0273-2289/02/98-100/0747/$11.25

Production of Coconut Aroma by Fungi Cultivation in Solid-State Fermentation

Álvaro Alberto de Aráujo,*[,1] Gláucia M. Pastore,[1] and Ralf G. Berger[2]

[1]Bioaroma Laboratory, Food Engineering School, UNICAMP, C.P. 6121, CEP 13083-970, Campinas, São Paulo, Brazil, E-mail: alvaro.dearaujo@libertysurf.fr; and [2]Institut für Lebensmittelchemie der Universität Hannover, 30453, Hannover, Germany

Abstract

The production of 6-pentyl-α-pyrone (6-PP), an unsaturated D-lactone with a strong coconut-like aroma was studied and compared with liquid and solid substrates. A fungi strain that produces coconut aroma compound was selected. The liquid medium of the submerged culture was used to impregnate a solid support of sugarcane bagasse in SSF (Solid State Fermentation). This substrate was adequate for growth and aroma production; the concentration obtained using SSF was higher than using liquid fermentation process. In the present work, it is demonstrated that, by solid-state-fermentation process, it is possible to produce 6-PP. The amount of 6-PP produced using a solid state substrate, following a 5 d culture, was 3 mg/g dry matter. Therefore, the amount of 6-PP produced during solid-state-fermentation process is higher than that reported in literature for submerged process.

Index Entries: Aroma production; coconut aroma; *Trichoderma*; solid-state fermentation; 6-PP; substrates.

Introduction

Extractions from natural raw materials and chemical synthesis are the conventional ways of producing flavor compounds, but they have their drawbacks. For example, agricultural production of aromatic plants is seasonal and limited quantities. The quality of essential oils is governed by uncontrollable factors, such as climatic and geographical conditions. Chemical synthesis leads to the so-called artificial compounds, which are not often appreciated by consumers, stated Gross and Asther *(1)*. Volatile

*Author to whom all correspondence and reprint requests should be addressed.

compounds production seems, according to Janssens et al. *(2)*, to be an interesting alternative route. A number of extensive reviews dealing with flavor generation by microorganisms are available in Latrasse et al. *(3)* and Welsh et al. *(4)*. In fact, the process is easily manageable and, moreover, legislation considers biotechnological aroma as natural substances.

It was Rifai *(5)* who first described and classified the different *Trichoderma* species, including some of them that produce a characteristic coconut aroma. 6-PP was first detected in *T. viride* by Collins and Halim *(6)* and its presence was related to coconut smell. Considerable work has been carried out on the optimization of the production of this lactone in a liquid medium by Zeppa et al. *(7)* and Satry et al. *(8)*; and on agar by Gervais and Serrette *(9)*.

The main purpose of this article is to test a strain that produces coconut aroma compound in solid-state-fermentation (SSF) and compare the aroma production in SSF with that reported in the literature for submerged fermentations.

Materials and Methods

Microbial Strain and Culture Media

Screening of 95 isolates was performed. Only one (#897) produced 6-PP. An isolated fungi strain 897 was cultured and maintained on potato dextrose agar (PDA), in Petri dishes. The cultures were incubated at 30°C. The 4-d-old mycelium obtained under these conditions was used for the inoculum of the biomass production. The strain was maintained on PDA at 4°C.

Biomass Production in Liquid Medium

The liquid medium (100 mL), containing malt extract (20 g/L) and glucose (10g/L), was poured into 500 mL Erlenmeyer flasks. The medium was autoclaved at 120°C for 15 min, before inoculation with 1 cm2 of mycelium-impregnated gel took place. It was incubated at 30°C for 72 h on a rotary shaker (150 rpm). The mycelium formed was separated from the medium by decantation and rinsed twice with physiological saline solution (0.9% NaCl). It was then suspended in 30 mL of the same saline solution, and 1 mL of this suspension was used to inoculate solid media.

Production of Aroma Compounds

The solid substrate, comprising 5 g sugarcane bagasse was placed into 500 mL flasks. It was impregnated with 25 mL of medium [glucose, 30.0 g/L; $(NH_4)_2SO_4$, 0.94 g/L; KH_2PO_4, 7.0 g/L; $Na_2HPO_4{\cdot}7H_2O$, 2.0 g/L; $MgSO_4{\cdot}7H_2O$, 1.5 g/L; $CaCl_2{\cdot}2H_2O$, 0.008 g/L; $FeCl_3{\cdot}6H_2O$, 0.008 g/L; $ZnSO_4{\cdot}7H_2O$, 0.0001 g/L]. The solid medium was autoclaved at 120 °C for 20 min. After cooling, the flasks were inoculated with 3 mL of mycelial cell suspension. The flasks were incubated at 30°C.

Extraction of Aroma Compounds

Samples (5 g) were removed from solid state cultures and placed into 250 mL flasks, with 50 mL distilled water. The aroma compounds were extracted from the samples with 10 mL dicloromethane. After extraction the mixture was dried with K_2SO_4.

Qualitative and Quantitative Analysis of Aroma Compounds

The aroma was characterized by the sniffing technique and the compounds were identified using a GC/MS Shimadzu QP 5000 with ionization by eletronic impact (70 eV), fitted with a DB WAX column (internal diameter: 0.25 mm, length: 30 m, film thickness: 0.25 μm). The splitless injector and detector temperatures were 250 and 280°C, respectively. The oven temperature was increased from 60 to 100°C, at a rate of 4°C/min, maintained at this temperature for 5 min and then increased again to 285°C at a rate of 15°C/min and maintained at this temperature for 10 min.

