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Cassava based foods: microbial fermentation by single starter culture towards cyanide reduction, protein enhancement and palatability

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Abstract

Cassava flour sample fermented with three pure starter cultures of Yeast *Saccharomyces cerevisiea*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. Three different inoculum level (0.25 ml, 0.50 ml, and 0.75 ml) were used. 20 gms of cassava samples were fermented to different times (24, 36 and 48 hrs). The samples were withdrew after each hrs of fermentation and subjected to analysis of pH, MC, CP, FC content of the samples. All fermented samples generally resulted in increased crude protein (CP) and decreased pH, free cyanide and moisture contents. The sample fermented with *L. plantarum* and *L. mesenterodes* for 36 and 48 hrs with 0.25 ml and 0.75 ml inoculums resulted in the highest pH reduction from 6.68 to 3.70, while the least pH reduction was recorded in sample fermented with *S. cereviseas* at inoculums level of 0.75 ml. The highest CP content increment were recorded on sample fermented by *S. cereviseas* for 48 hrs with inoculums level of 0.75 ml i.e from 0.71% unfermented to 4.58% fermented sample. The highest free cyanide (FC) reduction was recorded by *L. plantarum* (4.09 mg/g) at 24 hrs of 0.50 ml, followed by *L. mesenteroides* (4.67 mg/g) at 36 hrs of 0.75 ml of inoculum. While the least free cyanide reduction was recorded by *S. cereviseas* (111.62 mg/g) at 24 hrs of 0.25 ml of inoculum level. The FC content of all fermented sample at three fermentation time and inoculums level was significantly lower ($P < 0.05$) than the unfermented samples. The FC decreased from 197.19 mg/g to 4.09 mg/g upon fermentation.

Introduction

Cassava (*Manihot esculenta* crantz) is a staple food for over 500 million people in the developing world (Cock, 1985). It is one of the most drought-tolerant crops and capable of growing on marginal soils (Motto *et al.*, 1990). It encompasses high energy and starch producing tuber crop, but it is a poor source of protein. Cassava contains potentially toxic compounds, cyanogenic glucosides. If present in sufficient quantities, these compounds can cause acute cyanide poisoning and death in man and animals when consumed. The amount of these toxic compounds varies according to cultivars and growing conditions. As a result, predominantly cassava tuber diet can cause protein-energy malnutrition.

As cassava is the main staple root tuber in many developing countries, especially in West Africa, it is grown in more than 90 countries and ranks as the 6th most important source of energy in human diets worldwide and also the 4th supplier of energy after rice, sugar and corn/maize (Heuberger, 2005). Cassava is nutritionally a strategic famine crop and could support food security in areas of low rainfall. In some parts of Ethiopia, it has become a source of carbohydrate for low income consumers. Currently, the crop is widely cultivated in south western Gambella, particularly, in Mezengher zone, Godere *woreda* as a food source and is playing a significant role in alleviating the food crisis during harsh weather conditions. Locally the crop is called in its domestication area name "*ababure*" and it has been used in different food forms after passing through different processing methods.

Despite its importance as a good source of carbohydrate, cassava has four major drawbacks which limit its utilization as a food and feed (Kimaryo *et al*., 2000). These are low protein content, rapid postharvest deterioration and potential cyanide toxicity, deficiency in vitamins and mineral contents. In the same way Chauynarong *et al.* (2009) reported that major limitation of using cassava tuber meal in human food and animal feed is the low protein content and deficiency of essential amino acids. Among all the problems associated with cassava, the one that is of the greatest concern is that it contains cyannogenic glucosides. The two cyanogenic glucosides which are known in cassava are linamarin and lotaustralin. These compounds of cassava contain toxic antinutritional substances that interfere with digestion and uptake of

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nutrients (Wobeto *et al*., 2007).

Despite its importance as a food and feed in Godere *Woreda* of Mezengher Zone, southern Gambella, not much is known about the role of the fermentative microorganisms in cyanide reduction, improving the protein content, enhancing flavor and taste on locally processed cassava foods in the study area. Therefore this study is intended to evaluate the level of cyanide reduction and the extent in which improvement is made in the protein composition and palatability of cassava based foods using the fermentative activities of selected native microflora.

