Advance Journal of Food Science and Technology 3(6): 418-423, 2011
ISSN: 2042-4876

Submitted: August 27, 2011 Accepted: October 15, 2011 Published: December 25, 2011

Softening and Mineral Content of Cassava (*Manihot esculenta Crantz*) Leaves During the Fermentation to Produce *Ntoba mbodi*

S. Mokemiabeka, J. Dhellot, S.C. Kobawila, P. Diakabana, R.N. Ntietie Loukombo, A.G. Nyanga-Koumou and D. Louembe
Equipe Pluridisciplinaire en Alimentation et Nutrition (EPRAN), Pôle d'Excellence en Alimentation et Nutrition, Faculté des Sciences, BP 389, Brazzaville, Congo

Abstract: The aim of study is to investigate the softening of cassava leaves during the fermentation of *Ntoba mbodi* and the mineral content of cassava leaves fermented and unfermented. The softening of cassava leaves is a characteristic of fermentation to produce *Ntoba mbodi*. It is due to an enzymatic process. The activity of cellulase and polygalacturonase enzymes, very weak at the beginning of fermentation, increases sharply in 24 h and decreases thereafter. The pectinesterase and pectin lyase activity, already present at the beginning of fermentation, reaches the maximum after 24 h of fermentation for pectinesterase and 48 h for pectin lyase. The cellulase is mainly of microbial origin. The cassava leaves softening is gradual and becomes maximum at the end of fermentation. It is accompanied by a loss of water leading to a decrease in protein and magnesium content. The *Ntoba mbodi* is richer in ash than fresh cassava leaves. Although *Ntoba mbodi* is a vegetable rich in minerals, its sodium content is low. Thus, *Ntoba mbodi* can be recommended for a meal without peril.

Key words: *Bacillus*, cellulase, lactic acid bacteria, minerals, *Ntoba mbodi*, pectinesterase, pectin lyase, polygalacturonase

INTRODUCTION

Cassava leaves (*Manihot esculenta Crantz*) are processed by alkaline fermentation in fermented vegetable called *NStoba mbodi* which is consumed in Congo and Central Africa. Cassava leaves are rich in protein and minerals. They are rich in all essential amino acids except methionine and phenylalanine (Louembe *et al*., 2003) (Kobawila *et al*., 2003; Eggum, 1970; Ravindran and Etravindran, 1988; Rogers and Milner, 1963; Ross and Enriquez, 1969; Ayodeji, And Fasuyi, 2005; FAO, 1972; Bradbury, 1991).

From a nutritional standpoint, they contain in dry weight basis 17-34% protein and 16-26% fibres. The cassava leaves soften during fermentation, become darker and exhale a characteristic odor.

Microbiological analysis of fermented cassava leaves reveal the presence of lactic acid bacteria and *Micrococcus varians*, *Bacillus macerans*, *Bacillus subtilis*, Staphylococcus sciuri, *Staphylococcus*, whereas yeast and *Leuconostoc* are absent.

A diversity of genera and species of Lactic acid bacteria are found in *Ntoba-mbodi* showing the ability of Lactic acid bacteria to grow in alkaline pH. The dominant LAB strains in these samples are: Weissella confusa (45.3%), *Weissella cibaria* (7.6%), *Enterococcus casseliflavus* (16.9%), *Enterococcus faecium* (5.6%), *Enterococcus hirae* (3.7%), *Enterococcus faecalis* (3.7%), *Enterococcus avium* (1.8%) Lactobacillus plantarum (9.4%) and *Pediococcus pentosaceus* (5.6%). All isolates tested failed to show amylolytic and proteolytic activities. Positive pectinolytic activity was recorded for two strains of *Enterococcus faecalis* and one of Pediococcus pentosaceus (Ouoba *et al*., 2010).

The softening of cassava leaves is the main characteristic indicating the end of fermentation. It is caused, inter alia, by the cell wall degrading enzymes secreted by microorganisms among which *Bacillus macerans*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus xylosus* and *Erwinia* spp. must play an important role during the cassava leaves fermentation by their pectinolytic enzymes (Jayani *et al*., 2005; Louembe *et al*., 2003; Rexova-Benkova and Markovic, 1976) (Thibault, 1980).

