gels were stained with Coomassie blue.

Pulsed field gel electrophoresis of yeast isolates. The chromosomal DNA fingerprints of yeast isolates were determined by pulsed field gel electrophoresis (PFGE) as described by Donhauser *et al.* (1990) with some modifications. Pulsed field gel electrophoresis was run in a Gene Navigator™ system (Pharmacia) consisting of a Gene Navigator electrophoresis unit and a Pulsaphor GN Controller with Power Supply EP 3500 (Pharmacia) and a MultiTemp Cooling Unit (Pharmacia). The electrophoresis was run on an 8% agarose gel with running conditions of 150 V, pulse time 200 s for 24 h followed by a second phase of 100 V, pulse time 700 s for 28 h. The DNA marker used was CHEF DNA Site Markers–*H. Wingei* Chromosomes (Bio-Rad Laboratories GmbH, Munich, Germany).

Identification of moulds

Moulds were identified by examination of colony and cell morphology and detection of both intracellular and extracellular secondary metabolites using thin layer chromatography and high performance liquid chromatography (Pitt 1979; Frisvad *et al.* 1990). The cultures were compared with type cultures to assure correct identification.

For macroscopic examination isolates were cultured on Czapek yeast extract agar (CYA), malt extract agar and yeast extract sucrose agar (Singh *et al.* 1991). Plates were inoculated at three points from a suspension of conidia and other propagules picked up from a colony in a drop of 0·2% molten agar containing 0·05% detergent. Plates were incubated at 25°C for 7 d and examined visually by noting the colour of the colony surface and reverse, presence and colour of exudate and also by measuring the diameter of colonies.

Detection of internal and external metabolites by thin layer chromatography. Intra- and extracellular metabolites produced by isolates were determined by thin layer chromatography using the agar plug method (Filtenborg *et al.* 1983; Frisvad and Filtenborg 1990).

Secondary metabolites assay by high performance liquid chromatography. Secondary metabolites produced by the isolates were determined by high performance liquid chromatography according to Singh *et al.* (1991) in order to confirm the identity of the moulds and also to detect any mycotoxins produced by isolates. High performance liquid chromatography analysis was carried out in an HP 1090 High Performance Liquid Chromatograph (Hewlett Packard, Birkerød, Denmark) equipped with a diode array detector (Avondale Division, Avondale, PA, USA) using a 100×4 mm reversed phase 5 μm C_{18} column (Frisvad and Thrane 1987).

Determination of tissue-degrading enzymes and breakdown of cassava tissue

The ability of yeasts and moulds to produce tissue-degrading enzymes was investigated by assaying cultures for the production of cellulase, pectin esterase and polygalacturonase by methods described by Amoa-Awua and Jakobsen (1995). The ability of isolates to degrade cassava tissue was confirmed by directly inoculating sterile cassava slices with pure cultures as described by Amoa-Awua and Jakobsen (1995).

Tests for linamarase activity

Linamarase was determined as β-glucosidase activity (Ejiofor and Okafor 1981) using an API zym kit (bioMerieux).

RESULTS

Yeasts and moulds in inocula and fermenting cassava dough

With the exception of inoculum D, growth of all inocula samples on MA was composed entirely of yeasts present at levels of 10^5 cfu g^{-1} (Table 1). In inoculum D, the micropopulation contained substantial numbers of moulds in addition to yeasts as determined by colony appearance and cell morphology. It was noted that, during preparation, the inoculum D was usually covered with mould mycelium which was scraped off before the inoculum was used. One type of

Table 1 Dominating yeasts in cfu g^{-1} of inocula and fermenting cassava dough (agbelima)