Material and Methods

Samples were collected from Godere *Woreda* which is located in south western Ethiopia in Mezhenger Zone of Gambella region.

Experimental design

Completed randomized design with 3 x 3 x 3 factorial arrangements of treatments were used. The model included the use of three selected pure cultures of cassava fermenting microorganisms i.e. *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* and, each at 0.25 ml, 0.50 ml and 0.75 ml inoculums level and 3 fermentation times (24, 36 and 48 hrs).The non fermented cassava was used as a control for all fermentation experiments.

Sample preparation

The peeled cassava tubers (2 kgs) were cut into cylindrical pieces and steeped in 4 litter of sterile distilled water for 72 hours. The resulting soft cassava tubers were hand pulverized and sieved using a sieve of about 1.00 mm mash size. The sieved mash was allowed to sediment for 12 hours before the tap water was decanted. The sediment mash was then placed in jute bag and pressed to remove the water. The resulting wet product was further dried in a single layer at 65^{o}C for 48 hours in a cabinet dryer. The dried cake was then milled to powder by mortar and pestle. Finally the powder was kept in refrigerator at 4^{o}C until used for further analysis (Oyewole, 1991).The work was done at Haramaya University Pathology Laboratory.

Selection of starter microorganisms from fermented cassava

Three isolates which were dominant during the fermentation were selected. *L. plantarum* and *L. mesenteroides* and *S. cerevisiae*. The two bacterial isolates belong to lactic acid bacteria that are commonly isolated from foods. *L. plantarum* and *L. mesenteroides*, apart from being widely used in the preparation of fermented milks, have been reported as the predominant strains among isolates of traditional sour cassava fermentation (Figueroa *et al*., 1995). Similarly, *S. cerevisiae* is known industrially as important yeast used in the production of a variety of fermented foods. Besides, all the three isolates have no history of pathogencity (Colar, 1996). A similar procedure was employed in selection of starter cultures of fermented maize bread by previous researchers (Edem and Sanni, 2008).

Isolation and inocula preparation of isolates

Isolation and identification were carried out as described by Sharpe (1979) on the basis of Gram-staining, catalase test, cell and colony morphology, growth at 15^{o}C and 45^{o}C and other biochemical tests such as growth in 4% and 6% NaCl and carbohydrate fermentation patterns. Identification of *S. cerevisiae* was carried out based on morphological and physiological characteristics as per the standard yeast identification techniques used by Mossel *et al.* (1995).

The selected candidate starter cultures were harvested by aseptically adding 10 ml of sterile peptone water in to the respective agar slants. The resulting suspensions were adjusted with sterile peptone water using a spectrophotometer to give a concentration of 10^{6} – 10^{7} Cfu/ml and subsequently used as inocula.

Saccharomyces cerevisiae (S.C)

Growth medium containing yeast extract (1%), peptone (2 %), and glucose (2%) was prepared using three Erlenmeyer flasks of 250 ml capacity. Spore suspensions of *S. cerevisiae* were also prepared using sterilized peptone water in to the respective agar slants. The resulting suspensions were adjusted with sterile peptone water using a spectrophotometer to give a concentration of 10^{6} – 10^{7} cfu/ml and subsequently used as inocula. About 20 gm of cassava flour was then added into each of the three flasks and the moisture content was adjusted to about 25%. After autoclaving, the three flasks were inoculated with 0.25 ml, 0.5 ml, 0.75 ml of *S. cerevisiae* spore suspension and incubated at 25^{o}C (optimum growth temperature). Samples were then withdrawn for analysis after 24, 36 and 48 hrs of fermentation.

Lactobacillus plantarum (L.P) and Leuconostoc mesenteroides (L.M)

The growth medium used for slants of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* was MRS medium. 10 ml of sterile

peptone water was added to 18-24 hrs held culture slants of *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, followed by aseptic agar surface scrapping under vigorous shaking (Adeyel, 1986). From the resulting suspensions, 0.25 ml, 0.5 ml, and 0.75 ml of each of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were added aseptically to each of the two sets of three flasks containing 20 g of sterile cassava mush and allowed to ferment for 24, 36 and 48 hrs. The incubation temperature and the moisture contents were adjusted to 30ºC and 25%, respectively. After fermentation the water was pressed out and used for further analysis.

