Preservation of coffee pulp by ensilage: Influence of biological additives

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SUMMARY

Data on the influence of three biological additives on the ensilage of coffee pulp for its preservation show that the endogenous microflora of the coffee pulp is efficient enough to produce good quality silage, with acceptable levels of organic acid, dry matter loss and final pH. The use of inoculants, as biological additives, showed the efficiency of natural microflora grown on coffee pulp and the monoculture of *Lactobacillus plantarum* A6 in improving the physico-chemical characteristics of the silage, though commercial inoculum was not efficient, due to several reasons. Degradation of caffeine was absent in all the cases. Cellulases as a biological additive showed increased sugar production during ensilage. The results on the kinetics of different microflora development and physico-chemical characteristics during ensilage provide the insight into the microbiology and physiology of the process and point out a number of possibilities for improving the ensilage process as well as the quality of the silage.

Keywords: Coffee pulp, ensilage, endogenous microflora, biological additives, enzyme complex, inoculants, natural microflora inoculant, *Lactobacillus plantarum*, commercial inoculum, lactic acid, acetic acid, butyric acid, ethanol, caffeine, dry matter loss.

RESUME

Conservation de la pulpe de café par ensilage: Influence des additifs biologiques.

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La microflore lactique endogène de la pulpe de café séchée au soleil est suffisante pour amorcer un ensilage présentant des caractéristiques physico-chimiques finales acceptables. Cependant, l'apport de differents ferments biologiques (un pied de cuve de pulpe de café ensilée naturellement ; un ferment monosouche (*Lactobacillus plantarum* A6) ; un ferment commercial DpH4 (associant deux souches de bactéries lactiques à un complexe enzymatique) permet d'améliorer la qualité des ensilages : homogénéité de la population microbienne, augmentation du taux d'acide lactique, pas de production d'AGV indésirables ni d'éthanol. La présence de cellulases permet d'augmenter le taux de sucres reducteurs. Les suivis du développement des microflores et les résultats physico-chimiques obtenus au cours des ensilages démontrent une conservation rapide de la pulpe de café par acidification sans perte importante de matière sèche. L'ensilage est un bon procédé de conservation de la pulpe de café humide. La caféine n'a pas été dégradée dans tous les cas.

Mots clés: Pulpe de café, ensilage, microflore endogène, additifs biologiques, ferments, mélange d'enzymes, ferments naturels, *Lactobacillus plantarum*, ferments commerciaux, acide lactique, acide butyrique, ethanol, caféine, perte de matière sèche.

INTRODUCTION

Agro-industrial residues/wastes are generated in large quantities throughout the world. Their non-utilization results in loss of valuable nutrients and environmental pollution (Zuluaga, 1989). Their better utilization by biotechnological means assumes social, economic and industrial importance. Considering these facts, ORSTOM participated into a scientific collaboration with Universidad Autonoma Metropolitana (UAM), Mexico, for the development of biotechnological processes for better utilization of agro-industrial byproducts/wastes, especially the coffee pulp (Viniegra *et al*, 1991).

Coffee pulp, generated to the extent of 40% in the fermentation of coffee berries (Zuluaga, 1989), poses many problems in the coffee producing tropical countries. Its disposal in nature, without any treatment, causes severe environmental pollution,

due to putrefaction of organic matter (Zuluaga, 1989). Hence, the possibility of utilizing coffee pulp in the biotechnological processes for production of different metabolites was investigated thorought by Roussos *et al* (1993).

