CHAPTER 22

FERMENTATION IN CASSAVA BIOCONVERSION[1]

M. Raimbault, C. Ramírez Toro**, E. Giraud***, C. Soccol†, and G. Saucedo††*

Introduction

Cassava fermentation is traditionally practiced in the tropics. But both technology and product characteristics differ according to region and sociocultural conditions: *gari* in East and West Africa, *chikwangue* or *fufu* in Central Africa, and sour starch in Latin America. But they have in common the aim to eliminate the poisonous cyanide components and conserve cassava by lactic acidification.

The essential role of lactic acid bacteria in the three products was demonstrated by studies carried out by the Institut français de recherche scientifique pour le développement en coopération (ORSTOM) through the STD2 Program of the European Union (EU), otherwise known as "Improving the Quality of Traditional Foods Processed from Fermented Cassava" (Raimbault, 1992; Saucedo et al., 1990).

When producing *gari*, lactic acidification of cassava is rapid and detoxification is sometimes incomplete. Controlling through inoculation would improve quality. For *fufu* or *chikwangue*, retting is essential for texturing and detoxifying the cassava. Lactic acid fermentation is heterolactic, operating in association with secondary alcoholic and anaerobic fermentation to produce alcohol and organic acids such as butyrate, acetate, and propionate that develop special aromatic and organoleptic characteristics. As for *gari*, fermentation for sour starch (especially in Colombia and Brazil) is homolactic, but takes 3 or 4 weeks. Amylolytic lactic acid bacteria have been isolated from *chikwangue* by ORSTOM scientists and from sour starch by CIRAD scientists.

A. Brauman isolated a new strain, *Lactobacillus plantarum* A6, which was described by Giraud et al. (1991). Its physiological and enzymological characteristics for cultivation on cassava starch media, amylase production, and biochemical

* Institut français de recherche scientifique pour le développement en coopération (ORSTOM), stationed in Cali, Colombia.
** Laboratorio de Bioconversión, Departamento de Procesos Químicos y Biológicos, Facultad de Ingeniería, Universidad del Valle, Cali, Colombia.
*** ORSTOM, Montpellier, France.
† Laboratório de Procesos Biotecnologia, Departamento de Tecnologia Química, Faculdade de Engenharia, Universidade Federal de Paraná, Brazil.
†† Departamento de Biotecnología, Universidad Autónoma Metropolitana (UAM), Iztapalpa, Mexico.

1. No abstract was provided by the authors.

properties have now been described (Giraud et al., 1992; 1993a; 1993b).

ORSTOM scientists have been researching solid fermentation cultivation of fungi on cassava and amylaceous components for more than 10 years. Soccol et al. (1994) showed that protein enrichment is possible by cultivating various strains of *Rhizopus*, even on crude, nongelatinized cassava flours. Saucedo et al. (1992a; 1992b; 1992c) studied, at the ORSTOM Laboratory, Montpellier, the growth and alcohol fermentation of cassava starch in solid-state fermentation, using a highly promising amylolytic yeast.

Swedish and African researchers have described the beneficial effects of lactic acid fermentation on the prophylactic and keeping characteristics of those traditional foodstuffs made from fermented cassava, maize, and mixed cereals, and of baby foods. These foods tend to increase children's resistance to diarrhoea.

All these studies are being continued in new projects comprising the EU-STD3 Program. Other EU studies are being conducted on cassava quality, environment, physical processing, and transformation at a low industrial scale to take advantage of the economic and commercial opportunities in Latin America.

Solid-State Fermentation of Cassava and Starchy Products

For more than 15 years, an ORSTOM group has worked on a solid-state fermentation process for improving the protein content of cassava, potatoes, bananas, and other starchy commodities used for animal feed. Fungi, especially from the *Aspergillus* group, are used to transform starch and mineral salts into fungal proteins (Oriol et al., 1988a; 1988b; Raimbault and Alazard, 1980; Raimbault and Viniegra, 1991; Raimbault et al., 1985). Table 1 shows the overall changes in composition between the initial substrate and final products. Through such techniques a cassava-fermented product with an 18%-20% protein content (dry matter basis) was obtained.