The proximate composition of each sample of fermented cassava was determined using standard analytical procedures. The amount of free cyanide was calculated in milligram per gram of cassava based on AOAC (1995) method. The percentage moisture content of the sample was determined based on weight loss of water due to evaporation during drying in an oven at 130ºC for one hours until constant weight is obtained. The pH value of the flour samples were determined by using a digital pH meter (JENWAY-370, Burl World Scientific, England). Standardization (calibration) of the pH meter was done by using buffer solutions of pH 7 and 4. While crude protein was determined using the kjedahl method.

Sensory evaluation of the samples fermented with pure selected cultures and with no culture was done at the same time with equal amount of sample divided in labeled plastic trays. Then the samples were evaluated by assessors from Gambella ATVET College students of Meshenger zone. The samples were evaluated by 30 students. Evaluation was done on a five point hedonic scale with respect to color, odor, taste and overall acceptability following the methods of Larmond (1977).

Statistical analysis

All the measured variables were subjected to the analysis of variance for complete randomized design using SAS software. Three way ANOVA was used to compare results among fermented cassava and unfermented control. The least significance difference (LSD) at 5% was used to separate significant differences by different treatment means.

Results and Discussion

The effect of singles starter culture, size of inoculums, and fermentation time on pH, free cyanide, and crude protein content of fermented cassava

As shown on Figure 1, the cassava sample

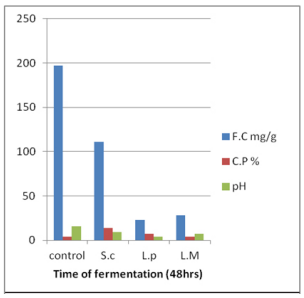

Figure 1. The effect of microorganisms and time of fermentation on F.C, C.P, and PH of fermented cassava

fermented with single starter culture at 48 hrs showed a pH change from 4.95 to 3.70. The mean pH of fermented cassava decreased from 6.68 in the non-fermented (control) to between 3.70 and 4.95 in cassava fermented with single starter cultures (Figure 1). This indicates that cassava fermentation by the action of a single species of micro-organisms can result in a significant reduction in pH. This result is in agreement with the report of Oyewole and Afolami (2000) who indicated acid production during fermentation as a result of the activities of lactic acid bacteria on the carbohydrate content of cassava root. The result was also in agreement with the results of Giraud *et al.* (1993) who reported that the use of *L. plantarum* strain as cassava fermentation starter for garri production caused lowering of the final pH change and a greater production of lactic acid. In this study, the observed mean pH value was lower than the ideal pH required (i.e. 5 and 6) for cyanogenic glycoside breakdown reported by White *et al.* (1994)

Addition of single starter culture, inoculum level and time of fermentation had a highly significant ($p < 0.001$) effect on free cyanide content of fermented cassava. As shown on Figure 1, the free cyanide content of all fermented cassava samples were reduced to lower levels in 48 hrs of fermentation. However, the extent of reduction varied with fermentation time, size of inoculum and type of microorganism. The free cyanide level dropped from 197.19 mg/g of non-fermented (control) cassava to 4.09 mg/g (a 97.92% reduction) after 24 hrs of fermentation with *L. plantarum* and an inoculum level of 0.50 ml. This indicated that it is possible to significantly reduce the residual HCN content of cassava through fermentation using appropriate microorganisms. The 4.09 mg/g free cyanide content obtained from samples fermented with L. plantarum was below the safe level recommended by FAO/WHO (1999). This finding suggests the need to use *L. plantarum* as the preferred cassava fermenting starter culture. The reduction in cyanide content could be attributed to the ability of the inoculated microorganism (*L. plantarum*) to

degrade cyanogenic glucosides. *L. plantarum* lowers the HCN content of cassava because of its ability to produce linamarase which can hydrolyze linamarin (a cyanogenic glucoside) (Guyot *et al.*, 1998).