Coffee pulp, as it is generated, contains 80-85% moisture (Bressani *et al*, 1972), in addition to appreciable quantities of sucrose, proteins, amino acids and other nutrients. All these factors and nutrients allow various microflora to develop quickly on the coffee pulp and the development of the microorganisms cause the putrefaction of coffee pulp (Gaime-Perraud *et al*, 1993 ; Roussos et al, 1995). It is also not practicable to utilize the coffee pulp immediately, after its generation during coffee berry treatment mainly because the season of coffee berry processing lasts for 3-5 months. During this season, the industry cannot divert attention to this waste, as its priority is focused on the quality of coffee seeds during the entire season. Moreover, quick dehydration of the coffee pulp is impracticable, considering the huge quantity of the waste, high energy requirement, larger capacity of machinery needed and heavy investment on space and building, not only for dehydration, but also for stocking of the dehydrated pulp, till its utilization.

Ensilage of coffee pulp, for its preservation and improvement of feed value, is one of the avenues for value-added utilization of coffee pulp. Ensilage, a quick anaerobic process involving lactic acid bacteria, has been extensively used for preservation of forage in the temperate regions. It allows the prevention of putrefaction of the forage with minimum degradation of organic matter. The process is quicker and it also improves the nutritive quality of the forage (Mc Donald *et al*, 1991).

A number of factors are of vital importance in obtaining a good silage. The substrate to be ensiled should have 30-40% dry matter, should be compactable to the desired level, amenable for anaerobiosis and contain utilizable sugars in sufficient quantities (Bertin, 1986). It must also have the colour, which is most nearer to the raw material, the fruity aroma and slightly acidic taste. In terms of chemical characteristics and achievement of the organic matter stability, the ensilage should involve a minimum loss of dry matter and the resulting silage should have a pH value lower than 4.5, higher than 3% lactic acid, but less than 0.5 and 0.3% acetic and butyric acid, respectively (Mc Donald *et al*, 1991).

A number of chemical and biological additives are mixed with the substrate for improving silage or reducing fermentation time. In the case of biological additives, a lactic acid bacterial inoculum is added, as a minimum of 10^5 lactic acid bacteria per g dry matter is required (Gouet, 1994) to convert the carbohydrates into lactic acid, but not into butyric acid. Enzymes are also added, when the rate of assimilation of sucrose by the endogenous lactic acid bacteria is slower (Bertin, 1986).

Ensilage is also practiced in tropical countries, despite the problems in terms of temperature, humidity and rains. Consequently, the rate of ensilage is slower, putrefaction is common and there is need to use a number of additives.

A number of reports have been ptoduced on ensilage of coffee pulp (Bohkenfor and Fonseca, 1974; Murillo, 1978; Carrizalez and Gonzalez, 1984). But, most of these are associated with the development of the ensilage technique or the effect of chemical additives on the process. For example, Murillo (1978) compared the silage of coffee pulp, obtained by natural microflora based fermentation, with that involving the use of molasses or organic acids as additives. After 90 days of ensilage, the loss of dry matter was as high as 26.8%, in the case of the use of organic acids as additive, though it allowed to attain a pH of less than 4.0. Caffeine content of the drained water was reported to increase significantly, in the case of the use of organic acids, probably because it became more soluble in acidic pH.

In the present studies, the ensilage of coffee pulp was investigated, with respect to the microbiology and biochemistry of the process, along with the evaluation of biological additives, for improving the process and also the quality of the silage. Accordingly, the studies involved a) allowing the endogenous lactic microflora to grow on coffee pulp for using the fermented mass as inoculum for the next batch, b) use of monoculture of *Lactobacillus plantarum* A6 as a biological additive and c) the use of commercial inoculum as yet another biological additive. The latter contained two lactic bacteria and an enzyme complex.

MATERIAL AND METHODS

In order to overcome the problem of variation, the same batch of coffee pulp was used throughout the whale experiment. The coffee pulp used was derived from berries of *Coffea arabica* and was obtained from a coffee processing industry from Xalapa, Mexico, where the coffee berries are processed using a wet fermentation method. The coffee pulp was sun-dried by the industry for 10 days to achieve less than 10% moisture and packed in plastic sacs. The dried coffee pulp was coarsely ground and sieved to select the particle size between 0.8 to 2.0 mm. It was hydrated to contain about 70% moisture, before ensilage.