More recently, Soccol et al. (1993a; 1993b), also at the ORSTOM Laboratory, obtained good results with the *Rhizopus* fungi, of special interest in traditionally fermented foods. In particular, they studied the effect of cooking before fermentation on the availability of starch, protein content, and the rate of starch's bioconversion into protein (Table 2). They found that a selected strain of *Rhizopus oryzae* could transform uncooked cassava, which contains only 1.68% protein, into a fermented cassava containing 10.89% protein.

Table 1. Effects of *Aspergillus niger* on protein and sugar contents of different starches (percentage of dry matter) after 30 h of fermentation in solid-state culture.

Substrate	Initial composition		Final composition	
	Proteins	Sugar	Proteins	Sugar
Cassava	2.5	90	18	30
Banana	6.4	80	20	25
Banana waste	6.5	72	17	33
Potato	5.1	90	20	35
Potato waste	5.1	65	18	28

Table 2. Growth of *Rhizopus oryzae* in solid-state cultivation on cassava granules after various cooking treatments.

Treatment[a]	Dry matter[b]		Total sugar[c]		Proteins[c]	
	Initial	Final	Initial	Final	Initial	Final
I	60.90	46.48	80.01	46.78	1.20	11.69
II	59.18	45.35	84.11	60.72	1.61	12.40
III	57.95	42.12	82.44	52.57	1.56	13.93
IV	55.63	43.88	82.49	56.62	1.47	11.89
V	45.57	37.88	82.04	56.62	1.68	10.89

a. Treatment:
I = Cassava autoclaved for 30 min at 120 °C, frozen, dried, and ground
II = Cassava flour (40% water) autoclaved for 30 min at 120 °C
III = Cassava flour (30% water) autoclaved for 30 min at 120 °C
IV = Cassava flour (30% water) vapor cooked for 30 min at 100 °C
V = Untreated crude cassava flour

b. g/100 g total weight.

c. g/100 g dry matter.

SOURCE: Soccol et al., 1994.

Table 3 shows results of amylase biosynthesis in solid or liquid culture, using raw or cooked cassava. The amount of glucoamylase was 10 to 15 times higher in solid than in liquid culture, and higher in raw starch medium than in cooked cassava.

This work is being continued in the EU-STD3 Program at the Bioconversion Laboratory of the Universidad del Valle, Cali, Colombia. It focuses on simplifying cassava processing by learning more about the specificity of *Rhizopus* strains in degrading the raw starch granule. But clean flours of raw cassava are needed. The common flours of cassava contain too much natural microflora to allow microbial studies with fungi; they must first be sterilized and (unfortunately) gelatinized. Ramírez et al. (1994) developed raw cassava flour with a very low content of bacteria and fungi, and little gelatinization.

To measure gelatinization, the simple method of Wotton et al. (1971) was adopted and a good correlation coefficient for the calibration curve was obtained. Table 4 shows the effect of thermic treatment and microwaves on starch gelatinization in cassava flour (water content typically lower than 10%). Where water content was very low, gelatinization was also low.

The same thermic treatment of dry cassava flour eliminated the natural microflora contained in raw flour, from 10^9 bacteria/g of dry flour to fewer than 10^3 bacteria/g after heating the flour for 30 min at 90 °C. With gelatinization limited to less than 5% under such conditions, obtaining clean, raw cassava flour is possible in the laboratory.

Figures 1 and 2 show the effects of various physical and thermic treatments on the bacteria content of cassava flour. Cassava flour will be used as a solid substrate for cultivating *Rhizopus* strains, and to compare the capacity of selected strains to grow on raw or gelatinized cassava starch.

Table 3. Effect of cooking and type of culture on the growth and amylases of various strains of *Rhizopus oryzae* cultivated on cassava granules.