A comparison of the reduced content of free cyanide in the yeast fermented sample and the unfermented control indicates that the use of *S. cerevisiae* as a starter culture in cassava fermentation will contribute significantly to reduce the free cyanide content. This is consistent with the observation of Amoa-Awua *et al.* (1997) which revealed that all yeasts and moulds identified in traditional cassava dough inocula exhibited linamarase activities and were therefore capable of degrading cyanogenic glycosides.

As indicated above, the degradation might be due to cyanophilic microorganisms that possess the enzymes linamarase, hydroxynitrile lyase and cyanide hydratase that catalyze the sequential degradation of cyanogenic glycosides into HCN which is subsequently converted into fomamide which is used as both a nitrogen and carbon source. However, the variations in the free cyanide concentration of the individual samples were attributed to differences in the type of microorganisms used, time of fermentation and the size of inoculum used. Additionally, the difference in free cyanide content within a given inocula is attributed to the reaction of acetone after degradation of linamarine with hydrogen cyanide from substrate to form aceton cyanohydrine and back to linamarine (Kwok, 2008).

The mean crude protein content of fermented cassava increased from 0.74% to 4.58% (3 folds increment) after 48 hrs of fermentation. The highest crude protein content (4.58%) was recorded in samples fermented with *S. cerevisiae* for 48 hrs at inoculum level of 0.50 ml followed by samples fermented with *L. plantarum* (4.31%) at inoculum level of 0.75 ml for 48 hrs. This indicated that *S. cerevisiae* had the highest capability to enrich the crude protein content of cassava products. This result is consistent with the results Oboh and Akindahunsi (2005) who reported that crude protein content in fermented cassava could be attributed to the ability of *S. cerevisiae* to secret some extra cellular enzymes such as amylases, linamarase and cellulase into cassava mash during their metabolic activities which could lead to yeast growth.

The crude protein content of cassava product shown in figure 1 (0.74 to 4.58%) was lower than that reported by Boonnop *et al.* (2009) who demonstrated that fermentation of cassava chips with *S. cerevisiae* could increase crude protein content from 2% to 32.4%. The difference could probably attribute to the variety of cassava used, agro-ecological conditions, and fermentation time. This finding is in agreement with the work of Belewu and Babalola (2009) who reported that the crude protein content of fermented cassava pulp was higher than the unfermented one. The increase in the crude protein content was due to the effect of microbial cell growth (MacDonald *et al.*, 1998). Of all the samples fermented with single starter culture, the sample that had been fermented with *S. cerevisiae* showed a significant increment (0.74% to 4.58%), followed by *L. plantarum* (0.95% to 4.31%) and *L. mesenteroides* (1.10% to 2.04%), respectively.

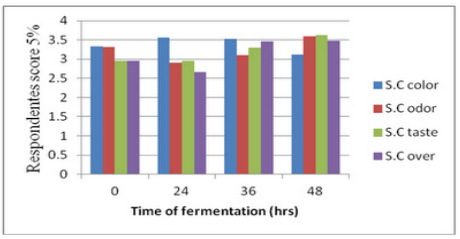

Figure 2.The effect of *Saccharomyces cerevisiae* (S.C) on color, taste, odor and overall acceptability of *chike.*

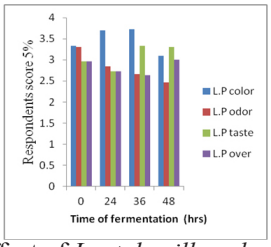

Figure 3. The effect of *Lactobacillus plantarum* (L.P) on color, taste, odor and overall acceptability of *chike.*

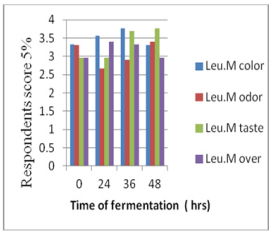

Figure 4. The effect of *Leuconostoc mesenteroides* (L.M) on color, taste, odor and overall acceptability of *chike.*

Sensory evaluation of cassava inoculated with single starter culture

Analysis of variance showed that the interaction effect of single starter culture, time of fermentation and addition of 0.75 ml of inoculum level had a significant ($P < 0.05$) difference on the odor and taste and highly significant ($P < 0.001$) difference on overall acceptability of *chike* (Figure 2, 3 and 4). In contrast, both the main and interaction effect of starter culture, fermentation time and addition of 0.75 ml of inoculum level made no significant ($p > 0.05$) difference on the color of chike.