These enzymes (cellulase (EC 3.2.1.4) and pectolytic enzymes) by degrading the pectins promote the softening observed in cassava leaves (Schleifer, 1986) (Ampe *et al*., 1995) and thereby improve the digestibility of vegetal organic matter.

This study is the study of the softening and the content of cassava leaves minerals in the production of *Ntoba mbodi*.

Corresponding Author: S. Mokemiabeka, Equipe Pluridisciplinaire en Alimentation et Nutrition (EPRAN), Pôle d'Excellence en Alimentation et Nutrition, Faculté des Sciences, BP 389, Brazzaville, Congo

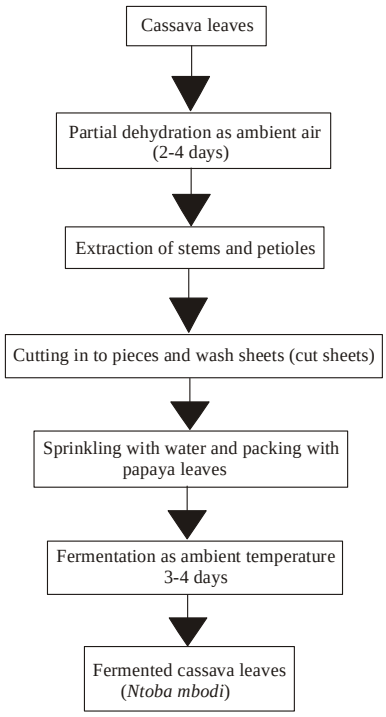

Fig. 1: Diagram of manufacturing process of *Ntoba mbodi*

MATERIALS AND METHODS

The study was conducted in 2008 at Brazzaville (University Marien Ngouabi, Congo), in collaboration with producers of *Ntoba mbodi*. Each experiment was performed in triplicate.

Production process of *Ntoba mbodi*: Cassava leaves used are aged from 2 weeks to 3 months. They are harvested in cassava plantations of Brazzaville, exposed to ambient air (2 days) and their petioles stripped. They are then cut into pieces and cleaned with water. After draining, they are wrapped in papaya leaves and put in a pot for fermentation (4 days). Leaves are fermented and processed into *Ntoba mbodi* (Fig. 1).

The process of *Ntoba mbodi* is represented in the diagram below (Fig. 1).

Determination of physical-chemical parameters during the fermentation:

Determination of pH: Every 24 h during the fermentation, 20 g of cassava leaves were removed and then ground in the homogenizer Waring blendor and finally suspended in 60 mL of distilled water. The pH was then measured using a pHmeter of the type HI 9321 Bioblock.

Appreciation of cassava leaves softening: The softening of cassava leaves during fermentation was appreciated every 24 h by touching with fingers by a panel of 5 members, all women, producers of *Ntoba mbodi*.

Dry matter content: The dry matter content was measured by the method described by AFNOR. A mass m_1 of each sample was added in a beaker and placed in an oven at 105ºC for 24 h.

Ash content: A weighed quantity of dry sample, obtained after drying in an oven at 105ºC, was cremated in the oven at 550ºC for 2 h until white ash was obtained.

Nitrogen and protein content: The determination of nitrogen was performed by the Kjedahl method. (AFNOR NF V 03-050) (AOAC Official Method 935.58). The protein content was obtained by multiplying the nitrogen by 6.25.

Determination of dissolved minerals: Assays were made by:

- Atomic absorption spectrophotometry for calcium (λ = 422.7 nm) and magnesium (λ = 285.2 nm)
- Spectrophotometry with flame emission for sodium (λ = 589 nm) and potassium (λ = 766.5 nm)
- Spectro-colorimetry for phosphorus

Determination of enzymes activity: Samples were taken every 24 h during 96 h of fermentation.