Strain of *Rhizopus*	Liquid-state culture[a]						Solid-state culture[a]					
	Raw cassava			Cooked cassava			Raw cassava			Cooked cassava		
	α–amylase (U/g DM)	Gluco-amylase (U/g DM)	Protein (g/100 g DM)	α–amylase (U/g DM)	Gluco-amylase (U/g DM)	Protein (g/100 g DM)	α–amylase (U/g DM)	Gluco-amylase (U/g DM)	Protein (g/100 g DM)	α–amylase (U/g DM)	Gluco-amylase (U/g DM)	Protein (g/100 g DM)
28168	42.20	9.60	3.90	157.20	3.10	10.00	39.30	55.30	10.60	178.40	46.22	12.30
34612	40.40	7.30	4.60	168.50	5.70	9.30	55.00	70.00	12.60	170.00	47.00	14.10
28627	76.00	7.80	4.00	145.40	3.30	9.60	98.00	108.00	11.40	167.00	37.00	13.80

a. DM = dry matter; U = enzyme units.

SOURCE: Soccol et al., 1994.

Table 4. Effect of temperature and microwaves on starch gelatinization of cassava flour.

Temperature	Time (min)	Gelatinization rate (%)[a]			
		Exp. 1	Exp. 2	Exp. 3	Mean
Test 1 (80% gel.)		75.439	84.063	88.911	82.80
Test 2 (20% gel.)		25.411	26.184	29.702	27.10
80 °C	60	3.529	3.444	2.714	3.23
85 °C	30	3.529	3.357	3.487	3.46
85 °C		3.444	3.486	3.444	3.46
90 °C	30	3.572	3.444	3.572	3.53
90 °C	60	9.454	9.064	9.107	9.21
95 °C	30	6.961	5.546	5.803	6.10
100 °C	30	4.965	4.602	4.001	4.52
105 °C	30	6.961	5.503	5.301	5.92
120 °C	30	4.816	4.730	4.473	4.67
140 °C	30	4.773	3.100	3.100	3.66
160 °C	30	3.529	3.487	4.301	3.77
Autoclaving (121 °C)	15	3.572	3.100	4.301	3.66
Microwaves (Pot. 70)	5	2.886	2.410	2.842	2.71
Microwaves (Pot. 100)	5	2.971	2.242	2.242	2.49
Microwaves (Pot. 30)	15	3.879	3.057	3.915	3.62

a. Exp. = Experiment. Mean is across the experiments.

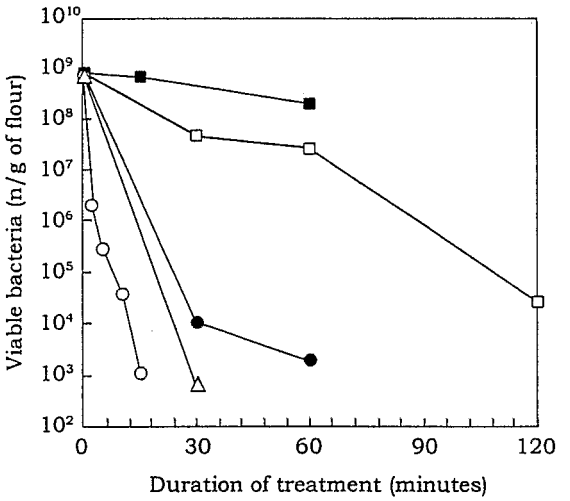

Figure 1. Total microflora (plate count analysis) in cassava flour, according to treatment. (■ = ultra-violet radiation; ○ = microwaves; □ = 80 °C; ● = 85 °C; △ = 90 °C.)

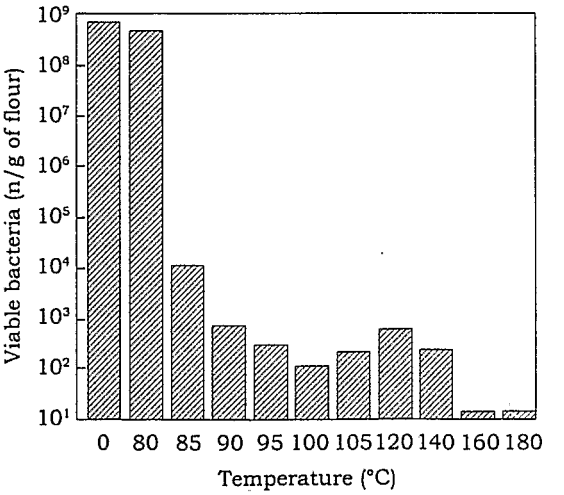

Figure 2. Effect of temperature on bacterial population in cassava flour.

