Chapter 39

A NOVEL APPROACH FOR THE PRODUCTION OF NATURAL AROMA COMPOUNDS USING COFFEE HUSK

M. Soares[1], A. Pandey[1a], P. Christen[2], M. Raimbault[3], C.R. Soccol[1]

[1]Laboratório de Processos Biotecnológicos, Departamento de Engenharia Química, Universidade Federal do Paraná, Curitiba, Brazil; [2]Laboratoire de Microbiologie IRD, IFR-BAIM, Université de Provence, ESIL, Case 925, 163 Avenue de Luminy, 13288 Marseille cedex 9, France; [3]Laboratoire de Biotechnologie, IRD, BP 5045; 34032 Montpellier cedex 1, France; [a]currently at Biotechnology Division, Regional Research Laboratory, Trivandrum-695 01, India

Running title: Production of aroma compounds

1. Introduction

Flavouring compounds have been traditionally obtained from plant sources but there are strong dependences on factors such as the influence of the weather, plant diseases etc... that are difficult to fully control. As a result, alternative sources were exploited and chemical synthesis was found very effective. Most of the available flavour compounds (84%) are now produced via chemical synthesis, although extraction from natural material continues. However, recent trends have demonstrated that consumers globally prefer foodstuff that can be labelled as natural. A directly viable alternative route for their production can be based on microbial processes. Often, the market prices of such compounds produced from natural materials has been very high. For example, synthetic 4-decalactone (the impact flavour compound of peach) costs US$ 150/kg, while the same substance extracted from a natural source costs about US$ 6000/kg (Janssens *et al.*, 1992). Several microbial species produce volatile fruity aromas. Fungi from the genus *Ceratocystis* produced a large diversity of fruit-like aromas (peach, pineapple, banana, citrus and rose), depending on the strain and culture conditions. Among this genus, *C. fimbriata* seems to be interesting because of its relatively rapid growth, its

T. Sera et al. (eds.), Coffee Biotechnology and Quality, 419–425.

good ability for spore production and the variety of aromas synthesized (Senemaud, 1988; Christen *et al.*, 1994).

One approach can be to use agro-industrial residues such as cassava bagasse, sugarcane bagasse, sugar beet pulp, coffee husk and coffee pulp, etc... (Pandey *et al.*, 1999a, b; Pandey and Soccol, 2000) and cultivate the micro-organisms in solid state fermentation (SSF). SSF has been termed as a potential tool for adding value to agro-industrial residues by production of organic acids, enzymes, secondary metabolites, amino acids, aroma compounds, etc... (Pandey, 1992, 1994; Soccol, 1996; Soccol and Krieger, 1998; Nampoothiri and Pandey, 1996; Pandey and Soccol, 1998; Pandey *et al.*, 1999c, d, e, f, 2000). Coffee husk is a fibrous mucilaginous material obtained during the processing of coffee cherries by dry process. It contains some amount of caffeine, tannins and polyphenols, which makes it relatively toxic. However, it is rich in organic matter, which makes it potentially interesting as a substrate for microbial processes for the production of added value compounds. Several alternative uses of the coffee husk have been tried. These include fertilizers, livestock feed, compost, etc... However, these applications utilize only a fraction of the available quantity and are not technically very efficient. Attempts have been made to detoxify it by degrading caffeine and tannins for its application as feed, and to use as substrate for the production of enzymes, organic acids, mushrooms, etc. (Woiciechowski *et al.*, 1999; Roussos *et al.*, 1994; Brand *et al.*, 1999, 2000). We attempted the production of fruity aroma compounds by SSF using pretreated coffee husk as substrate.

2. Micro-organism, substrate and SSF

A strain of *Ceratocystis fimbriata* CBS 374-83 was used. It was grown and periodically transferred on Potato-dextrose-agar (PDA) slants and stored at 4°C (Soares, 1998; Soares et al., 1999, 2000). Coffee husk, used as substrate, was sieved (0.4-0.8 mm particle size) and treated with hot water (Soares 1998). Fermentation was carried out in 250-ml Erlenmeyer flasks. For all experiments initial pH was 6.0 and moisture of the substrate was adjusted to 70%. The inoculum size was $1x10^7$ spores.g^{-1} dry matter and incubation temperature 30°C. Some experiments were performed to study the effect of supplementation of this substrate with glucose, leucine, soybean oil, and a nutrient salt solution in order to improve the volatile compound production.

3. Data analysis

Aroma compounds were separated and quantified by GC analysis according to Soares *et al.* (2000). The raw data were integrated in order to calculate the total volatiles (TV) accumulated during the fermentation (Soares *et al.*, 2000). The Gompertz model, a logistic like equation, was used to fit these integrated data, as previously described by other authors (Meraz *et al.*, 1992; Bramorski *et al.*, 1998). This model describes the dynamics of the production with respect to time. Data integration and non-linear Gompertz regression were made with KaleidaGraph program (Abelbeck Software, USA).

4. Fermentation profile of aroma compounds

Experiments using whole coffee husk, hot water treated coffee husk and the liquid extract obtained after the filtration of hot water treatment showed the superiority of the treated coffee husk for both fungus growth and aroma production (Soares *et al.*, 1999). This probably could be due to the removal of anti-physiological factors from the whole coffee husk during the treatment. Addition of glucose in coffee husk resulted in production of strong fruity aroma, which depended on the amount of glucose added (Soares *et al.*, 2000). Experimental results showed that although the fungal culture tolerated as high as 46% glucose concentration, lower concentrations of glucose appeared suitable for its growth and aroma producing activity. At the lowest experimental concentration of glucose (20%), TV production was fastest, and the maximum was about 28 μ mol/l/g DM after 40 h. At 35% glucose concentration, the rate of TV synthesis was slower and the maximum was about 24 μmol/l.g DM after 300 h. This could be due to a decrease in water activity of the substrate in relation to the high glucose concentration. It could also be partially due to catabolic repression due to the carbon source (Soares *et al.*, 1999, 2000). Although these results could not be considered as conclusive, they showed that different aroma compounds with different intensities could be produced with the addition of glucose to coffee husk. Another important aspect of this study was the requirement of a relatively shorter time to produce these compounds in comparison to earlier reports (Christen et al. 1997, Meza *et al.*, 1998) using cassava bagasse or apple pomace (5 and 4 days respectively). The amounts of TV reported in this study are superior to reported by Christen *et al.* (1997) with the same fungus grown on wheat bran or cassava bagasse.

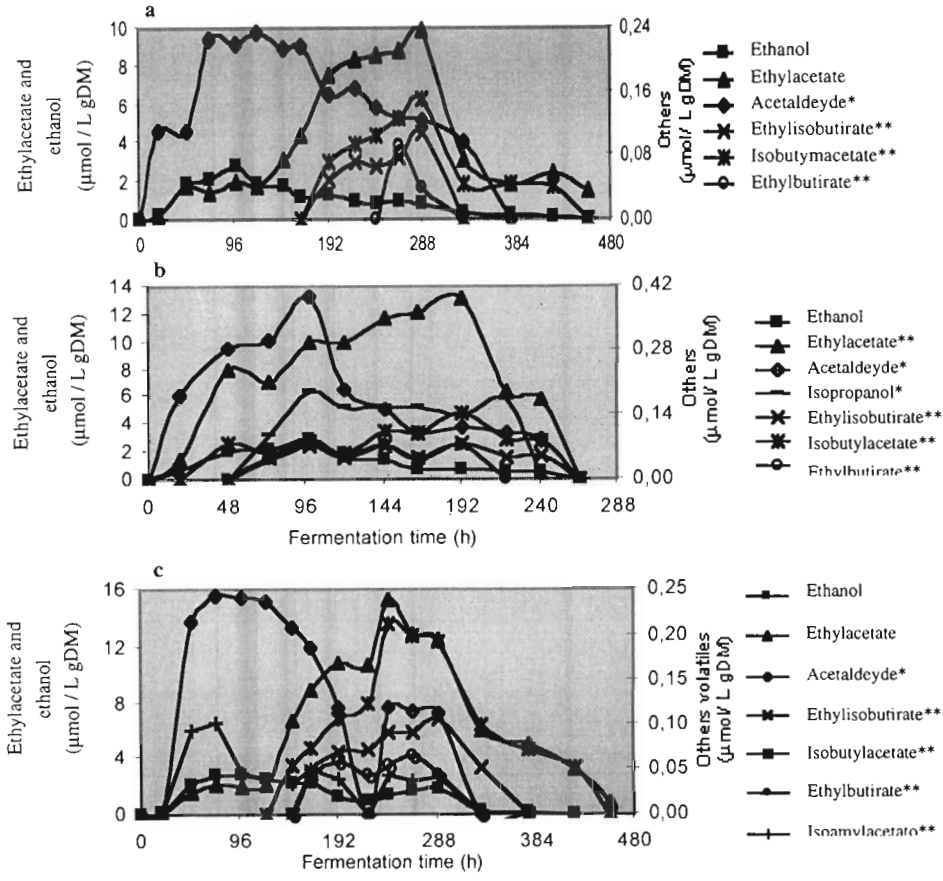

Figure 1. Dynamics of the major compounds in the headspace of the culture with 35% glucose (a), 20% glucose (b) and 35% glucose+leucine (c). * µmol ethanol equivalent.l^{-1}.g. $^{-1}$DM; **µmol ethyl acetate equivalent.l^{-1}.g. $^{-1}$DM)

Studies on the effect of supplementation with soybean oil showed no impact on aroma production, demonstrating that the fungus was not able to use soybean oil. Addition of saline solution drastically decreased the volatile production. Addition of leucine

improved TV production (Soares *et al.*, 2000). After the hot water treatment, the coffee husk lost about 30% in weight, which was mostly due to solublization of different minerals and salts present in it. Since hot water treatment or in other words, removal of these salts from the coffee husk improved the TV production, it could be considered logical only to observe such an effect on again supplementing the coffee husk with salts.

5. Compounds produced

Gas chromatography analysis allowed the identification of several compounds (Fig. 1). Under optimized conditions, a total of 13 compounds were produced which included alcohols (ethanol, isopropanol), aldehyde (acetaldehyde), ketones (2-heptanone, 2-octanone) and esters (ethyl acetate, ethyl isobutyrate, isobutyl acetate, ethyl butyrate, isoamyl acetate, propyl acetate, ethyl-3-hexanoate). Ethyl acetate was the prominent compound, followed by ethanol (Soares *et al.*, 1999, 2000).

Figure 1 shows the dynamics of the compounds measured in the headspace of the culture supplemented with glucose (20 and 35%) and with leucine. Addition of glucose (20%) reduced the fermentation time and increased the formation of isopropanol. Addition of leucine although did not alter the evolution of the compounds which was similar to control, it increased ester synthesis (Soares, 1998).

6. Conclusions

Coffee husk can be used as substrate in SSF for the cultivation of fungal strains to produce aroma compounds. Hot water treatment of the husk greatly improved the metabolic activity, particularly the synthesis of esters which is known to be a way for some micro-organisms to avoid a possible inhibition due to acids accumulation in the medium. The fungus was better adapted to this substrate than others mentioned in the literature.

7. Acknowledgements

Financial assistance from the European Union (grant no INCO DC: IC18*CT 970185) and PNP & D/CAFE- Coordenatior EMBRAPA, Brazil (Projeto no 07.1.99.057) is gratefully acknowledged. C. R. Soccol thanks CNPq, Brazil for a scholarship under Scientific Productivity Scheme.

8. References

Bramorski A., Christen P., Ramirez M., Soccol C.R., Revah S. (1998), Production of volatile compounds by the fungus *Rhizopus oryzae* during solid state cultivation on tropical agro-industrial substrates. Biotechnol. Letters, 20: 359-362

Brand D., Pandey A., Roussos S., Raimbault M., Soccol C.R. (1999), Biological detoxification of the coffee husk using filamentous fungi. Paper presented in III SIBAC, May 24-28, Londrina, Brazil, 1999, pp. 38

Brand D., Pandey A., Roussos S., Soccol C.R. (2000), Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system, Enzyme Microb Technol., accepted.

Christen P., Raimbault M. (1991), Optimization of culture medium for aroma production by *Ceratocystis fimbriata*. Biotechnol. Lett., 13: 521-526

Christen P., Villegas E., Revah S. (1994), Growth and aroma production by *Ceratocystis fimbriata*. Biotechnol. Lett., 16: 1183-1188

Christen P., Meza J.C., Revah S. (1997), Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: influence of the substrate type and the presence of precursors. Mycol. Res., 101: 911-919

Janssens L., De-Poorter H.L., Vandamme E.J., Schamp N.M. (1992), Production of flavours by micro-organisms. Process Biochem., 27: 195-215

Meraz M., Shirai K., Larralde P., Revah S. (1992), Studies on bacterial acidification process of cassava (*Manihot esculenta*). J. Sci. Food Agric., 60: 457-463

Meza J.C., Christen P., Revah S. (1998), Effect of added amino acids on the production of fruity aroma by *Ceratocystis fimbriata*, Sci. Alim., 18: 627-636.

Nampoothiri K.M., Pandey A. (1996), Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp., Biotechnol Letts, 16: 199-204

Pandey A. (1992), Recent developments in solid state fermentation, Process Biochem., 27:109-117

Pandey A. (1994), Solid state fermentation- an overview, In- Solid State Fermentation, A. Pandey (ed.), Wiley Eastern Publishers, New Delhi, pp. 3-10

Pandey A., Soccol C.R. (1998), Bioconversion of biomass- a case study of ligno-cellulosics bioconversions in solid state fermentation, Brazilian Arch. Biol. Technol., 41 (1998): 379-390

Pandey A., Nigam P., Vogel M. (1988), Simultaneous saccharification and protein enrichment fermentation of sugar beet pulp. Biotechnol. Letts., 10: 67-72

Pandey A., Soccol C.R., Nigam P., Soccol V.T., Vandenberghe L.P.S., Mohan R. (1999a), Biotechnological potential of agro-industrial residues: II Cassava bagasse. Biores. Technol., in press

Pandey A., Soccol C.R., Nigam P., Soccol V.T. (1999b), Biotechnological potential of agro-industrial residues: I Sugarcane bagasse. Biores. Technol., in press

Pandey A., Benjamin S., Soccol C.R., Nigam P., Krieger N., Soccol V.T. (1999c), The Realm of microbial lipases in biotechnology. Biotechnol. Appl. Biochem., 29: 119-131

Pandey A., Selvakumar P., Soccol C.R., Soccol V.T., Krieger N., Fontana J.D. (1999d), Recent developments in microbial inulinases - Its production, properties and industrial applications. Appl. Biochem. Biotechnol., 81: 35-52

Pandey A., Selvakumar P., Soccol C.R., Nigam P. (1999e), Solid state fermentation for the production of industrial enzymes, Curr. Sci., 77: 149-162

Pandey A., Azmi W., Singh J., Banerjee U.C. (1999f), Fermentation types and factors affecting it. In - Biotechnology: Food Fermentation. Vol. I, V. K. Joshi and A. Pandey (eds.), Educational Publishers & Distributors, New Delhi, pp. 383-426

Pandey A., Soccol C.R. (2000), Economic utilization of crop residues a futuristic approach. J. Sci. Ind. Res., 59: 12-22

Pandey A., Soccol C.R., Mitchell D. (2000), New developments in solid state fermentation. I- bioprocesses and products. Process Biochem., in press

Roussos S., Hannibal L., Aquiahuatl M.A., Trejo Hernandez M.R., Marakis S. (1994), Caffeine degradation by *Penicillium verrucosum* in solid state fermentation of coffee pulp: critical effect of additional inorganic and organic nitrogen sources. J. Food Sci. Technol., 31: 316-319

Senemaud C. (1988), Les champignons filamenteux producteurs d'arômes fruités. Etudes de faisabilité sur substrats agro-industriels. Ph.D. Thesis, Université de Bourgogne, France.

Soares M. (1998), Biossintese de metabolitos volateis frutais por *Pachysolen tannophilus* e *Ceratocystis fimbriata* pela fermentacao no estado solido da casca de cafe. Master's Thesis, Universidade Federal do Parana, Curitiba, Brazil

Soares M., Soccol C.R., Christen P., Pandey A., Raimbault M. (1999), Characterization of the fruity aroma compounds produced by *Ceratocystis fimbriata* in solid state fermentation of coffe husk. Paper presented in VI Seminar on Enzymatic Hydrolysis of Biomassa, Maringá, Brazil, 6-10 December, p.130

Soares M., Christen P., Pandey A., Soccol C.R. (2000), Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid state fermentation. Process Biochem., in press.

Soccol C.R. (1996), Biotechnology products from cassava root by solid state fermentation, J. Sci. Ind. Res., 55, 358-64

Soccol C.R., Krieger N. (1998), Brazilian experiments on valorisation of agro-industrial residues through solid state fermentation, In- Advances in Biotechnology, A. Pandey (ed.), Educational Publishers, New Delhi, pp. 25-36

Woiciechowski A.L., Soccol C.R., Ramos L.P., Pandey A. (1999), Experimental design to optimize the production of L- (+)-lactic acid from steam exploded wood hydrolysate using *Rhizopus oryzae*., Process Biochem., 34: 949-955

Soares M., Pandey A., Christen Pierre, Raimbault Maurice, Soccol C.R. (2000).

A novel approach for the production of natural aroma compounds using coffee husk.

In : Sera T. (ed.), Pandey A.(ed.), Roussos Sevastianos (ed.). Coffee biotechnology and quality.

Dordrecht : Kluwer, p. 419-425.

International Seminar on Biotechnology in the Coffee Agro-Industry, 3., Londrina (BRA), 1999/05/24-28.

ISBN 0-7923-6852-8.