Transformation Alimentaire du Manioc.
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Application of Biotechnology to cassava processing in Africa

Utilisation des biotechnologies à l'amélioration de la transformation du manioc en Afrique

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- Abstract -

Cassava (*Manihot esculenta*) is one of the most important food crops in Africa and many parts of the tropics. In Africa, fermentation is an important means of processing raw cassava root into food. The role of various microorganisms in fermentation processes have been shown to include that of detoxification, flavour development and preservation. This presentation reports on work on the traditional submerged fermentation of cassava and its optimization. The characteristics and role of lactic acid bacteria in cassava fermentation are also presented while efforts at developing appropriate starter cultures are reported. The need to improve the bacterial strains through biotechnological techniques are highlighted while future research needs and strategies for the biotechnological improvements in cassava processing are presented.

- Résumé -

Le manioc (*Manihot esculenta*) est l'une des plus importantes plantes alimentaires en Afrique et sous les tropiques. En Afrique, la fermentation est une opération importante de la transformation des racines brutes en aliments. Le rôle des microorganismes de la fermentation dans le processus de détoxication, dans le développement de la saveur et dans la conservation de l'aliment ont été confirmés.Cet article présente des travaux sur la fermentation traditionnelle par immersion dans l'eau des racines en vue de son optimisation. Les caractéristiques et le rôle des bactéries lactiques dans la fermentation du manioc sont également présentés ainsi que les efforts effectués pour développer des starters appropriés.

Le besoin d'améliorer davantage les souches bactériennes en utilisant des procédés biotechnologiques sont soulignés dans le même temps que sont présentées les priorités de recherche et les stratégies à mettre en oeuvre pour l'amélioration de la transformation du manioc par des méthodes biotechnologiques.

Introduction

Cassava (*Manihot esculenta Crantz*) is a major source of energy for millions of people in the tropics (de Bruijn and Fresco, 1989) and is also one single crop that is helping to alleviate food crisis problems in many war-torn and drought ravaged parts of Africa (Hahn and Keyser, 1985). Cassava is currently playing an important role in solving the food insecurity problems in these regions because it has a comparatively high biological efficiency of food-energy production and the ability to survive and grow under very adverse weather conditions (Cock, 1985).

In spite of these, cassava is often castigated as an "inferior food crop" (Kwatia, 1986); as "poor peoples crop" (Hahn and Keyser, 1985) and as a "dangerous crop" (Cheok, 1978). These myths on cassava were due to some limitations in the crop. The major "limitations" of cassava as food include the presence of toxic cyanogenic glucosides, low protein content and short post-harvest shelf-life. Traditionally, most of these constraints have been met through processing. Cassava is processed to remove or reduce the toxic glucosides, improve palatability as well as serve as a means of preservation (Nambissan and Sundaresan, 1985). Various village processing methods are known which include boiling, smoking, drying and fermentation. Fermentation of cassava is by far the most important and widely used means of processing cassava (Oyewole, 1992).

As of date, the age-old traditional processing of cassava is still being used. These practice is however, plagued with so many problems for which modern biotechnology offers the best solution. For example, traditional fermentation processes depend on chance inoculations from the environment. As a result, the fermentation period is rather long, the quality of the products varies from one processor to the other, or from one production batch to the other by the same processor, and from one season to the other. Improvements in cassava processing would help to reduce the duration of processing to economically viable limits, maximise the detoxification process and improve the physical and nutritional qualities of cassava products.

Biotechnology has been identified as a scientific tool that could be used to meet the current challenges in the traditional fermentation processing of cassava (Bokanga, 1992). This understanding guides our research on cassava fermentation. Our approach to cassava processing research involves investigations on the science of the traditional fermentation process, optimization, and improvement of the process and quality of the products through biotechnological techniques. This presentation will therefore highlight our current understanding on cassava fermentation and the role of biotechnology to its' improvement.

1. Cassava Fermentation Process

Cassava fermentation has been categorized into solid state and submerged fermentation processes (Oyewole, 1992).

1.1. Solid State Cassava Fermentation

The major feature of old state fermentation processing of cassava is that the cassava root is not soaked in water. There are two major variations in solid state fermentation of cassava.

The first is typified by the West African "gari" or the Brazilian "farinha de mandioca" production method where peeled cassava roots are grated, packed into polypropylene or jute sacks and subjected to pressure using heavy weights or hydraulic pressure for 3 to 5 days of fermentation (Okafor, 1977; Ofuya *et al.*, 1990). The fermented mass is further dewatered, sieved and roasted (garification) before consumption.

In the second variation cassava roots are not grated, but cut into pieces or sliced before being spread out in the open air or under the sun (Essers and Nout, 1989). The dried products are the milled into flour, cooked into a stiff dough before consumption with sauce.