Lactic Acid Fermentation of Cassava

Lactic acid fermentation is important for many traditional fermented foods, silage, and animal feed, and for recycling agroindustrial byproducts. Because of its acid, bacteriostatic, and bactericidal properties, fermentation prevents microorganisms, whether parasitic, saprophytic, or pathogenic, from breaking down vegetable material.

In tropical countries, lactic fermentation not only plays an important role in the traditional transformation of starchy foods, such as cassava, but also in the transformation and conservation of other foods, and fish and its byproducts. Two types of lactic fermentation exist:

(1) Homolactic, when more than 80% of total acidity and metabolites formed consists of lactic acid, and
(2) Heterolactic, when the percentage of acetic acid, propionic acid, and ethanol is more significant, and lactic acid represents 50%-80% of total acidity.

Lactic bacteria produce two types of lactic acid: L(+) and D(-). Only the L(+) form is assimilated by humans.

Previous studies, realized during the EU-STD2 Program in 1988-1991 (Raimbault, 1992), consisted of improving traditional fermented food made from cassava in Africa and Latin America. Three kinds of traditional foods were considered: *gari*, *chikwangue*, and sour starch. We demonstrated the essential role of lactic acid bacteria in all traditional processes.

Amylolytic lactic bacteria were isolated from fermented cassava. The first strain of *Lactobacillus plantarum* to be described as having very high amylolytic capacity was obtained from fermented cassava by A. Brauman in the Congo. Detailed physiological and biochemical characterization of this new strain is expected to be published soon by E. Giraud.

Mbugua and Njenga (1991) and Svanberg (1991a; 1991b), working in Tanzania and at the Uppsala University, respectively, have reported on the effect of lactic acid fermentation on the pathogen microflora content of traditional African foods.

Some of their results are reported in Table 5 and Figure 3, which show how lactic acid bacteria reduce the number of food-poisoning pathogens such as species of *Staphylococcus*, *Salmonella*, and *Shigella*, and *Escherichia coli*. High levels of such pathogens are sometimes found in traditional foods after processing under unhygienic conditions, especially those for malting maize during the rainy season in parts of tropical Africa.

Lactic fermentation of traditional foods reduces pathogenic bacteria from 10^8 to 10^3. The same authors also found a significant correlation between the resistance of young children to diarrhoea and eating acidified gruels.

We are bioconverting, through probiotics and bactericides, cassava flour and starch containing amylolytic lactic acid bacteria to isolate new strains from traditional foods. At the same time, we are broadening knowledge on the cultivation of lactic acid bacteria in starchy substrates. We hope such information will help elaborate new food and feed products.

Table 5. Effect of lactic acid fermentation on the content of pathogenic bacteria in traditional fermented foods in Africa.

Time (h)	Log number of bacteria/g food			
	Control	Nonfermented, acidified food	Fermented food	
			Flour (nonviable)	Gruel (viable)
Shigella flexneri				
0	6.8	6.7	6.4	6.0
3	6.6	5.8	5.1	4.0
7	7.0	4.2	5.5	3.3
24	7.0	4.1	3.7	2.7
Salmonella typhimurium				
0	8.5	8.1	8.3	7.7
3	8.0	6.7	6.0	7.1
7	7.9	5.3	4.4	6.3
24	8.9	4.0	2.0	2.0

SOURCE: Lorri and Svanberg, 1988.