	Inoculum and inoculated cassava dough			
	Inoculum A (blanched)	Inoculum B (roasted)	Inoculum C (warmed)	Inoculum D (thatch)
Inoculum				
Candida krusei	$3{\cdot}2 \times 10^5$	$5{\cdot}4 \times 10^5$	$4{\cdot}0 \times 10^5$	$4{\cdot}4 \times 10^4$
Candida tropicalis	$4{\cdot}0 \times 10^4$	$5{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^4$	$1{\cdot}3 \times 10^5$
Zygosaccharomyces spp.	$8{\cdot}0 \times 10^4$	ND	$1{\cdot}0 \times 10^5$	ND
Fermentation 0 h				
Candida krusei	$1{\cdot}3 \times 10^6$	$9{\cdot}5 \times 10^5$	$5{\cdot}6 \times 10^5$	ND
Candida tropicalis	$5{\cdot}5 \times 10^5$	$1{\cdot}0 \times 10^5$	$1{\cdot}0 \times 10^5$	ND
Zygosaccharomyces spp.	$1{\cdot}8 \times 10^5$	$3{\cdot}3 \times 10^5$	$1{\cdot}0 \times 10^5$	ND
Fermentation 24 h				
Candida krusei	$4{\cdot}5 \times 10^6$	$6{\cdot}0 \times 10^5$	$1{\cdot}6 \times 10^6$	$2{\cdot}1 \times 10^6$
Candida tropicalis	$1{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^5$	$1{\cdot}6 \times 10^6$	$1{\cdot}0 \times 10^5$
Zygosaccharomyces spp.	$1{\cdot}0 \times 10^5$	$1{\cdot}0 \times 10^6$	$1{\cdot}0 \times 10^5$	$1{\cdot}0 \times 10^5$
Fermentation 48 h				
Candida krusei	$4{\cdot}6 \times 10^7$	$1{\cdot}5 \times 10^6$	$1{\cdot}0 \times 10^6$	$1{\cdot}0 \times 10^5$
Candida tropicalis	$1{\cdot}0 \times 10^6$	$1{\cdot}5 \times 10^6$	$1{\cdot}0 \times 10^6$	$1{\cdot}0 \times 10^5$
Zygosaccharomyces spp.	$1{\cdot}0 \times 10^6$	$9{\cdot}0 \times 10^6$	$1{\cdot}0 \times 10^6$	$1{\cdot}0 \times 10^6$

ND, Not isolated.

mould was isolated in very low numbers in some samples of inoculum C (results not shown).

Growth of fermenting dough samples inoculated with inocula A, B or C was composed almost entirely of yeasts present at levels of 10^5–10^7 cfu g^{-1} throughout fermentation (Table 1). Growth of fermenting dough inoculated with the inoculum D contained substantial numbers of moulds in addition to yeasts, both of which were isolated throughout 48 h of fermentation (Tables 1 and 2).

Three main types of yeasts were isolated from the four different types of inocula and the four different types of fermenting dough. The most frequently isolated yeasts utilized only glucose, *N*-acetyl-glucosamine and DL-lactate out of the 32 carbohydrates tested in the ID 32 galleries and were identified as *Candida krusei*. The second type of yeasts utilized mainly glucose, galactose, sucrose, *N*-acetyl-glucosamine, cellobiose, maltose, trehalose, 2 keto-gluconate, α-methyl-D-glucoside, sorbitol, D-xylose, palatinose, melezitose, gluconate and aesculin and were identified as *C. tropicalis*. The third type of yeasts, *Zygosaccharomyces* spp., utilized only glucose in ID 32 galleries. The population of all three types of yeasts remained high throughout the fermentation of all four types of cassava dough and generally increased 10-fold during fermentation (Table 1).

The protein profiles of various isolates of the yeasts (*C. tropicalis*, *Zygosaccharomyces* spp. and *C. krusei* are presented in Fig. 1 and could be used to distinguish between the dif-

Table 2 The composition and population of moulds in cfu g^{-1} of fermenting cassava dough inoculated with inoculum D

	Inoculum D or fermenting cassava dough inoculated with inoculum D
Inoculum	
Penicillium sclerotiorum	$1{\cdot}0 \times 10^5$
Penicillium citrinum	$5{\cdot}0 \times 10^4$
Penicillium nodulum	$1{\cdot}0 \times 10^4$
Geotrichum candidum	$1{\cdot}0 \times 10^4$
Basidiomycetes	$> 10^4$
Fermentation 0 h	
Penicillium nodulum	$> 10^4$
Penicillium sclerotiorum	$1{\cdot}0 \times 10^4$
Penicillium citrinum	$1{\cdot}0 \times 10^4$
Geotrichum candidum	$1{\cdot}0 \times 10^4$
Basidiomycetes	$> 10^4$
Fermentation 24 h	
Penicillium nodulum	$7{\cdot}0 \times 10^4$
Penicillium sclerotiorum	$1{\cdot}4 \times 10^4$
Penicillium citrinum	$1{\cdot}0 \times 10^4$
Basidiomycetes	$> 10^4$
Fermentation 48 h	
Penicillium citrinum	$3{\cdot}2 \times 10^4$