Preparation of enzymatic extract: 5 g of fermented cassava leaves were weighed and then homogenized in 25 ml of buffer (appropriated for enzyme) and at a pH determined for the enzyme. The homogenate obtained was filtered and centrifuged at 30000 g for 10 min at 4ºC. The supernatant was used as enzymatic extract.

Determination of pectinesterase (EC 3.1.1.11): Enzymatic extract was made by homogenizing 5 g of fermented leaves in 25 mL of Tris-HCl 0.25 M NaCl 0.3 M, pH 8.0.

One mL of enzymatic extract was added to 5 mL of 1% pectin in 0.1 M NaCl. After mixing the pH was adjusted to 7.5. The mixture was incubated during 10 min at ambient temperature. After incubation, one to two drops of indicator solution were added and the mixture titrated with 0,02 M NaOH.

The activity was expressed in terms of ester hydrolyzed per min per mL of extract (Okolie *et al*., 1988).

Determination of cellulase (EC 3.2.1.4): The enzymatic extract was obtained by homogenizing 5 g of fermented leaves in 25 mL of phosphate buffer 0.02 M pH 6.5.

4.5 mL of 0.1% carboxymethylcellulose in phosphate buffer 0.02 M pH 6.5, were added to 0.5 mL of enzymatic extract and incubated at 35ºC for 1 h. The reaction was stopped by adding 5 mL of dinitrosalycilic acid (DNS).

The mixture was heated in boiling water for 5 min and cooled with tap water. Optical Density (OD) was read at 540 nm against the control prepared in the same way but without enzymatic extract.

The activity of the enzyme was expressed as reducing sugars (glucose) released (µmol/min.mL extract) (Oyewole and Odunfa, 1992).

Determination of polygalacturonase (EC 3.2.1.15): The enzymatic extract was performed by homogenizing 5 g of fermented leaves in 25 mL of 0.02 M phosphate buffer pH 6.5.

0.5 mL of enzymatic extract was added to 4.5 mL of pectic acid solution at 0.1% in 0.02 M phosphate buffer pH 6.5 and the mixture incubated for 1 h at 35ºC. Reaction was stopped by adding 5 mL of dinitrosalicylicacid reagent (DNS).

Mixture was heated in boiling water for 5 min then cooled in tap water. Optical Density (OD) was read at 540 nm against the control prepared in the same way but without enzymatic extract. The activity of the enzyme was expressed as reducing sugars (glucose) released (µmol/ min.mL extract) (Oyewole and odunfa, 1992).

Determination of pectin lyase (EC4.2.2.10): The enzymatic extract was obtained by homogenizing 5 g of fermented leaves in 25 mL of Tris - HCl 0.1 M pH 8.5. The thiobarbituric acid method was used for determination of pectin lyase activity.

0.25 mL of Tris - HCl 0.1 M pH 8.5 and 0.25 mL enzyme extract were added to 0.2 ml of 0.5% pectin solution in NaCl 0.1 M. Mixture was incubated at 37ºC for 1 h. At the end of incubation, 0.5 mL of 0.5 N HCl and 1 mL of thiobarbituric acid 0.01 M were added to 0.1 ml of incubated mixture and heat in boiling water bath for 45 min.

After heating, the mixture was cooled in ice for 30 min and let to decant for 24 h. The absorbance was read at 547 nm against a control which was prepared similarly but without enzymatic extract.

The unit of relative activity corresponds to an increase in Optical Density (OD) of 0.01/h. For the determination of the pectate lyase, pectic acid solution in 0.5% NaCl 0.1 M was used instead of thesolution of pectin (Kobawila, 2003).