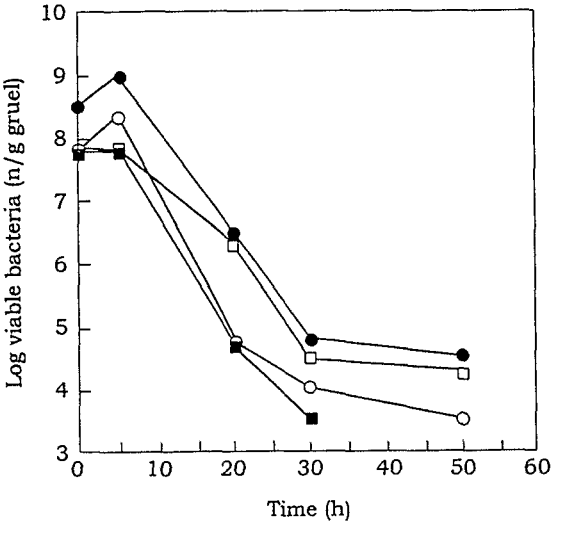

Figure 3. Evolution of pathogenic bacteria during the lactic fermentation of *uji*, a fermented cassava gruel (after Mbugua and Njenga, 1991). (□ = *Staphylococcus aureus*; ● = *Salmonella typhimurium*; ○ = *Escherichia coli*; ■ = *Shigella dysenteriae*.)

Alcoholic Fermentation of Cassava and Starch Products

Cassava is a potential producer of ethanol, considering its potentially high yields and low costs. Yet few reports concern the industrial application of cassava for ethanol production. This may be because, first, cassava cultivation yields relatively few, commercially significant byproducts, compared with, for example, sugarcane which yields enormous quantities of bagasse, a valuable source of energy for distillation. Second, cassava starch needs to be hydrolyzed into sugar for bioconversion into ethanol by the common *Saccharomyces cerevisiae*. This implies an additional, costly step.

For cassava to be an economically viable energy source, its processing costs must be reduced. Solid-state fermentation is one, simple, and new method of reducing costs: the use of an amylolytic yeast that eliminates hydrolysis.

At the ORSTOM Laboratory, Saucedo et al. (1992a; 1992c) developed a new process for the solid culture of an amylolytic yeast, *Schwanniomyces castelii* (Figure 4). The main advantage of this technique is its continuous recuperation of ethanol in a cold trap condenser. The gas produced in the reactor is pumped throughout the system, thus ensuring its continual removal from the medium

and limiting its toxic effects on the yeast's metabolism. The results obtained by Saucedo et al. (1992a; 1992b; 1992c) were promising, but the technology and feasibility of the process for commercial operation need further research.

Table 6 shows the results obtained by various authors on the potential of cassava as a substrate for ethanol production. The solid-state technique has to be carefully considered. Results obtained with the fungus *Rhizopus koji* are particularly significant. The potential of *Schwanniomyces* is also interesting because amylolytic yeast would be easier to control at the small-scale industrial level.

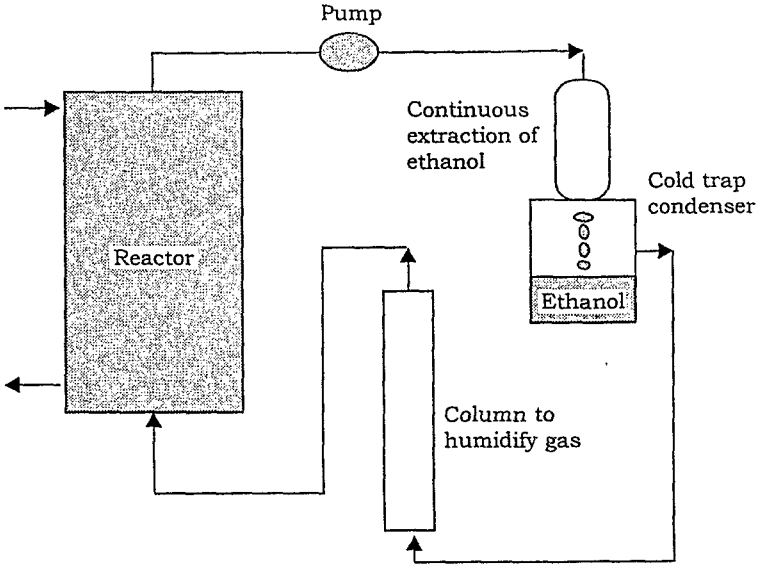

Figure 4. Producing ethanol through solid-substrate fermentation of cassava starch. The reactor contains a solid support impregnated with a starchy suspension and inoculated with the fermentation agent, an amylolytic yeast known as *Schwanniomyces castelii*. The resulting gas is pumped to a condenser where ethanol is extracted. The residual gas is sent to a humidifier.