1.2. Submerged Fermentation Processes

Cassava roots, peeled or unpeeled, whole or cut into pieces are soaked in water for the duration of fermentation (Oyewole and Odunfa, 1989). The duration of soaking varies with the weather, where relatively short periods (2-3 days) are used during the hot dry season and longer periods (4-7 days) during the cold raining season. The fermented roots may be wet-sieved and the mash cooked in boiling water to a stiff dough called fufu in Nigeria (Oyewole and Odunfa, 1989) or subjected to further processing which may include sieving, sundrying, smoking and milling into flour. The flour so obtained may then be cooked to stiff dough called lafun in Nigeria (Oyewole and Odunfa, 1988).

2. Biotechnological Investigations

The study on cassava fermentation have been solely devoted to the submerged fermentation process. Investigations have been carried out on:

1) The village fermentation process and its optimization
2) Lactic acid bacteria
3) Starter culture development.

2.1. Village Fermentation Process

The submerged fermentation of cassava to lafun and fufu is mainly an acidic fermentation process during which the pH of the cassava roots decreases from 6.5 - 6.9 to 3.8 - 4.1 after 84 hours of soaking in water.

A wide spectrum of microorganisms have been implicated in cassava fermentation. Oyewole and Odunfa (1988) isolated *Bacillus spp., Leuconostoc spp., Klebsiella spp., Corynebacterium spp., Lactobacillus spp., Aspergillus spp., Candida spp.*, and *Geotrichum spp.* A pattern of succession in microorganisms was found to take place during the submerged fermentation of cassava. *Bacillus spp., Corynebacterium spp., and Klebsiella spp.*, which were present at the beginning of fermentation decreased gradually as the process progressed because they could not withstand the increasing acidity of the medium. This first group of organisms were, however, found to play important roles in the fermentation process as most of these strains are capable of producing amylase enzymes needed for the initial breakdown of starch into sugars. The produced sugar is needed for the growth of other microbial groups and for acid production. In the submerged fermentation process, some moulds were occasionally encountered and when present, they disappeared after 36 hr of fermentation due to the low oxygen tension which develop in the soak-water. The latter period of the fermentation was dominated by yeasts and lactic acid bacteria.

Studies have been reported on the solid state fermentation of cassava (Okafor, 1977; Abe and Lindsay, 1978; Ngaba and Lee, 1979). In these studies, the spectrum of microorganisms implicated in the fermentation of grated cassava root for the production of gari were similar to those found by Oyewole and Odunfa, (1988) with lactic acid bacteria and yeasts dominating the latter periods of the process. However, the spectrum of bacteria is different for the solid state fermentation of ungrated cassava roots. Essers and Nout (1989) reported that moulds predominate in such products yielding dark-coloured, dry cassava pieces. The moulds found were *Rhizopus spp., Mucor spp., Penicillium spp.*, and *Fusarium spp.* The products of mould-fermented cassava have been reported to be safe (Thambirajah, 1989).

Submerged fermentation affects carbohydrate, protein and mineral contents of cassava roots (Oyewole and Odunfa, 1989). Fermentation also causes a reduction in starch content while the total soluble and reducing sugar levels are increased during the first 36 h and 24 h, respectively. Sugars are reduced during the latter periods of fermentation due to utilization by microrganisms and the conversion of sugars into organic acids. Fermentation also causes increases in cassava calcium levels (12%) with reductions in manganese (53%), potassium (71%) sodium (68%), iron (50%), copper (7%), zinc (85%) and phosphorus (67%) levels.

In the investigations on the optimization of submerged cassava fermentation through process control, the size to which the roots were cut was found to affect the rate of fermentation and the quality of product(Oyewole and Odunfa, 1992). A temperature range of 30-35°C was found best for the submerged fermentation process while a soaking period of not less than 60 h was appropriate to obtain a good quality product using the village fermentation method. In the same investigation, amylase and pectin-methyl esterase activities were reported to be involved in cassava root softening during the submerged fermentation process. A process has been developed to enrich fermented cassava with legume protein (Oyewole and Aibor, 1992). The developed scheme which resulted in an increase in the protein content of fermented cassava from 1.8% to 5.5% with cowpea and 8.2% with soybean, involved the addition of legume flour (20%) to fermenting cassava after 48 hrs and their co-fermentation for the remaining period of the fermentation.

2.2. The Lactic Acid Bacteria

Lactic acid bacteria are an important group of microorganisms which have been consistently isolated from fermenting cassava (Okafor, 1978; Abe and Lindsay, 1978; Ngaba and Lee, 1979; Oyewole and Odunfa, 1988). The involvement of more than one species of lactic acid bacteria during fermentation necessitated further studies on the spectrum of lactic acid bacterial flora in cassava fermentation (Oyewole and Odunfa, 1990). Different groups of lactic acid bacteria were isolated from fermenting cassava during "fufu" production and these included *Lactobacillus cellobiosus, L. bulgaricus, L. brevis, L. coprophilus, L. plantarum* and *Leuconostoc mesenteroides.* A succession trend was also established among the lactic acid bacteria with Lactobacillus plantarum being predominated during the last 36 hours of submerged fermentation.