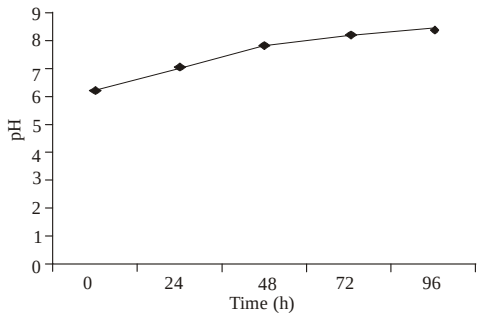

Fig. 2: Evolution of pH during the fermentation of *Ntoba mbodi*

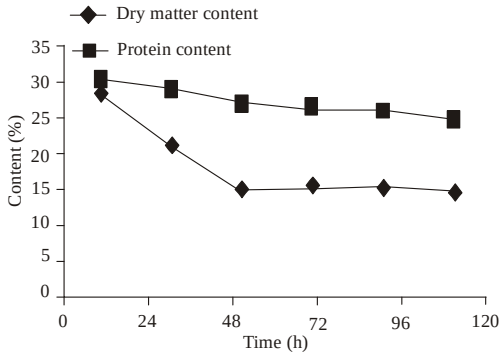

Fig. 3: Evolution of protein and dry matter content during the fermentation of cassava leaves in *Ntoba mbodi*

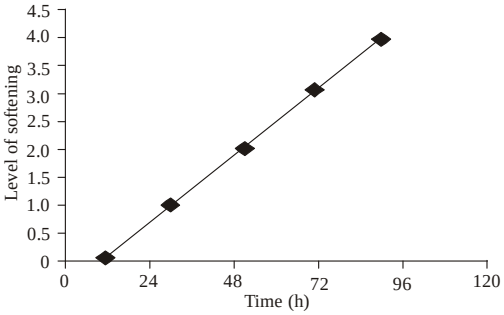

Fig. 4: Evolution of the softening of cassava leaves during the fermentation in *Ntoba mbodi*

RESULTS AND DISCUSSION

Evolution of physico-chemical parameters during the fermentation of *Ntoba mbodi*:

Evolution of pH during the fermentation of *Ntoba mbodi*: The pH, 6.2 at the beginning of fermentation, increased and became alkaline (pH 8.96) after 48 h of fermentation. Fermentation of *Ntoba mbodi* is alkaline fermentation (Fig. 2).

Evolution of protein and dry matter content during the fermentation of *Ntoba mbodi:* The protein content decreased weakly from 30.44 to 25% during the fermentation but the dry matter content diminishes strongly in 48 h of fermentation, then becomes stable (Fig. 3).

Evolution of the softening level during the fermentation of *Ntoba mbodi:* The softening increased progressively and becomes maximum after 96 h of fermentation, which indicates the end of fermentation (Fig. 4).

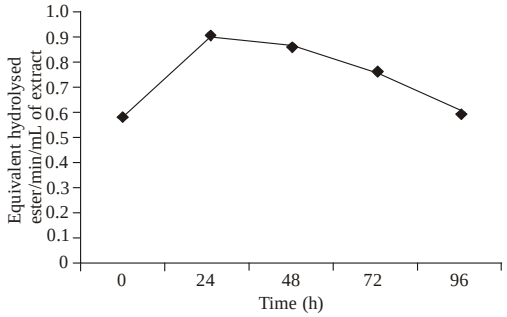

Fig. 5: Evolution of pectinesterase activity during the fermentation of *Ntoba mbodi*

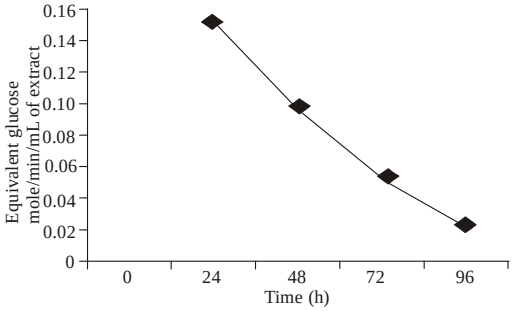

Fig. 6: Evolution of cellulase activity during the fermentation of *Ntoba mbodi*

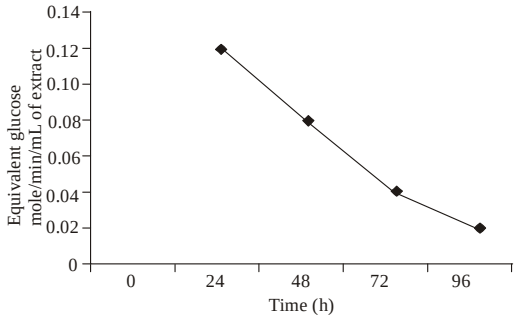

Fig. 7: Evolution of plygalacturonase activity

Five levels of appreciation were determinated by the panel of producers:

Level
0 : No softening (0)
1 : slight softening (+)
2 : little softening (++)
3 : strong softening (+++)
4 : perfect softening (++++)

Determination of enzyme activity:

Pectinesterase activity: The pectinesterase activity increased rapidly and reaches the maximum at 24 h of fermentation of cassava leaves in *Ntoba mbodi*. It decreased thereafter until the end of fermentation (Fig. 5).

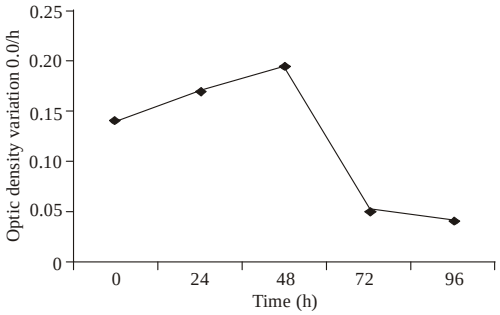

Fig. 8: Evolution of pectinelyase activity during the fermentation of *Ntoba mbodi*

Cellulase activity: Figure 5 shows that cellulase activity appears after 24 h of fermentation in the maximum position and then it decreases sharply until the end of fermentation (Fig. 6).

Polygalacturonase activity: Polygalacturonase activity showed an evolution similar to cellulase activity. It reached highest position in 24 h of fermentation. Thereafter, it decreased until the end of fermentation (96 hours) as shown in Fig. 7.

Pectin lyase activity: The pectin lyase activity progressively increased during the fermentation and reached a maximum after 48 h of fermentation. Thereafter, it fell in 24 h and varies little until the end of fermentation (Fig. 8).

Minerals content: Potassium, calcium, magnesium and ash levels were higher in *Ntoba mbodi* than in unfermented cassava leaves. However, nitrogen content was higher in leaves than in the unfermented *Ntoba mbodi*, the phosphorus and sodium content varied very slightly (Table 1).

Cellulase and polygalacturonase activities almost inexistent at the beginning of fermentation, increased to their maximum in 24 h of fermentation and decreased thereafter. However, the activity of pectinesterase and pectin lyase, already present in the early stage of fermentation, increased gradually and reached the maximum, respectively at 24 and 46 h of fermentation.

Pectin may undergo an enzymatic attack by polygalacturonase, pectate lyase, pectin lyase, rhamnoga lacturonase, pectin methy lesterase and pectinacety lesterase.

The results indicate that the activity of pectinesterase and pectin lyase precedes the action of cellulase and polygalacturonase, which comes subsequently to strengthen the action of pectinesteraseand pectin lyase.

Table1: Minerals content in fermented cassava leaves (*Ntoba mbodi*) and unfermented cassava leaves (*Manihot esculenta Crantz*)

Sample	Ca (%)	P (1/1000)	K (%)	Na (%)	Mg (%)	N (%)	Ash (%)
Unfermented cassava leaves	0.98	4.27	1.826	0	0.676	5.063	9.826
fermented cassava leaves *Ntoba mbodi*	1.376	4.52	2.363	0.013	0.913	4.446	13.29

Cellulase is mainly of microbial origin, which explains its very low level of activity at the beginning of the fermentation. Its activity increased strongly in 24 h with the beginning of cassava leaves softening during the fermentation.

The pectinesterase (EC 3.1.1.11), an ubiquitous enzyme associated to the cell wall, catalyzes the de-esterification of the methylesterified units of acid D-galactosiduronic in the pectic compounds producing substrates, more particularly pectic acids, for depolymerization enzymes. It operates principally by changing local pH leading to the alteration of the integrity of the cell wall.