Table 6. Comparison of various processes for ethanol production from cassava in liquid or solid substrate.

Process	Hydrolysis	Sugar (g/L)	Ethanol (g/L)	Recovered (g/L)	Theoretical (%)
Liquid substrate, using *S. cerevisiae* [a, b]	+	145	72.50	72.50	83.2
Solid substrate, using *S. cerevisiae* [b, c]	+	165	41.73	41.73	65.0
Solid substrate, using *Rhizopus koji* [d]	-	200	110.00	110.00	83.0
Solid substrate, using *Schw. castelii* [e, f]	-	300	68.40	212.60	64.0

a. Saraswati, 1988.
b. *S.* = *Saccharomyces.*
c. Jaleel et al., 1988.
d. Jujio et al., 1984.
e. Schw. = *Schwanniomyces.*
f. Saucedo et al., 1992a.

Conclusions on Bioconverting Cassava and Potential Products

To bioconvert cassava starch and flour to elaborate new products, ORSTOM, CIRAD, and collaborating institutes are emphasizing two approaches: solid-state fermentation, and lactic acid fermentation.

The first is of great interest because of its potential to simplify processes and reduce costs, and its large reactor volume. Both *Rhizopus* and *Schwanniomyces* (or other amylolytic) yeasts can be used in a solid-state cultivation process. This implies a three-phase reactor with a solid fiber support, a liquid phase containing the substrate in suspension and salts, and a gaseous phase for exchanging volatile components, that is, oxygen, water, and ethanol.

In lactic acid fermentation, we are investigating the culture control of amylolytic lactic acid bacteria in mixed and composite starters able to remain competitive in a natural, nonaxenic environment. The prophylactic role of lactic acid bacteria is also of great interest.

Finally, we are studying microorganisms able to degrade native cassava starches without need of gelatinization, as in in natural biotransformation and biodegradation. We will also study the amylolytic capacity of *Rhizopus* spp., yeasts, and lactic acid bacteria.

References

Giraud, E.; Brauman, A.; Kéléke, S.; Lelong, B.; and Raimbault, M. 1991. Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. Appl. Microbiol. Biotechnol. 36:379-383.

_____; Gosselin, L.; and Raimbault, M. 1992. Degradation of cassava linamarin by lactic acid bacteria. Biotechnol. Lett. 14(7):593-598.

_____; _____; Marín, B.; Parada, J. L.; and Raimbault, M. 1993a. Purification and characterization of an extracellular amylase from *lactobacillus plantarum* strain A6. J. Appl. Bacteriol. 75:276-282.

_____; _____; and Raimbault, M. 1993b. Production of a *Lactobacillus plantarum* starter with linamarase and amylase activities for cassava fermentation. J. Sci. Food Agric. 62:77-82.

Jaleel, S. A.; Srikanta, S.; Ghildyal, N. P.; and Lonsane, B. K. 1988. Simultaneous solid phase fermentation and saccharification of cassava fibrous residue for production of ethanol. Starch/Stärke 40(2):55-58.

Lorri, W. S. M. and Svanberg, U. 1988. Improved protein digestibility in cereal based weaning foods by lactic acid fermentation. Harare, Zimbabwe.

Mbugua, S. K. and Njenga, J. 1991. Antimicrobial properties of fermented UJI as a weaning food. In: Westby, A. and Reilly, P. J. A. (eds.). Traditional African foods: quality and nutrition. International Foundation of Science (IFS), Sweden. p. 63-67.

Oriol, E.; Raimbault, M.; Roussos, S.; and Viniegra-González, G. 1988a. Water and water activity in the solid state fermentation of cassava starch by *Aspergillus niger*. Appl. Microbiol. Biotech. 27:498-450.

_____; Schetino, B.; Viniegra-González, G.; and Raimbault, M. 1988b. Solid state culture of *Aspergillus niger* on support. J. Ferment. Technol. 66:1-6.