Plastic bottles with closed fitted caps were used as laboratory scale microsilos. Each plastic bottle was charged with 60 or 150 g moist coffee pulp. The moist material in the microsilos was compacted manually for removing the air from inter-particle spaces. The microsilos were incubated at darkness, at 30°C for one month and the samples were removed at desired intervals. Each microsilo constituted a sample.

The moist unsterilized coffee pulp was ensiled under four conditions, i.e., a) dry pulp was only hydrated with water without inoculation, b) dry pulp was hydrated with water and then mixed with natural inoculum provided from (a), c) dry pulp was hydrated and mixed with inoculum containing *Lactobacillus plantarum* A6, d) similar to (c) but inoculated with commercial inoculum. In all the cases, the media were inoculated, before charging into the microsilos, unless otherwise stated.

INOCULUM DETAILS

For development of natural microflora, the unsterilized dry pulp was hydrated and the endogenous microflora was allowed to grow in column fermenters at 30°C for 2 months in anaerobic conditions. This inoculum was added to obtain 10^4 lactic acid bacteria/g dry matter. In the case of monoculture studies, *L. plantarum* A6, isolated from cassava by the Laboratoire de Microbiologie, ORSTOM Centre, Brazzaville, Congo (Giraud *et al*, 1991), was used. The inoculum suspension contained 10^8 bacteria/g dry matter. The commercial inoculum contained two lactic acid bacterial species (10^{10} and 3.10^9 of *L. plantarum* and *Pediococcus acidilactici*, respectively, per g) and the enzyme cellulase (10.000 CMCases units/g). In this last case, the inoculation was done to achieve 10^5 cfu lactic acid bacteria/g dry matter.

TREATMENT OF THE SAMPLES AND ANALYSES

A schematic flow diagram of the sample treatment and analyses is given in Fig. 1. The sample was well mixed, 10% of the moist material was removed and mixed with sterile distilled water, containing 0.001% Tween 8O (dilution: 1/10). The mixture was homogenized at 8000 rpm for 4 min in Ultra-Turrax and used for estimating different microorganisms (Perraud-Gaime, 1995).

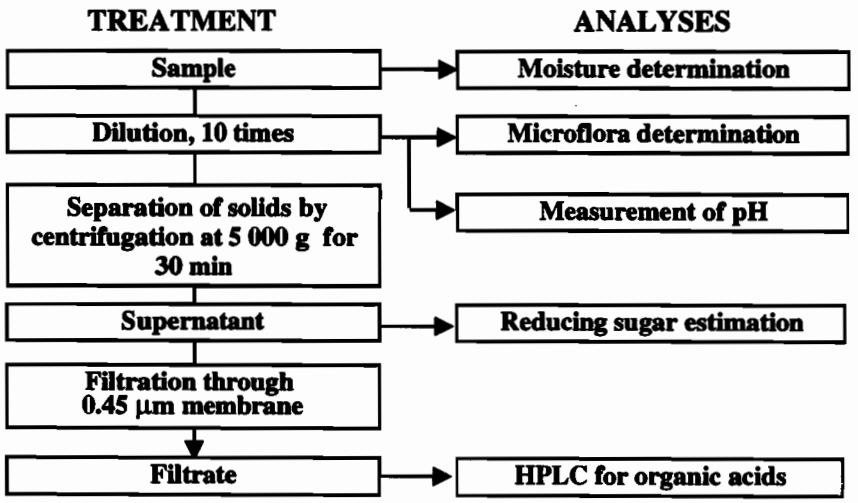

Fig. 1. Schematic diagram for treatment of the sample for microbiological and physico-chemical analyses.

Plate Count Agar was used for determining the total bacterial count, while Potato Dextrose Agar and Sabouraud Dextrose Agar were employed for determining total fungal and total yeast counts, respectively. In both of these media, chloramphenicol (0.25 g/L level) was added to prevent bacterial growth. For the determination of total anaerobes and lactobacilli, MRS agar was used in total anaerobic conditions in the anaerobic incubator (Anaerocult A, Merck). In all the above cases, the incubation temperature was 28°C.

Reducing sugars were estimated by using the dinitrosalicylic acid reagent, as in the method described by Miller (1959). Different organic acids were estimated by HPLC. The conditions employed and the operation of HPLC were described by Giraud *et al* (1991).

RESULTS

The microbial analysis of dry coffee pulp indicated the presence of endogenous microflora, which contained 10^4 lactic acid bacteria/g dry matter. Under favorable atmosphere, such as water and anaerobic conditions endogenous microflora started to grow and 2 months later, the lactic acid bacterial count increased from an initial of 10^4 to 2.10^5 /g dry matter (Fig. 2) in the ensiled coffee pulp based on natural microflora.

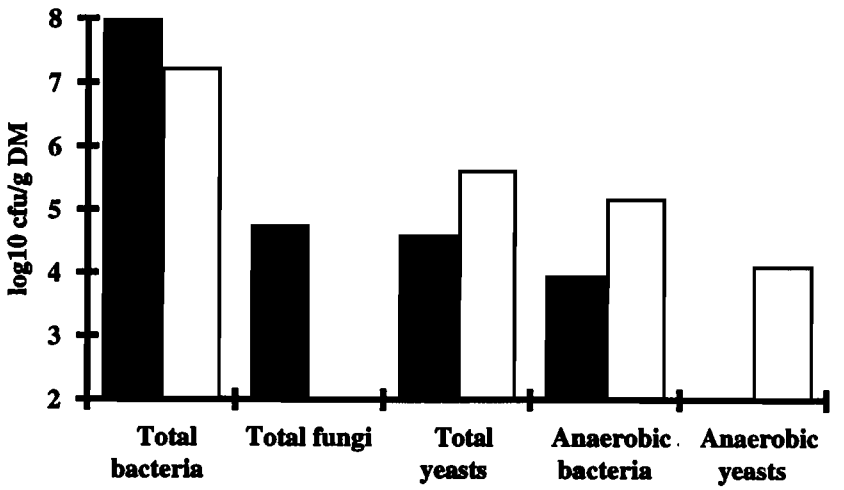

Fig. 2. Evolution of microflora in coffee pulp ensiled without any biological additive. n : Initial coffee pulp, o : Ensiled coffee pulp (2 months)

The microbiological studies indicated highly heterogeneous nature of the microflora, among which the principal genera was *Lactobacillus*. In the ensiled sample, fungi were totally absent, but yeast counts increased significantly, because of the anaerobic conditions prevailing during the ensilage.

The results of physico-chemical analysis of the coffee pulp, ensiled without additives, are presented in Table 1. The ensiled coffee pulp showed a good production of lactic acid (2.39%), a small production of acetic acid (0.29%) and total absence of butyric acid as well as ethanol. The loss of dry matter was very low (0.80%) and the pH was 3.9. However, there was negligible reduction in the caffeine content. These results indicated that ensilage of coffee pulp by natural microflora, without any chemical or biological additives, resulted in a satisfactory silage in many respects. But, the silage contained a lot of yeast, in addition to the presence of highly heterogeneous microflora, and hence it was not of good quality. Therefore, studies were undertaken to evaluate three different biological additives and their effect on the evolution of the microflora in the ensiled material, pH changes, production of organic acids and dry matter loss.

Table 1. Comparative physico-chemical characteristics of the coffee pulp ensiled using natural microflora and biological additives (Perraud-Gaime, 1995).

Parameters	Initial pulp	Ensilage of coffee pulp (28 days)			
		Without additives	Natural microflora	*L. plantarum* A6	Commercial inoculum
Moisture	62,55	61,63	66,78	66,78	66,19
pH	4,44	3,90	3,91	3,92	4,11
DM losses (%)	-	0,80	1,73	1,41	0,38
Lactic acid (%DM)	0,00	2,39	3,35	2,14	0,08
Acetic acid (%DM)	0,00	0,29	0,68	0,48	0,05
Reducing sugars (%DM)	4,72	4,85	4,56	3,67	8,32
Caffeine (%DM)	1,04	0,95	1,02	0,93	0,90

ENSILAGE WITH BIOLOGICAL ADDITIVES

The development of microflora in three ensilage processes is presented in Fig. 3. The microbial population was dominated by aerobic bacteria during the first two days of ensilage. By this time, the lactic acid bacteria grew sufficiently and their number was more or less equal to that of the total aerobic bacteria during the rest of the fermentation period, but only in the case of ensilage with additives of natural microflora or monoculture of *L. plantarum*. In the case of a commercial inoculum, aerobic bacteria continued to be dominant throughout the fermentation period of 30 days.

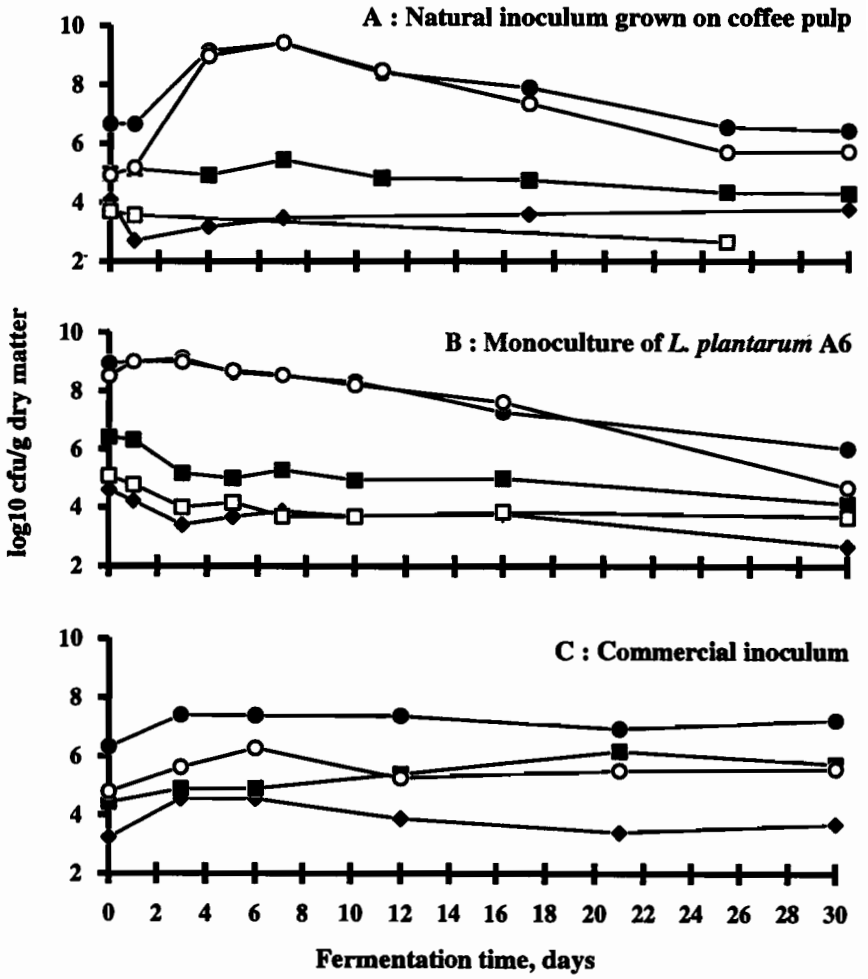

Fig. 3. Development of microflora during the course of ensilage of the coffee pulp with three different biological additives (A, B, C) during 30 days (Perraud-Gaime, 1995). l : Total bacteria, u : Total fungi, n : Total yeasts, m : Total anaerobic bacteria, o : Total anaerobic yeasts.

In ensilage with the addition of natural microflora and monoculture of *L. plantarum*, the initial lactic acid bacterial count of $1.5x10^4$ and $2x10^8$ per g dry matter, respectively, increased to 10^9-10^{10} per g dry matter in about 2-4 days (Figs. 3A, B). In contrast, the lactic acid bacterial population increased from 10^5 to merely about 10^6 even by 6 days, in the case of the commercial inoculum (Fig. 3C). The counts of lactic acid bacteria started decreazsng after 10 days and became stable at 10^5 per g dry matter by the end of ensilage process in all the cases.

Similarly, decrease in the population of the fungi started from the first day of ensilage, but only in the case of ensilage with inoculum of natural microflora and monoculture of *L. plantarum*. The number of total yeasts in the system did not change in the case of use the *L. plantarum* monoculture. Similarly, the anaerobic yeasts, which represent about 10% of the total yeasts, did not change in the medium with addition of natural microflora, in contrast to their decrease in the medium with monoculture of *L. plantarum*. It is of interest to point out that the microscopic examination of the samples revealed that the fungi are present in spore forms, as no vegetative cells were seen. In the case of the commercial inoculum, the picture in terms of the number of yeasts and fungi is totally different than that observed with the other two biological additives. For example, there was no change in fungi during the initial period and these started declining only after 10 days. However, the total yeast count increased slightly. Anaerobic yeasts could not be counted as their number was less than 10^2 per g dry matter.

The evolution of the kinetics of different parameters of ensilage (pH, lactic acid, acetic acid, and sucrose reduction) is presented in Fig. 4, while Table 1 gives the physico-chemical analysis of the coffee pulp ensiled for 28 days.

These results indicate that the quality of the ensiled coffee pulp is satisfactory when natural microflora or monoculture of *L. plantarum* were used as biological additives. The production of lactic acid, in these cases, correlates with the pH changes and these values indicate a good silage. Moreover, the acetic acid formed is also much lower (Fig. 4). In contrast, the silage, made by using commercial inoculum as a biological additive, is of inferior quality, probably because of the poor development of lactic acid bacteria in the system and consequent lower production of lactic acid.

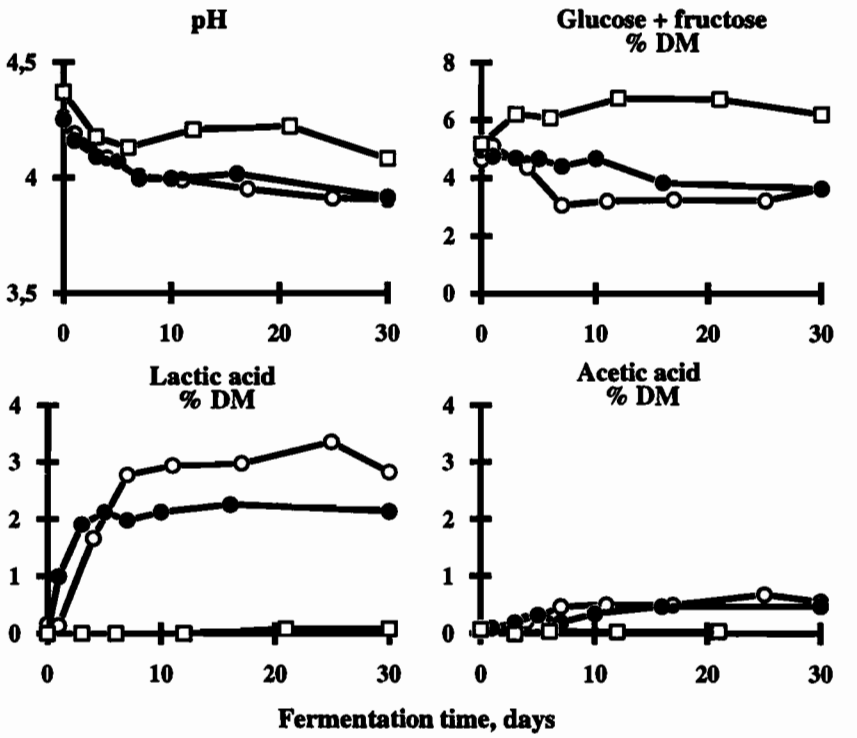

Fig. 4. Kinetics of the changes in physico-chemical characteristics of the coffee pulp ensiled for 30 days with the use of the different biological additives (Perraud-Gaime, 1995). m : Natural microflora grown on coffee pulp, l : Monoculture of *L. plantarum* A6, o : Commercial inoculum.

DISCUSSION

Macroscopic examination of the final product permits to state that the sillage of coffee pulp, obtained during this study, is satisfactory, except in the case of use of commercial inoculum as a biological additive. Of course, the inoculum rate in the case of the commercial inoculum was the lowest among all the experiments, but it was used at that level, as recommended by the society that produces it commercially. The physico-chemical analyses of the coffee pulp ensiled under different conditions (Table 1) support the above statement, as lactic acid is more than 2%, the final pH is less than 4.0, dry matter loss is less than 2%, acetic acid is less than 0.7% and butyric acid as well as ethanol are absent.

The effect of the inoculum as a biological additive has been reported to be positive by Weinberg *et al* (1993), when the endogenous lactic acid bacterial count of the substrate to be ensiled is between 10^2 to 10^4 per g dry matter. The ensilage process becomes slower otherwise and the use of inoculum makes it faster.

In the case of ensilage of dry coffee pulp in laboratory scale microsilos, after hydration and inoculation with the monoculture of *L. plantarum*, the differences in the quality are minor, when compared to the use of inoculum consisting of natural microflora, in spite of the initial lactic acid bacterial count of $2x10^8$ per g dry matter in the former case, opposed to that of $1.5x10^4$ per g dry matter in the latter case. However, it is of interest to note that the lactic acid culture in the former case is homogeneouscompared to the heterogeneous nature of the microflora, in the case of use of natural microflora as an inoculant. Such massive inoculation, in the case of monoculture of *L. plantarum*, allows production of lactic acid at a faster rate, with consequent rapid drop in the pH value.

Total endogenous microflora of the dried pulp was 10^7 per g dry matter and was dominated by total bacteria and, hence, problems will be encountered, when the lactic acid inoculum is low, despite the presence of endogenous lactic acid bacteria in the dry coffee pulp. This may be the reason for unsatisfactory quality of the silage, in the case of use of the commercial inoculum. The level of this inoculum, used in the present studies, was probably lower for the substrate used, i.e., coffee pulp, as this inoculum is made for use in ensilage of forage. Poor development of natural and inoculated lactic acid bacterial population, in the case of use of the commercial inoculum, may also be due to the antagonism between different population of the ensilage coffee pulp. Moreover, the commercial inoculum has been manufactured for use in temperate regions. It is of interest to note that the silage quality was good, when the commercial inoculum was used at a level of 10^8 lactic acid bateria per g dry matter (Perraud-Gaime, 1995).

It has been reported that the endogenous lactic acid microflora of dry coffee pulp is highly heterogeneous (Perraud-Gaime, 1995). In spite of this fact, the use of natural microflora of the coffee pulp gives a good silage. This may be explained by the fact that the endogenous lactic acid flora of coffee pulp have been selected by the substrate itself. Therefore, these are more adapted to grow on the coffee pulp, than the other lactic acid bacterial inoculants grown on liquid media, using sucrose, glucose or lactose as substrate.

The evolution of volatile organic acids is an important indicator of the quality of the silage. It has been reported that the final concentrations of lactic acid should be 3 to 13% (Catchpoole and Henzell, 1981). The silage is also stated to be good, when the production of lactic acid starts the very first day of ensilage, reaches the maximum level by 6 days and then stabilizes (Luis and Ramirez, 1985). In the present studies,

this has been the case, when natural microflora and monoculture of *L. plantarum* were used as biological additives. In both cases, production of lactic acid correlated with pH changes.

Acetic acid is known as an anti-myiotic agent inhibiting the development of fungi and yeasts (Moon, 1983). Therefore, the presence of acetic acid in silage has positive effects. Moreover, the absence of propionic and butyric acids in the silage indicates that endogenous lactic acid flora is mostly homofermentative, while the heterofermentative lactic acid microflora on dry coffee pulp is either absent or represents a minority.

It has been reported that the substrate should have a minimum of 12% reducing sugars, in order to obtain good silage (Demarquilly, 1985). In the case of coffee pulp, the quantity of reducing sugars present is lower, i.e., about 5% (Table 1), but it does not appear to be of any consequences, as the ensiled coffee pulp has the pH value of about 3.9 and, hence, silage is good. As the initial pH of the moist coffee pulp is lower, i.e., 4.3 (Table 1), the quantity of reducing sugars presents in the coffee pulp is probably sufficient to lower the pH to the desirable level. Moreover, the level of reducing sugar does not reduce significantly during the ensilage, as the residual reducing sugar present in the silage is between 3.67 to 4.56% on a dry matter basis (Table 1, Fig. 4). The data, thus, indicate that the production of lactic acid in ensilage of coffee pulp is probably due to degradation of complex sugars, such as hemicelluloses and pentosans (Pettersson and Lindgren, 1990).

Consequently, it is not necessary to supplement the coffee pulp by sugars, such as molasses. It is worth pointing out that Murillo (1974), Ferrer (1984) and Porres *et al* (1993) have used molasses as chemical additive, but the improvement in the quality of the silage was negligible. It seems more reasonable to use biological additives, such as a lactic acid bacterial inoculant and an enzyme complex, to achieve higher degradation of the constituents of the cellulosic components of the vegatative cell-wall (Vanbelle *et al*, 1994). This observation is supported by production of sugars in the silage, in the case of use of the commercial inoculum (Fig. 4). Data, thus, indicate that the conditions (acidic pH, temperature and contact of cellulases with substrate) employed in the present studies are favourable and optimum for the cellulolytic activity present in the commercial inoculum. In fact, the residual sugars in the silage can lead to the production of a higher quantity of lactic acid, using a higher inoculum.

However in all cases, negligible degradation of caffeine was observed in the present studies (Table 1). Moreover, the loss of dry matter was very low without production of water drainage during ensilage (Table 1). These results indicate that the caffeine is not degraded by the lactic acid bacteria. This is in contrast to the report that the caffeine is degraded during the ensilage of coffee pulp, with the use of natural

microflora and added lactic acid as a bacterial inoculum (Porres *et al*, 1993). It, thus, appears that the loss of caffeine observed by these workers is actually not due to the activity of lactic acid bacteria. It was probably due to increased solubility of caffeine in water at low pH values. Hence, the evacuation of water drains during ensilage reduced the caffeine content of the pulp. Studies to support these facts are contemplated.

CONCLUSION

Data allow us to conclude that ensilage is a good technique for preservation of wet coffee pulp. The endogenous lactic acid flora of dry coffee pulp is sufficient enough to produce a good quality of silage. However, addition of biological additives, such as lactic acid bacterial inoculants and enzymes, allows the improvement of the quality of the silage, in terms of augmentation of lactic acid production, without concomitant production of volatile organic acids and ethanol. Caffeine is not degraded during the process and hence it is necessary to decaffeinate the coffee pulp with appropriate fungi by solid state fermentation (Perraud-Gaime and Roussos, 1995), if the ensiled coffee pulp is to be used for animal feeding, as caffeine has antiphysiological effects (Bressani *et al*, 1972).

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