The result of sensory evaluation carried out

on *chike* (product made from cooked cassava) fermented with three single starter culture, in three different fermentation times with the addition of 0.75 ml of inoculums size. The panelists preferred sample fermented with *S. cerevisiae* for 48 hrs with 0.75 ml of inoculum size. They rated the odor of *chike* produced under this treatment condition as the best giving it a score of 3.60 (72%) (Figure 2). The microbial activities which increased as fermentation continued might have accounted for the perceived differences in the odor of the product fermented for different lengths of fermentation time. In line with this finding Torner *et al.* (1992) reported that *S. cerevisiae* was able to produce compounds such as organic acids, alcohols aldehydes and carbonyls which have imparted appealing flavor to the fermenting cassava.

The panelists rated the sample fermented with *L. mesenteroides* for 48 hrs at an inoculum level of 0.75 ml as having the best taste with the score of 3.76 (75.2%) (Figure 4) . This might be possibly attributed to the fact that *L. mesenteroides* converts the sugars in fermenting substrate (primarily glucose and fructose) to lactic acid, acetic acid, ethanol, CO_2 and other flavor compounds (Lu *et al*., 2010).

In terms of overall acceptability, compared to samples fermented with other combinations of treatments and unfermented samples, the panelists rated 3.48 (69.6%) and showed preference for the samples fermented with *S. cerevisiae* for 48 hrs with the addition of 0.75 ml inoculum level as indicated on Figure 2. This might be due to improvement of the organoleptic property of the product by *S. cerevisiae.* This finding is in agreement with Sanni (1993) who indicated about the role of *S. cerevisiae* in fermented foods and beverages showing that besides having many beneficial effects it also improves the flavor, texture, overall acceptance and the shelf-life of the products. In general, the sensory evaluation of *chike* showed that all the pure cultures of isolates had varying contributions to odor, taste. Over all fermentation with *S. cerevisiae* played a major role in enhancing odor and overall acceptability and *L. mesenteroides* only in the taste of chike.

Conclusion

The effect of single starter culture, time of fermentation and inoculum level had shown significant ($p < 0.05$) difference on pH, free cyanide, crude protein and moisture content of fermented cassava. From inoculated pure single starter cultures, the two lactic acid bacteria (*L. plantarum* and *L. mesenteroides*) resulted in reduced pH value from 6.68 (unfermented/control) to 3.70 and 3.71 (fermented) samples .at the end of fermentation with inoculum size of 0.50 ml and 0.75 ml, similarly 97.92% and 97.76% reduction in the amount of free cyanide was observed in samples fermented by the two lactic acid bacteria, i.e. *L. plantarum* and *L. mesenteroides*, respectively. Whereas sample fermented by *S. cerevisiae* was identified as more efficient in improving the crude protein content of cassava from 0.71% (unfermented) to 4.58% after fermentation.The sensory evaluation of *chike* in this study showed that cassava fermented with single starter culture of *S. cerevisiae* was more preferred by panelists in terms of odor and overall acceptability, while cassava fermented by *L. mesenteroides* was preferred in improving the taste of the product.

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References

Association of Official Analytical Chemists (AOAC). 1995. Official method of analysis. 16th Ed. Arlington, Virginia, U. S. A.

Adeyele, S. 1986. Microbiological studies on guinea corn fermentation for Ogi-baba production. Ibadan, Nigeria: University of Ibadan, PhD dissertation.

Amoa-Awua, W. K. A., Frisvad, L. M., Sefa-Dedeh, S. and Jakobsen, M. 1997. Contribution of moulds and yeasts to the fermentation of cassava dough. Journal of Applied Microbiology 83: 288- 296.

Belewu, M. A. and Babalola, F. T. 2009. Nutrient enrichment of waste agricultural residues after solid state fermentation using *Rhizopus oligasporus*. Journal of Applied Bioscience 13: 695-699.

Boonnop, K., Wanapat, M., Nontaso, N. and Wanapat, S. 2009. Enriching nutritive value of cassava root by yeast fermentation. Science of food and Agriculture. Piracicaba, Braz. 66: 616-620.