Raimbault, M. 1992. Etudes physiologiques et génétiques des bactéries lactiques dans les fermentations traditionnelles du manioc. Final report CEE/STD2, no. TS2A-00226. Institut français de recherche scientifique pour le développement en coopération (ORSTOM), Montpellier, France. p. 1-53. (Internal document.)

_____ and Alazard, D. 1980. Culture method to study fungal growth in solid fermentation. Eur. J. Appl. Microbiol. Biotechnol. 9:199-209.

_____ and Viniegra, G. 1991. In: Chahal, D. S. (ed.). Modern and traditional aspects of solid state fermentation in food, feed and fuel from biomass. p. 153-163.

_____; Revah, S.; Pina, F.; and Villalobos, P. 1985. Protein enrichment of cassava by solid substrate fermentation using molds isolated from traditional foods. J. Ferment. Technol. 63(4):395-399.

Ramírez, C.; de Stouvenel, A.; and Raimbault, M. 1994. Effect of physical treatments on microflora content in cassava flour. Poster presented at the International Meeting on Cassava Flour and Starch, held in January 1994 at Cali, Colombia.

Saraswati. 1988. The experience of pilot plant of ethanol from cassava in Indonesia. Regional Workshop on Upgrading of Cassava/Cassava Wastes by Appropriate Biotechnologies, Bangkok, Thailand, 1987. Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. p. 41-49.

Saucedo, G.; González, P.; Revah, S.; Viniegra, G.; and Raimbault, M. 1990. Effect of *Lactobacilli* inoculation on cassava (*Manihot esculenta*) silage: fermentation pattern and kinetic analysis. J. Sci. Food Agric. 50:467-477.

_____; Lonsane, B. K.; Navarro, J. M.; Roussos, S.; and Raimbault, M. 1992a. Potential of using a single fermenter for biomass build-up, starch hydrolysis and ethanol production: solid state fermentation system involving *Schwanniomyces castelii*. Appl. Biochem. Biotechnol. 36:47-61.

_____; _____; _____; _____; and _____. 1992b. Importance of medium pH in solid state fermentation system for growth of *Schwanniomyces castelii*. Lett. Appl. Microbiol. 15:164-167.

_____; _____; and Raimbault, M. 1992c. Maintenance of heat and water balance as scale-up criterion for production of ethanol by *Schwanniomyces castelii* in solid state fermentation system. Process Biochem. 27:97-107.

Soccol, C.; Iloki, I.; Marín, B.; and Raimbault, M. 1994. Comparative production of alpha-amylase, glucoamylase and protein enrichment of raw and cooked cassava by *Rhizopus* strains in submerged and solid state fermentations. J. Food Sci. Technol. 31:320-332.

_____; Marín, B.; Roussos, S.; and Raimbault, M. 1993a. Scanning electron microscopy of the development of *Rhizopus arrhizus* on raw cassava by solid state fermentation. Micol. Neotrop. Apl. 6:27-39.

_____; Rodríguez, J.; Marín, B.; Roussos, S.; and Raimbault, M. 1993b. Growth kinetics of *Rhizopus arrhizus* in solid state fermentation of treated cassava. Biotechnol. Tech. 7(8):563-568.

Svanberg, U. 1991a. Lactic fermentation of cereal-based weaning gruels and improved nutritional quality. In: Westby, A. and Reilly, P. J. A. (eds.). Traditional African foods: quality and nutrition. International Foundation of Science (IFS), Sweden. p. 53-60.

_____. 1991b. The potential role of fermented cereal gruels in reduction of diarrhoea among young children. In: Westby, A. and Reilly, P. J. A. (eds.). Traditional African foods: quality and nutrition. International Foundation of Science (IFS), Sweden. p. 33-38.

Wotton, M.; Weedon, D.; and Munck, N. 1971. A rapid method for estimation of starch gelatinization in processed foods. Food Technol. Aust. 23:612-614.

ISBN 958-9439-88-8

Cassava Flour and Starch:

Progress in Research and Development

Edited by: D. Dufour
G. M. O'Brien
Rupert Best

CIRAD

Centre
de coopération
internationale
en recherche
agronomique
pour le
développement

Département
des systèmes
agroalimentaires
et ruraux
CIRAD-SAR

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture