Chapter 35

DEVELOPMENT OF BIOPROCESSES FOR THE CONSERVATION, DETOXIFICATION AND VALUE-ADDITION OF COFFEE PULP AND COFFEE HUSK

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1. Introduction

A collaborative effort by research teams from France, the UK, Mexico and Brazil funded by the European Union INCO-DC programme (contract no IC18*CT970185) was initiated with the global objective of recycling the coffee pulp and coffee husk by biotechnological processes. Mexico alone produces more than 300,000 metric tons of

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grain coffee each year using 'wet-processing' technology resulting coffee pulp as the waste residue. The Brazilian State of Parana produced 220,000 metric tons of grain coffee in 1998 using 'dry-processing' technology resulting coffee husk as the waste residue. Considering the great volume of this agro-industrial waste, its high biodegradability and its potential as feed source for animals, a proposal was set-up to stabilize fresh pulp by the lactic acid silage technique, as it is being produced, during the crop season. However, Recalcitrant and Toxic Compounds (RTC) such as caffeine, tannins and polyphenols need to be removed. RTC's actually represent a strong limitation on the use of coffee pulp/husk as a nutritive source for animal feeding purposes (Bressani, 1979). They also cause serious problems of environmental contamination (Violle *et al.*, 1995; Zambrano-Franco *et al.*, 1999). Ensiling of coffee pulp and its further utilization could avoid the rapid degradation of the material, which quickly becomes a very significant source of contamination of water in coffee growing areas (Zuluaga, 1989). The stabilized and detoxified coffee pulp/husk will be further utilized after the crop season as animal feed or for mushroom, fungal metabolites, or enzymes production (Perraud-Gaime, 1995; Ramirez-Martinez, 1999; Pandey and Soccol, 2000).

Recently, several mechanical improvements of coffee processing have been made in Colombia and Central America such as the use of 'dry' (no water added) depulpers and demucilagers and the re-design of water currents for minimal washing of fresh coffee beans (Alvarez-Gallo, 1991, 1995; Avallone, 1999). Thus, a new kind of coffee pulp is produced enriched with soluble compounds, mainly sugars. Those improvements have been shown to save a great deal of process water but they raise questions about the quality of the main product (beans) and the by-product (coffee pulp). The first question to answer involved local observations, mass balances and sampling of products and by-products in order to make a technical assessment of such improvements in terms of future market value of these coffee derivatives, together with an economical appraisal of the impact of water saving techniques. Special reference should be made to the cost of spent water treatment, as compared to the traditional system which involves great amounts of water for de-pulping, fermentation and washing operations (Urquiza, 1989; Viniegra-Gonzalez, 1993; Violle *et al.*, 1995; Zambrano-Franco *et al.*, 1999).

Coffee pulp produced by the new dry process is rich in soluble sugars, rots easily and is difficult to handle. Furthermore, it contains anti-physiological and anti-nutritional compounds such as caffeine, tannins and a large variety of phenolic compounds (Elias,

1978; Bressani, 1979). There is, therefore, a need for a new technology that can handle, detoxify and keep large amounts of coffee pulp using simple farm machinery at a minimal cost, but should also improve the nutritional quality and market value of the final product.

Recent work as described in following sections indicated that such an alternative could be an induced ensiling technique with a mixed inoculum of lactic bacteria and non pathogenic filamentous fungi followed by a two step fermentation process: a) aerobic step (solid-state fungal culture to detoxify and improve the nutritive value of the material), b) anaerobic step (silage fermentation to keep the material in good shape). This way, fungal organisms would be activated under forced aeration and silage could be kept for several months before animal consumption or before further industrial processing (sun or oven drying). Alternatively, pectinase and cellulase crude extracts could be obtained from the fermented mash, leaving a residual material with possible probiotic value for ruminant or fish consumption (Favela *et al.*, 1989; Tapia *et al.*, 1989; Roussos *et al.*, 1998). This new technical alternative for processing and storing coffee pulp ought to be tested at pilot scale in terms of mass balance, engineering feasibility and changes in biochemical and chemical composition of the product (Saucedo-Castañeda *et al.*, 1999).

Castillo *et al.* (1993) showed that the traditional wet process uses 6-23 m^3 of water per ton of fresh cherries, which corresponded to 30-114 m^3 of water per ton of green beans. This water is used mostly in de-pulping, fermentation, washing and transportation of cherries or coffee beans. The new 'dry' process has been shown to reduce water use to the level of only 2 m^3 of water per ton of cherries if transport of materials is mechanical and also if wash water is properly recycled (Vásquez-Morera, 1993; Alvarez-Gallo, 1995). Spent waters can be treated by anaerobic digestion with significant BOD reduction (Jacquet, 1993; Zambrano-Franco *et al.*, 1999), as previously shown by Violle *et al.* (1995) in Mexico. Apparently, the dry process does not affect the final quality of coffee beans (Alvarez-Gallo, 1995) but little published information is available on the fine chemistry of coffee beans and coffee pulp derived from this new process (Avallone, 1999).

Recent work done in the Biotechnology Laboratory of IRD, Montpellier (France) and UAM-I (Mexico) has shown that it could be possible to keep and improve the biochemical quality of coffee pulp by using a mixture of selected strains of lactic bacteria and filamentous fungi. Solid-state fermentation (SSF) of this material yielded a

decaffeinated product which could be dried or re-ensiled (Roussos *et al.*, 1989; Perraud-Gaime and Roussos, 1997a). Some HPLC measurements suggested that a major fraction of phenolic compounds was broken down (Perraud-Gaime, 1995). On the other hand, work by Antier *et al.* (1993 a,b) has shown that coffee pulp could be an excellent substrate for pectinase production by selected strains of *Aspergillus niger* (Boccas *et al.*, 1994). The solid residue after such fermentation has been found to have a probiotic effect when assayed *in vitro* (Tapia *et al.*, 1988). This probiotic effect was apparently linked to a water soluble enhancement growth factor present in fungal biomass acting on rumen cellulolytic bacteria (Campos-Montiel and Viniegra-Gonzalez, 1995; Soto-Cruz *et al.*, 1999).

2. Benefits

As with many other tropical agricultural products, green coffee beans agro-industry also suffers large international price fluctuations of the selling product *i. e.* coffee beans. In particular, the period during 1978-92 has been catastrophic for coffee price, which sunk to its lowest levels in 1989. This commercial problem is being worsened by the progressive increase in production costs. There has also been an increase in the payments for the right to use water, associated with the cost of treating wastewater. The commercial crisis has not affected large companies, which are strong and diversified. However, small and medium enterprises, wich are weak and vulnerable, have disappeared (Viniegra-Gonzalez, 1989). The consequences of the crisis have been felt in all coffee sectors and particularly in the research sector. In the latter part of the crisis period (1987-92), the number of coffee research oriented workers decreased in Latin America. Several national research institutes have reduced their size or closed down. For example in Colombia, Centro Nacional de Investigaciones sobre el Cafe (CENICAFE) was reduced to half its previous size, and in Mexico, Instituto Mexicano del Cafe (INMECAFE) totally disappeared. What could be done to cope up with financial entrepreneurial problems and protect the environment at the same time? How can one support the remaining research teams in subjects related to coffee agro-industry? Experts suggest to make a new and improve old 'beneficio' technology. The main thrust is to present alternatives in order to upgrade and diversify the products obtained from all the organic residues derived from agro-industrial cherry transformation. This way, coffee pulp/husk will become one of the selling products from this industry. Also, utilisation of

the upgraded by-products should be carried out in such a manner to help reduce the volume of spent water and the net cost of wastewater treatment. Storage of fresh ensiled and decaffeinated pulp would help in solving its toxicity aspect, together with season to season availability (Porres et al., 1993; Perraud-Gaime and Roussos, 1997b). Thus, the silage product could be kept without immediate drying. Later on during the season, the product could be oven or sun dried without imposing any strains on overloaded machinery and hand labour.

Nutritional tests done on ruminants and fish would help in evaluating the commercial value of the upgraded coffee pulp/husk (Ramirez-Martinez, 1999). Basic studies on the enzymology of silage and SSF could help in selecting the best performing microbial strains to be used for silage and detoxification purposes. It would also be helpful to isolate new strains of lactic acid bacteria, filamentous fungi and edible mushrooms with specific features such as probiotic and enzymatic activities or with other new properties related to a better market value (Denis, 1996; Soares, 1998; Soares *et al.*, 1999, 2000; Brand 1999; Brand *et al.*, 2000; Hakil, 1999; Fan, 1999; Fan et al., 1999a, b, c, 2000a, b; Suzuki-Lopez, 1999). These are some of the planned BIOPULCA project goals.

3. Lactic acid starters for coffee pulp silage

The overall aim of the investigation on lactic acid starters for coffee pulp silage was to study the biodiversity of lactic acid bacteria (LAB) from coffee biotopes and to isolate, characterize and select LAB for the formulation of starters for coffee pulp silage associated with detoxifying fungal enzymes. Furthermore, this work involved co-operation regarding technology associated with the engineering and scale-up basis of controlled coffee pulp silage and detoxification by means of SSF as well as the testing of applications of this bioprocess (Perraud-Gaime et al., 1997a, b).

3.1. ISOLATION AND SCREENING OF LAB FROM COFFEE BIOTOPES

Bacterial strains were isolated from fresh coffee pulp. Fermentation profiles were studied in order to select suitable strains. Each strain was submitted to biochemical tests for precise phenotypic characterization. Among the studied strains, 62% were LAB, out of which 38% were further tested in silage studies. Most of the isolated strains were homo-fermentative with less than 5% being hetero-fermentative. An interesting aspect to point

out was that 20% of the isolated strains were able to grow in the presence of tannic acid. It has yet to be determined whether these bacterial strains would be able to degrade hydrolysable tannin (Suzuki-Lopez, 1999).

3.2. LAB SCALE STUDIES OF SILAGE FROM INOCULATED COFFEE PULP

Various silage conditions were tested on laboratory scale with an inoculation of between 300-1000g of fresh coffee pulp. In Mexico, a preliminary assay to inoculate fresh pulp with one homo-lactic natural strain (*Lactobacillus paracasei*) was compared with the natural uninoculated silage. Both the trials were satisfactory, but ethanol and acetic acid were detected besides lactic acid. Inoculated silage caused the production of acetic acid, but did not avoid ethanol accumulation, probably due to acidic tolerant yeast development. New assays would be tried at lab scale in Mexico with other selected strains (*L. plantarum* strains) to improve acidification kinetics of silage and to limit ethanol production (Romano-Machado *et al.*, 1999).

4. Fungal enzymes for coffee pulp silage/detoxification

The production of fungal enzymes as decaffeinases and tannases was obtained in SSF with coffee pulp or impregnated polyurethane foam as solid substrates (Denis *et al.*, 1998; Hakil, 1999; Hakil *et al.*, 1998, 1999). Large-scale enzyme production could be obtained by the development of a continuous solid-state process (Van de Lagemaat *et al.*, 1999). Continuous SSF is carried out in a counter-current reactor adapted for this purpose and a rotating cylinder equipped with mixing baffles (Roussos and Pyle, 1998). The produced enzymes could be useful in the detoxification of the pulp.

Several fungal strains known to be GRAS (generally recognized as safe) were tested for their ability to produce tannases (Van de Lagemaat *et al.*, 1999). Screening of these fungi for tannase was necessary since originally fungi were only screened for caffeinases (Hakil *et al.*, 1999; Aguilar et al., 1999; Romano-Machado *et al.*, 1999). Tannases from the seven most productive strains were isolated on polyacrylamide gels and identified (gel activity assay). *Penicillium frequentans* and *Aspergillus phoenicis* were the most productive strains and would be used for tannase production with continuous reactors. A large-scale purification process is proposed to be developed after sufficient amounts of the enzyme are produced in continuous processes. Monitoring of biomass and tannic

acid concentration would be necessary in both batch and continuous fermentations and methods have been developed for this.

4.1. PILOT PLANT PRODUCTION OF FUNGAL ENZYMES BY SSF

Recent experimental evidence indicated that it was possible to degrade caffeine and tannin (polyphenolic compounds) simultaneously by using a single strain of filamentous fungi. It is worth noticing that these results could allow the production of detoxifying enzymes in only one fermentation step (Saucedo-Castañeda *et al.*, 1999).

4.1.1. Process engineering studies for scale-up of continuous bioreactors and process modelling for enzyme production in SSF

Two different types of laboratory scale prototype reactors have been built with the specific aim to develop reactors, which can operate continuously with solid substrates and without inoculation of the feed. In consequence it is important to have sufficient back mixing within the reactor to be able to operate with a sterile feed. The screw reactor ran successfully with sterile feed for around ten days before the experiment had to be stopped (Van de Lagemaat *et al.*, 1999).This device is very promising and a full programme of experimental work began in Autumn 1999. The other design is a rotating baffled cylinder, which ran continuously for 28 days. We believe this period as the longest time a truly continuous sterile SSF has operated work on this design is also continuing. We now have also made considerable progress on modelling these systems. We have developed mathematical models for the solids mixing behaviour and also a new model for the behaviour of the system as a reactor. Together these represent very encouraging progress in our aim to develop and run truly continuous solid substrate reactors.

5. Mushroom cultivation on raw wastes of coffee pulp

Studies were carried out by Fan *et al.* (1999a, b, 2000a) to compare the growth and activity of *Pleurotus* sp. on different residues of coffee industry; viz. coffee husk, coffee leaves and coffee spent ground in solid state fermentation (SSF). It was found that in coffee husk, protein levels increased with the time during fermentation; during first 15 days of fermentation, protein increased by 6.8%. After this period, although there was

further increase, it was slow (1.2% during the last 10 days fermentation). Fibre contents decreased 6% during first 15 days of fermentation. This also decreased further with time course of fermentation; the decrease was slow (1.1% during the last 10 days fermentation). Better increase in protein contents were noted when the substrate had 60-65% moisture in comparison to that at 45-55% and 70-75%. With 60-65% moisture maximum protein content was 8.2% and minimum fibre was 5% after 15 days fermentation. Studies on the effect of inoculum size showed that protein increased from 7.6-8.3% when the inoculation rate was 15%. It was significant to note that the concentration of caffeine decreased after colonization and fructification (60 days) up to 60% on average, but caffeine was actually not degraded by the fungus. Tannins also decreased in fermented coffee husk, up to 79% on average.

When coffee leaves were used as substrate, protein increased with time of fermentation. During the first 15 days of fermentation, protein increased by 7.6%. In this case also, the pattern was similar to that of coffee husk and during the last 10 days period, the increase was only 1.7%. The fibre contents also decreased in a similar pattern (decreasing with the time of fermentation by 6.9% during the first 15 days fermentation). A 65-70% substrate moisture was found best than 45-60% and 75% when maximum protein was 10.2% and the content of fibres was minimum. The inoculum rate of 15% was found most suitable in coffee-leaf SSF, which resulted in 9.4% protein after 15 days of fermentation. Correspondingly, the fibre contents decreased to 7%. Higher inoculum size was not effective. SSF using coffee spent ground as substrate also revealed a similar pattern.

SSF was also performed using *Flammulina velutipes*. When coffee husk was used as the substrate, the biological efficiency reached 55%. Experiments carried out to evaluate the efficiency of *F. velutipes* in submerged fermentation using the extract of coffee husk showed that yeast extract ($2g.l^{-1}$) was the best nitrogen source, resulting in 1.8 $mg.ml^{-1}$ biomass after 20 days. During the fermentation, the pH increased from 7.0 to 8.8.

6. Pre-treatment of coffee husk

Thermal hydrolysis of coffee husk was applied as the treatment method to separate efficiently its carbohydrate components (Woiciechowski *et al.*, 1999). Temperature and time of reaction and chemical catalysts were the variables used to optimize the hydrolysis. Temperatures tested were 100, 120 and 140°C for 5, 10 and 15 minutes for

dry coffee husk treatment and acid chemical hydrolysis. Results obtained for reducing sugars recovered in the hydrolysate were submitted to a statistical treatment, testing two factors (temperature and time) at three levels. Results showed that the concentration of reducing sugars was almost similar with or without acid catalysis. Thus, in view of the economical and environmental aspects, acid hydrolysis was not recommended. Due to the severe conditions of temperature and acid concentration, during processing sugars was degraded, producing toxic compounds such as furfural. These compounds are harmful for micro-organisms and affect fermentative processes. Best results were obtained with aqueous hydrolysis at 121°C for 15 min, which resulted in 240-g of reducing sugar.kg^{-1} of coffee husk. Further work on using the hydrolysate for fungal cultivation is being carried out.

7. Production of gibberellic acid from coffee husk

Gibberellic acid (GA_3) is a plant hormone widely used in the agro-industry. It is produced as a secondary metabolite by specific fungi. Coffee husk was used as carbon source for its production. Five strains of *Gibberella fujikuroi* and one of its imperfect states, *Fusarium moniliforme* were screened for their efficiency to produce GA_3 in SSF and liquid fermentation (SmF). SmF was carried out using aqueous extract of coffee husk. Results showed the superiority of SSF for GA_3 production. *G. fujikuroi* LPB-06, which gave the best performance was chosen for further studies. Fermentation was carried out in 250-ml Erlenmeyer flasks by inoculating the substrate at 15% v/w. The GA_3 isolation was done by acidifying the fermented mash to pH 2.5 and then extracting with ethyl acetate. The organic layer was separated and analyzed by high performance liquid chromatography. All the studies utilized statistical experimental designs. In order to enhance substrate utilization, different pre-treatments were applied using KOH at different concentrations and varied time of treatment. Best results were obtained when coffee husk was pre-treated with 5g.l^{-1} KOH for 45 min in aqueous solution, which resulted in about 100 mg GA_3.kg^{-1} coffee husk. Further studies to optimize the nutritional conditions of the strain are being carried out (Machado *et al.*, 1999).

8. Nutritional quality of processed coffee pulp

8.1. IN AQUACULTURE

Fish farming could be a way to produce animal protein using locally available feedstuff but taking into account current market limitations. Some work has been done on feeding coffee pulp to Tilapia (Garcia and Baynes, 1974), carp and catfish (*Clarius mossenbicus*) as indicated by Christensen (1981). The level of coffee pulp used in the experimental diets was close to 33% without negative effects on the growth rate and yields of the fish. Lagooning may be also used as a secondary water treatment process, after anaerobic primary treatment of spent waters in the coffee mill.

8.2. RUMINANT NUTRITION

Proximate composition of coffee pulp shows a relative low nutritive value due to high levels of wall materials (60%) and lignin (15%) and also due to the presence of caffeine, tannins and chlorogenic acid. Therefore, the use of raw coffee pulp has been suggested to be lower than 20% in ruminant diets (Ruiz and Ruiz, 1977; Vargas *et al.*, 1982; Abate and Pfeffer, 1986). High raw coffee pulp intake has been associated with negative nitrogen balance because of diuretic effect of caffeine (Cabezas *et al.*, 1977). Coffee pulp silage seems to correct this problem probably because of caffeine leaching in the silage liquor (Cabezas *et al.*, 1976; Ramirez-Martinez, 1999). On the other hand, solid-state culture of fungal organisms such as *Penicillium roquefortii* or *Aspergillus niger* may reduce to less than 1% the level of caffeine in coffee pulp, leaving a probiotic activity in the fungal biomass as indicated above (Tapia *et al.*, 1988; Campos-Montiel and Viniegra-Gonzalez, 1995). Therefore, despite the nutritional limitations of raw coffee pulp, solid-state fungal culture and ensiling may increase the ruminant nutritional and market value of this material. This is an interesting feature, which remains to be tested *in vivo*.

9. Summary and conclusions

The objective of Biopulca project, European INCO-DC project N°IC18*CT970185, is to recycle coffee pulp and coffee husk by biotechnological processes using lactic acid bacteria (LAB) for fresh coffee pulp transformation by silage, followed by the use of selected filamentous fungi capable of producing specific enzymes (caffeinases, tannases) in order to detoxify this tropical agro-industrial waste. It is to be kept in mind that the pulp generated from green coffee production accounts for nearly 50% in volume of the total yearly production. Further objectives are

a) to transform fresh coffee pulp into a stable and detoxified lactic acid silage product,
b) to use lactic acid silage as food or feed purposes and as substrate for solid-state fermentation of fungal metabolites or enzymes production,
c) to use coffee industry residues for mushroom and metabolites production,
d) to transform highly toxic compounds (caffeine, tannins) by biotechnological processes,
e) to diversify people's activity and employment in rural coffee-growing areas.

As seen above, a large number of attempts are being made to eliminate coffee pulp or husk, which, at the present time, are a pollutant in Latin America. As soon as such lab-scale studies show a definitive potential, industrial applications can be attempted. Whether coffee by-products are used for animal feed , for mushrooms, enzymes or metabolites production, the result should ultimately be seen through a diversification of peoples activities through new employment potential in rural coffee-growing areas. It is hoped that in the years to come, coffee by-products, initially discarted, will be transformed into high added value products, turning coffee pulp or husk, for example, into a commodity, rather than a waste.

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