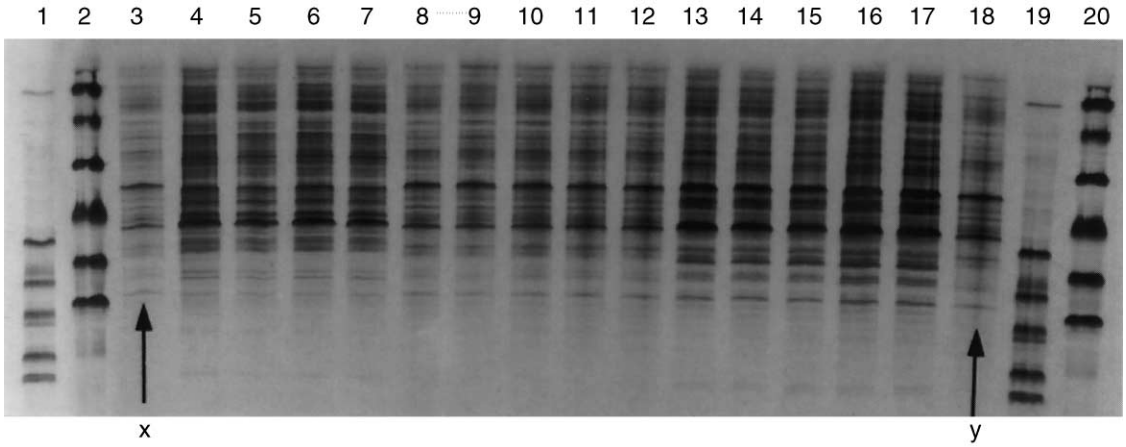

Fig. 1 SDS-whole cell protein fingerprints of yeasts isolated from fermenting agbelima (cassava dough) run on 8–18% gradient gel. Lanes: 1 and 19, high molecular weight protein standard; 2 and 20, low molecular weight protein standard; 3, R4-M5 (unknown isolate, x); 4–7, *Candida tropicalis*; 8–12, *Zygosaccharomyces* spp.; 13–17, *Candida krusei*; 18, T2-M1 (unknown isolate, y)

ferent yeast species and to support the identification based upon patterns of fermentation and assimilation of carbon compounds. The different species could be more easily distinguished on the 8–18% gradient gel than on 15% homogeneous gels (results not shown). In Fig. 1, unidentified yeast strains were run in tracks 3 and 18 (labelled x and y) and could be identified as *Zygosaccharomyces* spp. by comparison with the profiles of the known species.

The chromosomal DNA fingerprints of the yeasts are shown in Fig. 2. The differences between the chromosomal DNA fingerprints of the different yeast species were very profound and could be used to identify the different species. The size and number of chromosomes found agree with published information for *Candida* spp. (Doi *et al.* 1992) and *Zygosaccharomyces* spp. (Török *et al.* 1993). The DNA profiles also seem to discriminate between strains within the species, as most clearly demonstrated for the large chromosomes of *C. krusei* isolates (Fig. 2, lanes 11–15).

Of the four types of inocula and fermenting dough examined, moulds formed an important part of the biota of only inoculum D and fermenting dough inoculated with this type of inoculum. However, the population of moulds varied markedly in the different samples of inoculum D and subsequently fermented dough samples. This is probably because at the end of preparation of inoculum D, the fungal mycelium usually covering the product is carefully scraped off and the inoculum washed before use. The inoculum D samples examined in these studies were collected after they had been cleaned by the processors and were ready to be used to inoculate cassava dough.

Several different types of moulds were isolated in samples of inoculum D and subsequently inoculated fermenting

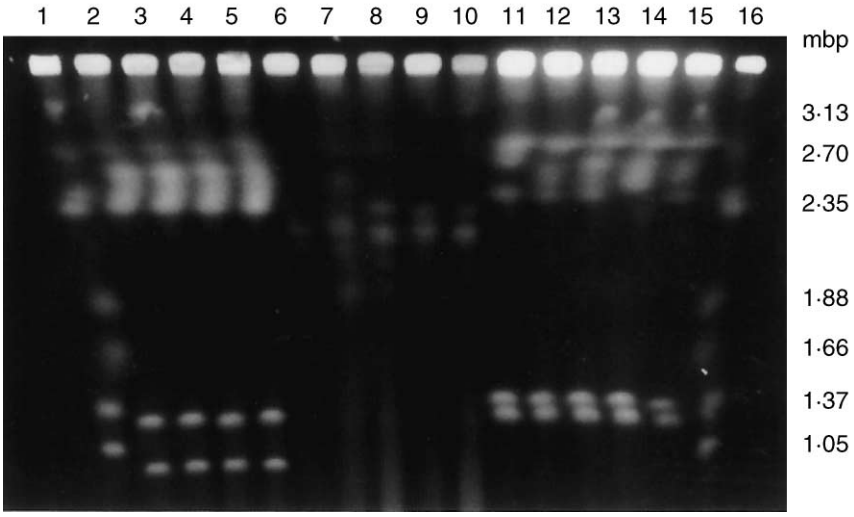

Fig. 2 Chromosomal DNA profiles (pulsed field gel electrophoresis) of yeasts isolated from fermenting agbelima (cassava dough). Lanes: 1 and 16, DNA marker; 2–5, *Candida tropicalis*; 6–10, *Zygosaccharomyces* spp.; 11–15, *Candida krusei*

dough. The moulds were usually present in the inoculum and during the first 24 h of fermentation and most species were not isolated in the final product (Table 2).

The most frequently occurring moulds had green colonies with a white border and yellow colony reverse with a red or yellowish red core when grown on CYA. These colonies attained a diameter of about 3·7 cm after 7 d incubation at 25°C in complete darkness and produced the secondary metabolites sclerotiorin, rotiorin and 7-epi-sclerotiorin and were identified as *Penicillium sclerotiorum*. They were present at levels of 10^5 cfu g^{-1} in the inoculum and were also detected during the first 24 h of fermentation (Table 2). A second type of mould identified as *P. nodulum* had green colonies with a white outline and yellow colony reverse with diameters of about 2·1 cm. They produced some unknown compounds as secondary metabolites and were present at levels of 10^4 cfu g^{-1} in the inoculum and were detected during the first 24 h of fermentation. A third type of mould produced a clear exudate on the surface of its green colonies with a white outline and had yellow colony reverse with diameters of about 2·8 cm. They produced citrinin and were identified as *P. citrinum*. This was the only mould detected at the end of fermentation. A fourth type of mould, *G. candidum*, had off-white colonies with an off-white colony reverse of diameter 4·4 cm and produced pyroclavine. They were present in the inoculum at 10^4 cfu g^{-1} and only isolated at the beginning of fermentation. Another mould tentatively identified as a basidiomycete had white fluffy colonies with an off-white colony reverse and diameters of about 2·0 cm. It was present in low numbers in the inoculum and during the first 24 h of fermentation. A mould identified by colony morphology as a *Rhizopus* spp. was isolated in very low numbers in inoculum D but not in the inoculated fermenting dough. *Aspergillus flavus*, also identified by colony morphology, was isolated in one of the three samples of inoculum D examined (results not shown).

No moulds were isolated in inocula A or B nor in samples of fermenting dough inoculated with these types of inocula. *Geotrichum candidum* was the only mould isolated from inoculum C and was also present during the initial stages of fermentation of the dough inoculated with this type of inoculum. This mould was not found in the final product. Similar results were obtained on the three other occasions of sampling from production sites (results not shown).

Breakdown of cassava tissue by isolates

As seen from Table 3, *C. tropicalis* and some strains of *Zygosaccharomyces* spp. showed weak cellulase activity and were able to break down cassava tissue on prolonged incubation. However, none of the isolates of *C. krusei*, the most frequently isolated yeast from agbelima, were able to disintegrate cassava tissue or tested positive for the production of cellulase. All yeast strains examined produced polygalacturonase but none produced pectin esterase. All moulds examined were able to produce cellulase and also to break down cassava tissue but showed no polygalacturonase or pectin esterase activity with the exception of a *Rhizopus* spp. which tested positive for pectin esterase (Table 3). The ability of the *Rhizopus* spp. to break down cassava tissue was not determined because the

Table 3 Production of cassava tissue-degrading enzymes by yeasts and moulds isolated from cassava dough inocula

Isolate	Cellulase	Polygalacturonase	Pectin esterase	Softening of cassava tissue
Yeasts*				
Candida tropicalis	+‡	+	−	+
Candida krusei	−	+	−	−
Zygosaccharomyces spp.	−§	+	−	+‖
Moulds†				
Penicillium sclerotiorum	+	−	−	+
Penicillium citrinum	+	−	−	+
Penicillium nodulum	+	−	−	+
Geotrichum candidum	+	−	−	+
Rhizopus spp.	+	−	+	ND
Basidiomycetes	ND	−	−	+

* Five strains of each species of yeast examined.
† With the exception of *Rhizopus* spp. and Basidiomycetes spp., four isolates of each mould examined.
‡ Some strains weakly positive.
§ Some strains hydrolysed cassava tissue.
‖ Most strains weakly positive.
ND, Not determined.

profuse mycelial growth of the *Rhizopus* spp. made it difficult to control the experiment.

Linamarase activity

In this study the detection of β-glucosidase activity was interpreted as an indication of linamarase activity (Okafor and Ejiofor 1986). Of the yeasts, only *C. tropicalis* tested positive for the production of β-glucosidase whilst all moulds, with the exception of *G. candidum*, exhibited linamarase activity at high levels, most strains producing at levels that could hydrolyse from 30 to over 40 nmol of the substrate as estimated from the API zym kit (results not shown). All *Penicillium* species in particular produced high levels of β-glucosidase.

DISCUSSION

Traditional cassava processors use inoculum to ferment cassava dough during agbelima production because the added inoculum has the ability to break down the texture of cassava dough yielding a smooth textured dough. In the use of inoculum D, the present study has shown that the moulds *P. sclerotiorum*, *P. nodulum*, *P. citrinum*, *G. candidum* and, to some extent, the yeasts *C. tropicalis* and *Zygosaccharomyces* spp., are the main agents responsible for the breakdown of cassava tissue during the fermentation of cassava dough. The contribution of yeasts to the modification of cassava texture, in comparison to the moulds, has been assigned a secondary role because the yeast isolates showed weak production of cellulase as estimated from the diameter of the clearing zones around colonies during tests for cellulase activity. They could also hydrolyse cassava tissue only on prolonged incubation.

Since fermentation of cassava dough into agbelima is carried out under fairly anaerobic conditions and mycelial growth does not proliferate through the medium during fermentation, it can be argued that, in the use of inoculum D, most of the cellulase enzymes are elaborated by the moulds during the preparation of inoculum and are therefore present in the inoculum before its use. This supports the observation by traditional cassava processors that if too high a ratio of inoculum to cassava dough is used it results in the liquefaction of cassava dough within a few hours of fermentation which is considered undesirable.

Tudor and Board (1993), in their review, list *C. tropicalis*, *C. krusei* and several species of *Zygosaccharomyces* as food spoilage yeasts but, in the present work, *C. tropicalis* and *Zygosaccharomyces* spp. have been shown to play a positive role in cassava fermentation by contributing to the modification of the texture of the product. *Candida* species have also been shown by other workers to exert a positive influence on food fermentation by contributing to the development of flavour in various products (Oyewole and Odunfa 1990; Jespersen *et al*. 1994).

In the present work *C. krusei* was the yeast isolated in the highest numbers in all four types of dough. Surprisingly, however, *C. krusei* was unable to hydrolyse cassava tissue whilst *C. tropicalis* and *Zygosaccharomyces* spp., which were present at lower levels, were able to modify the texture of cassava during fermentation. The isolation of *C. krusei* and *C. tropicalis* in the present work is in agreement with the findings of Adjei (1990) who isolated *Candida* spp. from a traditional cassava dough inoculum.

The usefulness of SDS-PAGE determinations of protein patterns and PFGE analysis for determination of chromosome profiles has been demonstrated in this study. They may even discriminate between yeasts at subspecies level (Fig. 2) and they are rapid and more easily interpreted than the conventional identification techniques. Previous studies (Jespersen *et al*. 1994) demonstrated the value of using PFGE for the characterization of isolates of *Saccharomyces cerevisiae* from fermented maize in West Africa.

Since cellulase activity appears to be the mechanism through which degradation of cassava tissue is achieved during fermentation, it is not surprising that moulds were found as a dominant biota in one type of inoculum examined in this study. Wood, which is made up mainly of cellulose in addition to hemicellulase and lignin, is mainly decayed by aerobic fungi (Coughlan 1990). In what is described as a partial list of cellulolytic fungi, Ljungdahl and Eriksson (1985) named 60 different species. Padmaja and Balagopal (1985) found that *r. oryzae*, one of the moulds isolated from inoculum D in the present work, elaborated cellulases capable of degrading cassava tuber cellulose.

The dominant mould isolated from inoculum D, *P. sclerotiorum*, produced the secondary metabolite sclerotiorin, which has been demonstrated to have inhibitory activity against the binding of HIV gp120-CD4 envelope protein with a CD4 molecule on the surface of most susceptible cells during the entry of the HIV virus in AIDS infection (Omura *et al*. 1993). One of the isolated moulds, *P. citrinum*, however, produces the mycotoxin citrinin. Although *Aspergillus flavus*, which is often responsible for the production of aflatoxins, was isolated in one of three samples of inoculum D examined, previous examinations (unpublished results) did not detect aflatoxin in three market samples of agbelima.

This work and previous studies by the present authors (Amoa-Awua and Jakobsen 1995; Amoa-Awua *et al*. 1996) have shown that four principal activities occur when traditional inoculum is used to ferment cassava dough into agbelima. (i) During fermentation the coarse texture of cassava dough is broken down through the hydrolysis of cassava tuber cellulose by microbial cellulase. The cellulolytic microorganisms responsible for the breakdown of cassava dough texture in four different types of traditional inocula are summarized in Table 4. (ii) A souring of cassava dough occurs through the production of lactic and acetic acids by the

Table 4 Micro-organisms responsible for the breakdown of cassava tissue during the fermentation of cassava dough into agbelima

Fermenting dough inoculated with			
Inoculum A (blanched)	Inoculum B (roasted)	Inoculum C (warmed)	Inoculum D (thatch)
Bacillus subtilis *Bacillus* spp. *Candida tropicalis* *Zygosaccharomyces* spp.	*Bacillus subtilis* *Bacillus* spp. *Candida tropicalis* *Zygosaccharomyces* spp.	*Bacillus subtilis* *Bacillus* spp. *Candida tropicalis* *Zygosaccharomyces* spp.	*Penicillium sclerotiorum* *Penicillium citrinum* *Penicillium nodulum* *Geotrichum candidum* *Rhizopus* spp. *Candida tropicalis* *Zygosaccharomyces* spp.

lactic acid bacteria, *Lact. plantarum*, *Lact. brevis* and *Leuc. mesenteroides*. (iii) There is a reduction in the level of cyanogenic glucosides present in cassava leading to partial detoxification of the product. Even though the detoxification may be attributed to the hydrolysis of the cyanogenic glucosides by endogenous cassava linamarase, the major lactic acid bacteria, yeasts and moulds involved in agbelima fermentation all produce linamarase capable of breaking down the cyanogenic glucosides. Detoxification of cassava is enhanced in the presence of the traditional inoculum because of the breakdown of cassava tissue resulting in more intimate contact between the cyanogenic glucosides and linamarase. (iv) Volatile aroma compounds, mainly 1-propanol, isoamylalcohol, ethylacetate, 3-methyl-1-butanol acetoin and a non-identified low molecular weight alcohol, are produced by lactic acid bacteria and yeasts during the fermentation.

All the microbial and biochemical activities which occur during the fermentation of cassava dough into agbelima in the presence of traditional inoculum are summarized schematically in Fig. 3.

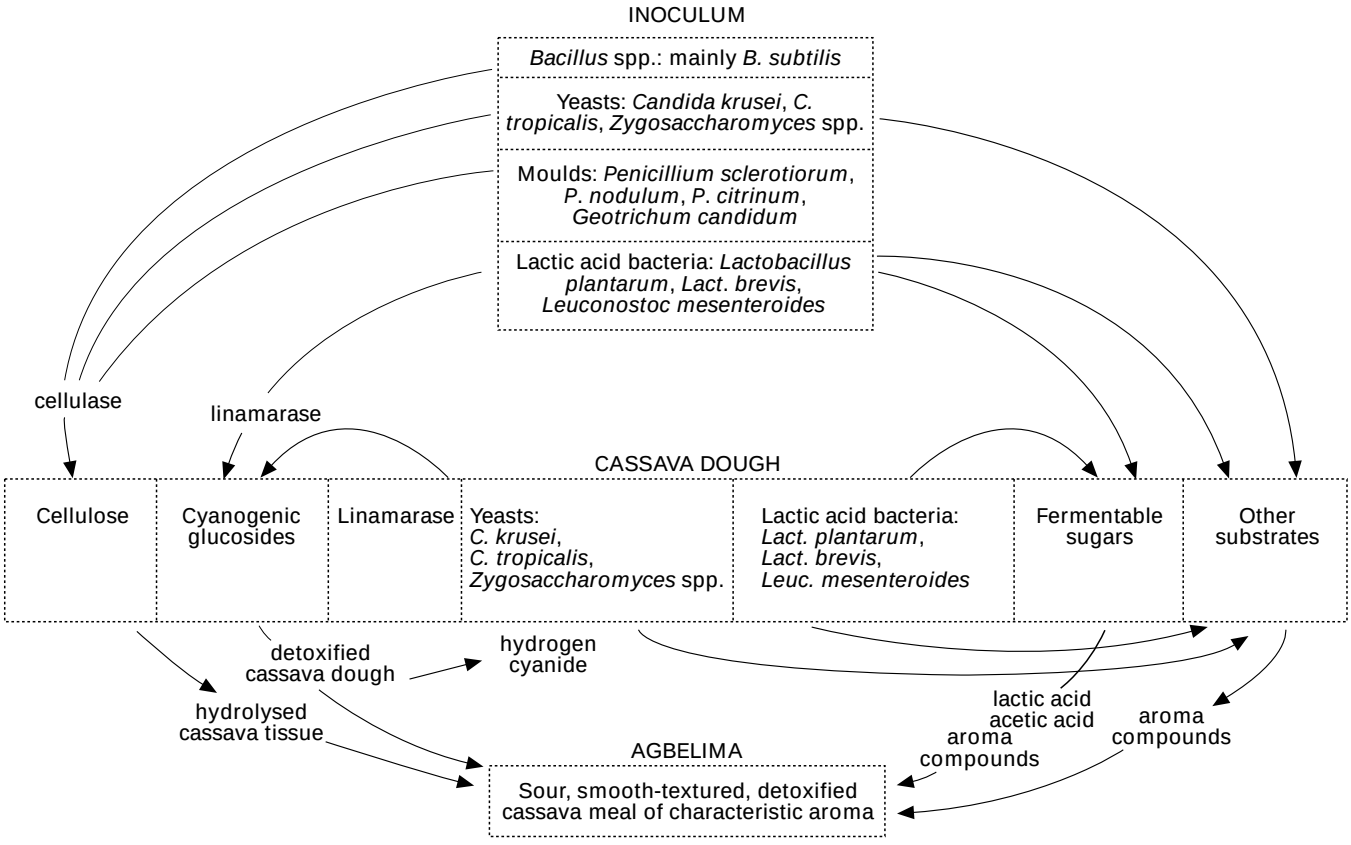

Fig. 3 Fermentation of cassava into agbelima

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