Chauynarong, N., Elangovan, A. V. and Iji, P. A. 2009. The potential of cassava products in diets for poultry. World Poultry Science journal 65: 23-35.

Cock, J.H. 1985. Cassava: New potential for a neglected crop. Boulder, Colorado, USA. Westview Press.

Colar, C. 1996. Review: Biochemical and technological assessment of the metabolism of pure and mixed cultures of yeast and lactic acid bacteria in bread-making applications. Food Science and technology International 2: 349-356

Edema, M. O. and Sanni, A. I. 2008. "Functional Properties of Selected Starter Cultures in Sour Maize Bread" Food Microbiology 25(4): 616-625.

Food and Agriculture Organization of the United Nations / World Health Organization. 1991. Joint FAO/WHO food standards programme. In: Codex Alimentarius Commission XII (Suppl. 4). Rome

Figueroa, C., Davila, A. M. and Pourquie, J. 1995. Lactic acid bacteria of the sour cassava starch fermentation. Letters in Applied Microbiology 21:126-130.

Giraud, E., Champailler, A. R. and Raimbult, R. 1994. Degradation of raw starch by a wild amylolytic strain of *Lactobacillus platarum.* Applied Environmental Microbiology 60:319-323.

Guyot, J. M. 1998. *Lactobacillus manihotivorans* species. nov, a new starch-hydrolysing lactic acid bacterium isolated during cassava sour starch fermentation. International Journal of System Bacteriology 48: 1101-1109.

Heuberger, C. 2005. Cyanide content of cassava and fermented products with focus on tieke and attieke garba. Diss. ETH No. Zurich, Swiss: Federal Institute of Technology, BSc thesis.

Kimaryo, V. M., Massawi, G. A., Olasupo, N. A. and Holzapfel, W. H. 2000. The use of a starter culture in the fermentation of cassava for the production of 'Kivunde', a traditional Tanzanian food product. International Journal of Food Microbiology 56: 179–190.

Larmond, E. 1977. Laboratory method of sensory evaluation of foods. Canada publication. Department of Agriculture, Canada, 1-13.

Lu, Z., Altermann, E. and Breidt, F. 2010. Studying the dynamics of microbial populations during food fermentation Appied Environmental Microbiology 76: 1955-1966.

Motto, H.L., Daines, R.H., Chucks, D.M. and Motto, C.K. 1990. Environmental Science and Techology 4(3): 234-237.

Mossel, D. A. A., Lorry, J. E. L., Strick, C. B. and Baird, R. M. 1995. Essentials of Microbiology of foods. John Willey and sons Ltd, IDOSI 43-44.

Oboh, G. and Akindahunsi, A. A. 2005. Nutritional and toxicological evaluation of *Saccharomyces cerevisiae* fermented cassava flour. Journal of Food Composition and Analysis 18:731-738.

Oyewole, O. B. 1991. Fermentation of cassava for Lafun production. Food Laboratory News 17:29-31.

Oyewole, O. B. and Afolami, O. A. 2000. Quality and preference of different cassava varieties for Lafun Production. Journal of Food Science Technology 6(1):12-13.

Sanni, A.I. 1993. The need for optimization of African fermented foods and beverages. International Journal of Food Microbiology 18: 85-95.

Sharpe, M. E. 1979. Identification of lactic acid bacteria. In identification methods for microbiologist 2nd ed. Skinner F A and Livelock D W. Soc. Applied Bacterial Technology Series, Press. London. Academy 14: 233-259.

Torner, M. J., Martinez-Anaya, M. A., Antuna, B. and Benedito, C.1992 Headspace favour compounds produced by yeasts and lactobacilli during fermentation of preferments and bread dough. International Journal Food Microbiology 15:145-152.

White, W., McMahon, J. and Sayre, R. 1994. Regulation of cyanogensin cassava. Acta Horticulture 375:69-77.

Wobeto, C., Corrêa, A.D., De Abreu, C.M.P., Dos Santos, C.D. and Pereira, H.V. 2007. Antinutrients in the cassava (*Manihot esculenta* Crantz) leaf powder at three ages of the plant. Cienc Tecnol Aliment Campinas 27:108–112.