A study was carried out on the role and activities of *Lactobacilli spp.* in cassava fermentation studied.

The role of the *Lactobacilli spp.* in starch hydrolysis and cassava detoxification were investigated. A total of 43 *lactobacilli* strains isolated at different times during cassava fermentation were screened for their abilities to hydrolyse starch and linamarin which is the main cyanogenic glucoside in cassava. Twenty-four of the isolates were able to hydrolyse linamarin and most of these (83%) belonged to the *Lactobacillus plantarum* group. The linamarase enzyme responsible for linamarin breakdown produced by one of the *Lactobacillus plantarum* strains (*GL 721*) were purified and characterized. Optimum linamarase activity was obtained at pH 5-7 with a temperature range of 30-40°C. The physiological properties of linamarase elaborated by the *L. plantarum* strain were

similar to those produced endogenously by cassava plant materials (Yeoh, 1989). This study confirms that cassava detoxification during submerged fermentation, where the roots were not grated, involves enzymes from both the plant material and microorganisms (Oyewole and Odunfa, 1991).

When screened for amylase production, over 80% of the *lactobacilli* strains were able to hydrolyse starch. *L. plantarum* strain GL 721 optimum amylase activity was found at pH 5.8 and temperature 30-40°C.

Due to the detoxifying and amylolytic characteristics of the *Lactobacilli* strains isolated from fermenting cassava, this group of microrganisms has been identified as appropriate for development of starter cultures for cassava fermentation.

A part from selecting microorganisms with multiple characteristics for starter culture development, it was necessary to carry out genetic studies on the selected strains. However, because of the importance of plasmids in bacteria genetic studies, the *lactobacilli* strains were screened for their presence. Plasmids of various sizes were found to be present in 27% of the *lactobacilli* strains screened. The sizes of the plasmids range from 2.1 - 52 Kb. However, no correlation was found between the possession of plasmids and the ability of the isolates to hydrolyse linamarin or starch.

3. Starter Cultures Development

Four different microorganisms (*Bacillus subtilis, Klebsiella spp., Lactobacillus plantarum* and *Candida krusei*) were singly inoculated into sterilized cassava tubers as single inoculum for cassava fermentation (Oyewole, 1990). The role of the single inocula in natural fermentation was identified as acid production. The highest level of acid production was recorded for cassava roots inoculated with *Lactobacillus plantarum* while those inoculated with *Bacillus subtilis* showed the highest rate of softening. However, all roots inoculated with each of the organisms showed variable degrees of softening. The characteristics flavour of fermented cassava was noted to be highest in roots inoculated with *Candida krusei.* The different microrganisms had specific and complementary roles during cassava fermentation.

In a similar manner, *Lactobacillus plantarum (GL 721)* was also investigated as a single starter culture for cassava fermentation since it had hydrolytic action on cyanogenic glucoside and starch. Oyewole and Odunfa, (1991) reported that, within the first 36 h of fermentation, the rate of acidification was relatively lower in *L. plantarum (GL 721)* than in the natural process. During this period, the pH for *L. plantarum (GL 721)* fermentation stayed above 5.0 while in the natural process it was 4.4-4.7.Thereafter, acid production stabilised and increased rapidly to normal level at the end of fermentation.

Further work still need to be carried out on the development of appropriate starter cultures for cassava fermentation processes. Starter cultures will help to standardise the processing, optimise the microbial activities in the detoxification and acid production stages to the extent that the duration of processing may be reduced to levels that will meet today's time constraints. There is still the need to develop appropriate carriers for the starter cultures.

Future Challenges

In spite of various studies on cassava fermentation, the age-old technique is still being practised. Fermentation is still relatively long and the quality of the products variable. The current knowledge on microorganisms needs to be translated into packages that will benefit cassava processors.

In this study, a single lactic acid bacteria with multiple capacities (starch hydrolysis, acid production and detoxifying enzyme production) was selected. The strain was appropriate for starter culture development. Further investigations are needed on the starter cultures in various carriers and under different environmental and storage conditions.

Strains for starter culture development could be improved through the genetic and molecular studies. Cassava fermenting microorganisms and their enzymes could be engineered to carry out specific desired functions or improved through modern biotechnological studies. There is the prospect of genetically improving the nutritional quality of cassava through the use of protein enriching microorganisms. The problems of cassava wastes needs to be challenged with biotechnological solutions.

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