The action of these pectinolytic enzymes causes softening of cassava leaves leading to the end of fermentation. This softening phenomenon is also observed during the retting of cassava roots, flax, hemp (Chesson, 1978; Avrova *et al*., 1981; Jauneau, 1985; Rosemberg and De Franca, 1967; Tanner *et al*., 1993) and the softening of fruits (Benchabane *et al*., 2006; Abu - Sarra and Abu - Goukh, 1992; Carrington *et al*., 1993; Cutillasituttalde *et al*., 1993; Knegt *et al*., 1988).

The pectinesterase action on the wall components of plant cells can produce two effects diametrically opposed:

- The first is a contribution by stiffening the cell wall, producing blocks of unesterified carboxylic groups which can interact with calcium ions forming a pectate gel.
- The second being the release of protons that can stimulate the activity of cell wall hydrolases contributing to the loss of the cell wall.

Thus this second effect explains the rapid increase of polygalacturonase activity in 24 h fermentation.

It has been shown that some isoforms enzymes of vegetal pectinesterase may show the two mechanisms and that such mechanisms are driven by changes in pH. The optimum pH in higher plants is usually between 7 and 8 when it is usually lower in fungi and bacteria.

The decreased amount of nitrogen during the fermentation of *Ntoba mbodi* is due in part to solubilization and volatilization of hydrogen cyanide (Kobawila *et al*., 2005) and to the departure of some proteins in the loss water accompanying the softening of cassava leaves during the fermentation. Similarly, the loss of water causes the decreasing of magnesium concentration as a result of the degradation of the chlorophyll structure during the fermentation.

Increase of the quantity of ash is related to the increase in microbial mass, the result of microbial synthesis. The *Ntoba mbodi* can be considered a vegetable rich in minerals because the *Ntoba mbodi* is richer in ash than fresh cassava leaves. In addition, its low sodium content is an added bonus for a diet that requires less sodium. Indeed, a high sodium diet can cause cardiovascular and renal diseases.

CONCLUSION

The softening of cassava leaves marks the end of the fermentation of *Ntoba mbodi* and implies a progressive breakdown of cell walls under the action of pectic and cellulosic enzymes whose maximum activity is reached within 24 h of fermentation for cellulase and polygalacturonase and 48 h for pectinesterase and pectin lyase. The results show that the fermented cassava leaves *Ntoba mbodi* can be used as an additional source of protein and minerals for humans because of their wealth in these products.

REFERENCES

Abu-Sarra, F.A. and A.A. Abu-Goukh, 1992. Changes in pectin esterase, polygalacturonase and cellulase activity during mango fruit ripening. J. Horticultural Sci., 4(67): 561-568.

Ampe, F., S. Keleke, H. Robert and A. Brauman, 1995. The role of pectin degrading enzymes during cassava retting. In: T. Agbor-Egbe, A. Brauman, D. Griffon and S. Trèche, (Eds.), Transformation Alimentaire Du Manioc, Editions ORSTOM, pp: 331-344.

Avrova, N.P., I.K. Zubko and E.G. Alekseeva, 1981. Fermentation products and activity of pectolytic enzymes in strains of Clostridium felsineum differing in rate of spore formation. Microbiologiya, 50(30): 458-466.

Ayodeji, O.F., 2005. Nutritient composition and processing effects on cassava leaft (*Manihot esculenta Crantz*). Antinutriments Pak. J. Nutr., 4(1): 37-42.

Benchabane, A., S. Abbeddou, M.M. BellaL and J.F.Thibault, 2006. Evolution des pectines et des activités polygalacturonases au cours de la maturation de date Deglet-nour. Sci. Des Aliments, 26(3): 233-246.

Bradbury, J.H., 1991. Properties and analysis of antinutritional factors in foods. Food J., 6(4): 123-128.

Carrington, C.M.S., L.C. Greve and J.J.M. Labavitch, 1993. Cell all metabolism in repining fruit. Effect of antiense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. Plant Physiol., 2(103): 429-434.

Chesson, A., 1978. The maceration of linen flax under anaerobic conditions. J. Appl. Bacteriol, 45: 219-230.

Cutillasituttalde, A., T. Zarra and E.P. Lorences 1993. Metabolism of cell wall polysaccharides from persimmon fruit-pectin solubilization during fruit ripening occurs in apparent absence of polygalacturonase activity. Physiol. Plantarum, 2(89): 369-375.

Eggum, B.O., 1970. The protein quality of cassava leaves. Br. J. Nutr., 24: 761-768.

FAO, et Us Dept. Health and welfare, 1972. Food composition table for use in East Africa.

Jauneau, A., 1985. Contribution à la connaissance de la microflore pectinolytique: Application au rouissage du lin. Thèse, Sciences, Université de Rouen.

Jayani, R.S., S. Saxena and R. Gupta, 2005. Microbial pectinolytic enzymes: A review. Process Biochem. 40: 2931-2944.

Knegt E., E. Vermeer and J. Bruinsma, 1988. Conversion of the polygalacturonase iso enzymes from ripening fruits. Physiologia Plantarum, nnaissance de la microflore pectinolytique: Application au rouissage du lin. Thèse, Sciences, Université de Rouen, 72: 108-114.

Kobawila, S.C., 2003. Les produits fermentés d'Afrique Centrale: Cas du Congo. Thèse, Doctorat d'Etat, UFR.S.V.T. Université De Ouagadougou.

Kobawila, S.C., D.Louembe, S.Keleke and A.S. Traore, 2003. Aspects physico-chimiques et biochimiques de la fermentation des feuilles en *Ntoba mbodi*. Procédé Biologique et Alimentaire, 1(1): 106-109.

Kobawila, S.C., D. Louembe, S. Keleke, J. Hounhouigan and C. Gamba, 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and *Ntoba mbodi*, two food products from Congo. Afr. J. Biotechnol., 4(7): 689-696.

Louembe, D., S.C. Kobawila, G. Bouanga Kalou and S. Kéléké, 2003. Etude microbiologique des feuilles fermentées de manioc: *Ntoba mbodi*. Tropicultura, 21(3): 106-111.

Okolie, P., N. Ugochukwu and N. Emmanuel, 1988. Changes in activities of cell wall degrading enzymes during fermentation of cassava *(Manihot esculenta Crantz)* with *Citrobacter freundii*. J. Sci. Food Agric., 44: 51-61.

Ouoba, L.I.I., C.A.G. Nyanga-Koumou, C. Parkouda, H. Sawadogo, S.C. Kobawila, S. Keleke, B. Diawara, D. Louembe and J.P. Sutherland, 2010. Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. J. Appl. Microbiol., 108(6): 2019-2029.

Oyewole and SA OB Odunfa., 1992, Extracellular enzyme activities during cassava fermentation for 'fufu' production. World J. Microbiol. Biotechnol., 8: 71-72.

Ravindran, G. and V. Etravindran, 1988. Changes in the nutritional composition of cassava (*Manihot esculenta Crantz*) leaves during maturity. Food Chem., 27: 299-309.

Rexova-Benkova, L. and O. Markovic, 1976. Pectic enzymes. Adv. Carbohydr. Chem. Biochem., 33: 323-385.

Rogers, D., J. Milner and M. Amino, 1963. Acid profile of manioc leaf protein in relation to nutritive value. Econ. Bot., 17: 211-216.

Rosemberg J.A. and F.P. Defranca, 1967. Importance of galacturonic acid in controlling the retting of flax. Appl. Microbiol., 15(3): 484-486.

ROSS E. and F.O. Enriquez, 1969. The nutritive value of cassava leaf meal. Poult. Sci., 48: 846-853.

Schleifer, K.H., 1986. Gram-Positive Cocci. In: Peter, H.A.S., S.M., Nicholas Sharpe and M. Elisabeth, (Eds.), Bergey's Manuel of Systematic Bacteriology, Williams et Wilkins. Baltimore, 7: 999-1103.

Tanner, R.D., A. Prokop and R.K. Bajpai, 1993. Removal of fibers from vines by solid state fermentation. Enzymatic degradation: a composition of flax and kudzu retting. Biotechnology Adv., 3(11): 635-643.

Thibault, J.F., 1980. Biochimie Appliqué, Gautier-Villars, Paris, pp: 323-385.