Chapitre 41

ADDING VALUE TO COFFEE SOLID BY-PRODUCTS THROUGH BIOTECHNOLOGY

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1. Introduction

Coffee is a universal drink with millions of tons consumed throughout the world. As a result of the brewing of coffee, only 6% by weight of the berry harvested in the coffee field ends up in the cups, leaving 94% as waste, which includes by-products from the process as well as water from the drying of the seeds (Zuluaga, 1989). Although a small amount of these wastes are utilized partially as described below, most of this causes environmental pollution. From 100 g of fresh berries, around 29% of the dry weight constitutes coffee pulp, 12% husk, 4% mucilage and 55% are the coffee seeds (Bressani, 1978). Some typical yields resulting from 100 kg ripe cherries of Canephora (robusta) coffee by different processing methods are: a) wet processing, 22 kg of traded coffee; b) dry processing, 40-45 kg dry berries, 22 kg traded coffee. The by-products include 56-60 kg fresh pulp or 12-15 kg dried pulp, 3-5 kg of parche and 20 kg husk. One hundred kilogram of ripe cherries from arabica coffee using wet process results in 39 kg fresh pulp or 16 kg of dry pulp, 22 kg mucilage, 39 kg wet coffee parche (at 20% moisture) or 20 kg of processed coffee. Whatever the treatment may be, the final yield of processed coffee is around 20% (Zuluaga, 1989, Coste, 1989).

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Potential uses of the husk obtained through dry process include animal feed, composting and extraction of caffeine. Animal feed trials on husk and parches showed that a daily supplement of 10-20% did not affect the feed digestibility. It husk could also be used for caffeine extraction as it contains 0.4-0.7% of the alkaloid. Composting of the by-products would be useful in increasing the carbon and nitrogen contents of soil. At present, these by-products are burned at the sites of production, mainly for economical reasons. The energy value of these products (3,600 $kcal.kg^{-1}$ husk) is high making combustion profitable but only when the combustion is carried out at the production sites. However, the combustion of the husk produces highly corrosive gases and vapours that deteriorate equipment and affect the quality of the seeds. Due to this reason, the husk is used for indirect heating. Besides, dried coffee husk can become a serious pollutant if it re-humidifies accidentally.

The enormous residual volume of by-products generated during coffee processing has sparked a number of research projects geared towards their potential use which include animal and fish feeding, earthworms culture substrate, mushroom *Pleurotus* culture, aerobic compost etc. Roussos et al (1993) investigated biotechnological processes for the production of different metabolites utilizing coffee pulp. High level of humidity (80-85%) of coffee pulp was found inducive for the development of natural micro-flora and its rapid putrefaction (Gaime-Perraud *et al.*, 1993).

2. Chemical composition of coffee pulp

Coffee pulp differs in chemical composition according to the variety of the coffee cultivar, the stage of maturity of the fruit, the type of treatment applied and the production site. Carbohydrates account for 68% of dry matter, fibres 15%, ashes 6.5% and proteins 9% (Elias, 1978). Sixty percent of total nitrogen present in coffee pulp comes from amino acids. Amino acid composition of coffee pulp is very similar to that of soy or cotton flour but contains more valine and lysine and less leucine, tyrosine and phenylalanine than maize flour. The main amino acids present in coffee pulp are lysine, threonine, tyrosine, valine and phenylalanine with methionine and isoleucine as minor constituents (Bressani *et al.*, 1972). There are variations in the percentages of caffeine, tannins, chlorogenic acid and caffeic acid (0.6-1.3, 1.8-8.6, 0.2-3.2, 0.3-2.6 % DM, respectively). Each of these compounds may directly or indirectly have a toxic or anti-physiological effect (Clifford and Ramirez-Martinez, 1991). Condensation of tannins starts a few hours after fruit harvest and intensifies in the presence of water and heat. Condensed tannins increase through polymerization of anthocyanidins. Studies by Velez *et al.* (1985) demonstrated that phenolic compounds from coffee pulp have the capacity to bind proteins with maximal interaction at pH 5. De Rozo *et al.* (1985) observed that phenolic compounds decreased considerably the capacity for iron absorption, even when used as a food supply comprising only 10% of coffee pulp. Coffee pulp may contain

other substances, for example potassium that alone or in synergy with other compounds account for the anti-nutritional effects observed when used as animal feed. The high concentration of potassium could contribute to the anti-nutritional effect of the pulp as animal feed by modifying the ionic equilibrium of tissues (Campabadal, 1987).
Cellulose and lignin contents of coffee pulp suggest that coffee pulp could be a better feed source than citrus fruit skin, presently used as animal feed. However, the fibre content may be an obstacle for use of the fresh pulp as a feed source for monogastrics. D-fructose makes up 50% of the monosaccharides present while D-glucose accounts for 30% of the lyophilized pulp. The remaining 20% are represented by saccharose and galactose. Inositol content is negligible (Zuluaga, 1989).

3. Uses of coffee pulp

3. 1. ANIMAL FEED

Research has primarily been focused on upgrading and utilizing coffee pulp in order to obtain an ensiled or dried product suitable for animal consumption (Bressani *et al*, 1974). Coffee pulp could also serve as a substrate for caffeine extraction with residual products being used as animal feed. Dried pulp accounts for 10% proteins and a little less than 25% fibres. However, its low digestibility allows for only partial substitution as a daily diet. Its high fibre and phenolic compound contents as well as the presence of caffeine also strongly diminish its digestibility, especially with monogastrics, resulting weight hair losses.
Gomez-Brenes (1978) treated coffee pulp with a 1.2-3% calcium hydroxide solution. He observed a decrease in tannin content but without significant change in caffeine, chlorogenic acid or caffeic acid. It was concluded that such a base treatment did not enhance the nutritional value of coffee pulp. Peñaloza *et al.* (1985) grew *Aspergillus niger* in a solid state fermentation (SSF) process and achieved 200% increase in protein content with a significant decrease in fibre content, cellulose and hemicellulose. Despite the fact that tannins and caffeine were unaffected by the fermentation, the nutritional quality of the product was improved when tested as chicken feed. Aquiahuatl *et al.* (1988) isolated 350 fungal strains from soil, leaves or fruit from coffee growing regions. Among these, eight (two *Penicillium* and six *Aspergillus*) presented a high capacity to degrade caffeine in liquid synthetic medium. When tested in SSF using coffee pulp as substrate, all the strains degraded caffeine without any exogenous nitrogen supplementation (Perraud-Gaime, 1995). Roussos *et al.* (1994) and Hakil *et al.* (1998) also reported caffeine degradation by these strains. Boccas *et al.* (1994) reported high levels of pectinases from these strains.

3.2. ORGANIC FERTILIZER

Due to high levels of nitrogen, phosphorus and potassium as well as the presence of organic matter, coffee pulp can be used as soil fertilizer or soil conditioner. Various studies demonstrated that coffee pulp could be a good fertilizer agent, especially on coffee plantations. Such an alternative is presently used on production sites on a small scale. There are, however, two major drawbacks in this: the high water content of the pulp and the price of labour involved. It seems that composting the pulp is necessary to prevent rapid exothermic fermentation of fresh pulp, when stacked at the base of coffee explants. Distribution of the pulp over large plantations is also not cost effective.

3.3. SUBSTRATE FOR BIOGAS PRODUCTION

Coffee pulp has been tested as a substrate in anaerobic fermentation processes for the production of biogas (Calle, 1974a; Blanes, 1982). These studies had the advantage of proposing simple technological solutions, adapted to small size installations within production sites. Pre-treating the pulp by aerating or ensiling along with a good inoculum could be useful to effectively start-up the process. Presence of tannins, caffeine, chlorogenic acid or caffeic acid was termed harmful for production along with the observed drop in pH (Morales and Chacon, 1981).

3.4. MICROBIAL CULTIVATION AND ENZYME PRODUCTION

In view of the fact that citrus fruit used for the industrial production of pectin contain only 1.5-3.5% pectin, high pectin content (33% dry wt.) of the mucilage make it attractive substrate for pectin production. Coffee pulp has potential as a substrate for inducible enzymes production such as pectinases or cellulases (Favela *et al.*, 1989; Boccas *et al.*, 1994). However, up to now, pectic enzyme titres obtained from various fermentations of either coffee pulp itself or from pectin-rich waters derived from the process are lower than those of commercial preparations. Coffee pulp has also been used to cultivate yeast and other micro-organisms (Calle, 1974b; Penaloza *et al.*, 1985).

3.5. PRODUCTION OF EDIBLE FUNGI

Studies on the production of edible fungi (*Pleurotus ostreatus*) grown on coffee pulp have shown satisfactory results with industrial potential (Guzman and Martinez-Carrera, 1985; Rolz *et al.*, 1988; Martinez-Carrera *et al.*, 1989). Residual pulp resulting from the production process could be used as animal feed or as fertilizer (Martinez-*Carrera et al.*, 1989).

3.6. EARTHWORM PRODUCTION

The culture of the earthworm (*Eisenia foetida*, Sav.) on fresh coffee pulp has been envisaged as an alternative (Davila and Arango, 1991). Advantages include a decrease in decomposition time of the pulp, a decrease in its contaminating constituents, an easy set-up with commercially interesting final products. The worms are used to feed fish or chickens and the humus obtained is of better quality than the fertilizing properties of direct fresh coffee pulp addition as previously proposed (Salazar and Mestre, 1991).

4. Microbial flora present in coffee pulp

Coffee pulp (as well as the husk) contains a wide variety of natural micro-flora (bacteria, yeast, and filamentous fungi) which varies in concentration from $7.0x10^5$-$1.1x10^8$ Colony Forming Units (CFU).g^{-1} of dry matter. Samples obtained from the "wet process" contained the highest population of micro-organisms, between 17 and 160 times higher than from samples resulting respectively from the dry process or semi-humid (dry de-pulping). Yeast dominated the population when lyophilized samples (obtained immediately after de-pulping) were analysed. Filamentous fungi predominated samples from coffee husk (Gaime-Perraud, 1995, Roussos *et al.*, 1995).

Natural micro-flora evolves extremely rapidly, resulting in the need for an effective and reliable conservation method. Ensiling would be the method of choice to stabilize coffee pulp through natural micro-flora development of lactic acid bacteria. Bertin and Hellings (1985) advocated a level of 10^5 lactic bacteria per gram of dry matter in order to obtain a satisfactory ensiled product. Anaerobic bacteria from coffee pulp grown on MRS medium accounted for 3.10^4 bacteria per gram of dry matter.

5. Silage: a conservation technique

Ensiling of coffee pulp for its preservation and improvement of feed value is one of the avenues for value-added utilization of coffee pulp. Silage making is based on natural fermentation whereby lactic acid bacteria (LAB) ferment water-soluble carbohydrates to organic acids, mainly lactic acid, under anaerobic conditions. As a result the pH decreases, inhibiting detrimental anaerobes, thereby preserving moist forage. The aim of ensiling is to minimize loss of dry matter as well as nutritious value. It also prevents the development of an undesirable microbial population that would otherwise produce compounds with adverse effects when fed to animals. Other effects such as a better distribution of amino acids is attributed to silage (Weinberg and Muck, 1996). Silage techniques have been developed empirically over the centuries to stock and preserve products of both plant and animal origin. A number of physical, chemical and microbiological factors are of vital importance in obtaining good silage. The substrate to

be ensiled should have 30-40% dry matter, compatible to the desired level, amenable for anaerobiosis and contain utilizable sugars in sufficient quantity (up to 13% DM). It also must have the colour, which is nearest to the raw material, fruity aroma and slightly acidic taste. In terms of chemical characteristics and to achieve stability of the organic matter, ensiling should involve a minimum loss of dry matter and the resulting silage should have a pH value lower than 4.5, with over 3% lactic acid, but with less than 0.5 and 0.3% acetic and butyric acids, respectively, and a ratio of ammonical nitrogen over total nitrogen of 10 (Mc Donald *et al.*, 1991). The silage process can be compared to a three-component system: plant substrate, enzymes, and bacteria, in which each player has a key role in the success or failure of the silage (Bertin and Hellings, 1985). LAB develops within the mass of the substrate to be ensiled. They transform soluble sugars, producing lactic acid, a natural preserving agent. These optional anaerobes produce either exclusively lactic acid (strict homo-fermentative bacteria) or lactic acid and acetic acid (optional homo-fermentative bacteria) or lactic acid and acetic acid along with ethanol, butyric acid or CO_2 (strict hetero-fermentative bacteria) (Dellaglio *et al.*, 1994). They are particularly demanding micro-organisms. In addition to fermentable sugars, these bacteria require specific amino acids, vitamins and oligo-elements. LAB can inhibit growth of other micro-organisms through the production of organic acids such as acetic and propionic (Moon, 1983), hydrogen peroxide or bacteriocins such as nisin (Beliard and Thuault, 1989).

Success of a silage depends greatly on the presence of an adequate micro-flora, a quick reduction in pH, and a high production of lactic acid to preserve the substrate by blocking the activity of intracellular enzymes and by inhibiting the proliferation of unwanted micro-organisms (McDonald *et al.*, 1991). Enterobacteria, yeast and fungi represent in general a large proportion of the initial micro-flora present in the substrate to be ensiled (Pahlow, 1991). These micro-organisms, if given a chance, compete with LAB for the fermentable sugar sources. However, anaerobic bacteria such as *Clostridium*, which are strict anaerobes, can multiply rapidly as soon as oxygen becomes scarce, producing toxins (Woolford, 1984). A proposed solution, therefore, could be to add an inoculum of LAB (both homo-fermentative and hetero-fermentative) at the beginning of the silage process in order to inhibit naturally occurring and unwanted micro-organisms.

Plant, bacterial and often fungal enzymes are able to depolymerize the plant cell wall. They liberate soluble sugars within the product being ensiled. These sugars can then be metabolized by LAB. The available literature on the effects of different additives is contradictory. The results depend greatly on the nature of the substrate, its chemical composition as well as on the type of additive, its composition and concentration. The effects of enzyme addition along with lactic acid bacterial starters gives mixed results regarding animal performance (Vanbelle *et al.*, 1994).

5.1. SILAGE OF COFFEE PULP

Most of the reports concerning coffee pulp silage deal with the development of ensiling techniques or with the effect of chemical additives on the processed pulp (Daqui, 1975 ; Murillo, 1978; Carrizales and Ferrer, 1984). In general, an important loss in dry matter has generally been observed. The quality of the silage has also not been satisfactory. Ensiling does exist in tropical countries, in spite of the problems in terms of temperature and humidity. Consequently, the rate of ensiling is slow, putrefaction is common and chemical additives are used (where biological ones should be).
In order to enhance silage quality of coffee pulp, it could be useful to add various types of forage such as those of corn or sorghum depending on availability and price. Coffee pulp has similar nutritious value to those of tropical forage of good quality. Ensiled pulp is better than the dried coffee pulp as far as nutritional value is concerned but shows poor *in vitro* digestibility. The improvement of the acceptability of coffee pulp after silage could be enhanced by a decrease of caffeine and tannin contents. Porres *et al.* (1993) reported caffeine reduction during silage, which most probably was due to its solublization in silage liquids. De Menezes *et al.* (1993) reported that LAB degraded tannins during the fermentation.

5.2. LACTIC ACID STARTERS FOR COFFEE PULP SILAGE

With an aim to study the biodiversity of LAB from coffee biotopes, they were isolated, characterized for preparing the formulation of starters for coffee pulp silage. Due to the presence of toxic compounds such as polyphenols and caffeine, starters could be associated with detoxifying fungal enzymes. Starters were tested on fresh coffee pulp (0.3-1 kg). Samples of arabica coffee pulp were collected at a production site in Coatepec, Veracruz, Mexico during October 1997 until March 1998. This site is equipped with a de-pulping apparatus of the Penagos type. Micro-organisms were isolated from four silage of two kg each of coffee pulp. Two silage resulted from natural endogenous micro-flora found in the pulp and two were prepared by inoculating the pulp using part of previous silage. One hundred and fifty bacterial strains and 20 yeast strains were isolated on MRS and MRS + coffee medium. The selected strains were characterized by HPLC, identified by API, APIZYM and RFLP biodiversity approach.
Fresh pulp was inoculated with one homo-lactic natural strain and the ensiled product was compared to the natural uninoculated silage. Both lab trials were satisfactory, but ethanol and acetic acid were detected besides lactic acid. Inoculation caused the elimination of acetic acid production, but did not avoid ethanol production, probably due to acidic tolerant yeast development. Further studies are underway in Mexico with different homo-fermentative strains associated with a hetero-fermentative strain in order to improve acidification kinetics of silage and to prevent ethanol production.

6. Summary

In tropical countries, coffee industry produces various by-products (coffee pulp, mucilage, parches, husk) which are under-utilized and are a source of environmental pollution. These by-products represent around 50% (dry matter) of the world coffee beans production. Coffee pulp is rich in carbohydrates, amino acids, minerals, and various other nutrients. Nevertheless, the high level of humidity (80-87%) of coffee pulp induces the development of natural micro-flora and causes rapid putrefaction resulting in its transformation from by-product to waste. Coffee pulp offers could be used as solid substrate in biotechnological processes such as animal feed, mushroom and earthworm production, organic fertilizer and micro-organism growth for enzyme production. Due to time constraints during the coffee season, silage of fresh coffee pulp could be the best solution for conservation of the pulp. However, little information exists in inter-tropical regions concerning silage in general, and coffee pulp silage in particular. The global outlook points towards the necessity to elaborate a lactic acid starter specifically for coffee pulp silage.

7. Conclusions

At the present moment, biotechnological innovations and applications offer the greatest potential for agro-industrial by-products. Using a short term outlook, it could be possible to obtain a detoxified product ready for animal feed. It could also be possible to obtain high added value products such as enzymes or secondary metabolites from coffee pulp. However, it must be kept in mind that the utilization of coffee pulp as well as the mucilage depends on a number of factors such as the amount of product, type of treatment, seasonal and regional distribution, humidity, efficient stocking of the product and commercial potential. In recent publications concerning potential industrial applications of coffee by-products, there is an obvious lack of economic and feasibility studies. There is a need to estimate beneficial effects (especially environmentally friendly ones) of the transformed substrates in order to prevent the generation of new sources of pollution.

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