Quantitative analysis of 6-PP was carried out using the internal calibration method, with γ-undecalactone (99% Acros) as the internal standard.

Dry Matter Measurement

Dry matter was determined by weight difference; 2–3 g fermented substrate was weighed and then dried to constant weight at 105°C.

Results

The microorganism selected was identified as a fungi *Trichoderma* genera by taxonomic studies. The microphotography (magnification 1000×) of Fig. 1 was taken with light microscope after properly staining the mycelia and the spores with blue cotton. It reveals that this strain is a *Trichoderma* sp.

The 6-PP was identified by its mass spectra obtained from solid fermentation (Fig. 2) of fungal cultures. The data were compared to the previous studies of Bonnarme et al. *(10)* on the production of 6-PP in liquid culture. Electron impact mass spectral data indicated a molecular ion peak ($M+$) at m/z 166. The formula is $C_{10}H_{14}O_2$. The biosynthesis was accompanied by the production of other compounds, however, 6-PP remained by far the main component.

The compounds produced by culturing *Trichoderma* sp. for 5 d were extracted and quantified, using the internal calibration method. The amount of 6-PP recovered was 3.0 ± 0.5 mg/g dry matter (DM). If it is assumed that the fermented product contained 75% moisture, it could be estimated that about 940 mg of 6-PP was produced per liter of liquid solution adsorbed on the substrate. In liquid culture, the maximum concentration recorded in the literature, using Amberlite XAD-2 as adsorbent, was 248 ppm, which makes possible to overcome growth inhibition by 6-PP. A 6-PP concentration of 90 to 110 mg/L would be enough to

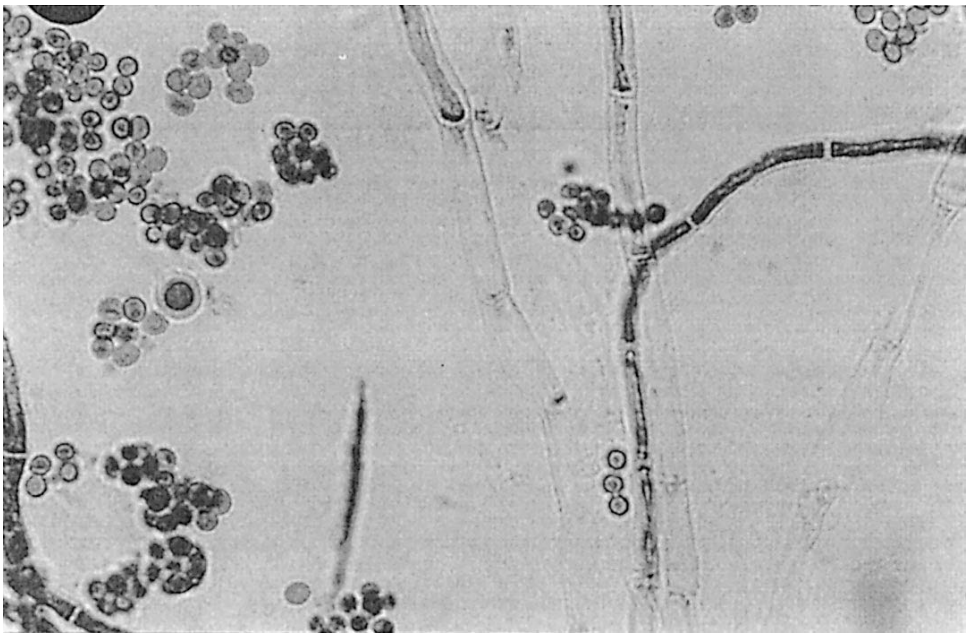

Fig. 1. Microphotograph of mycelia and spores of isolate 897 after 7 d of culture on PDA Petri dishes plates.

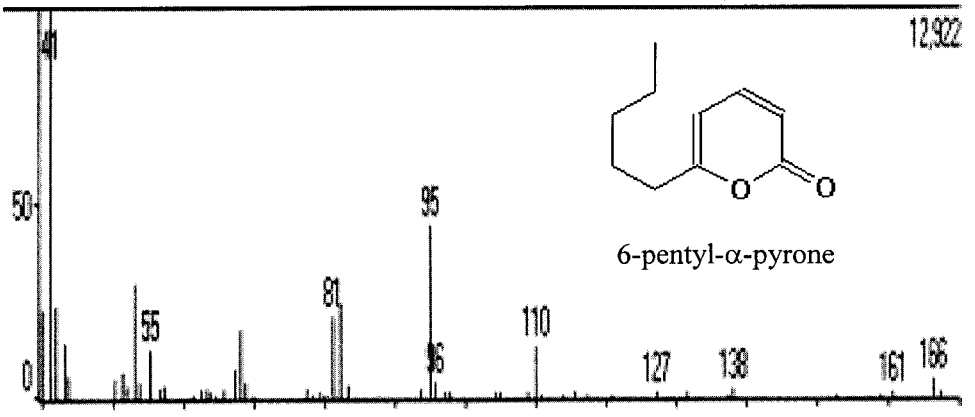

Fig. 2. Mass spectra of 6-PP from fungal solid state culture and structure of the molecule.

inhibit growth and prevent further 6-PP production, according to Prapulha et al. *(11)*. It can be concluded that the 6-PP concentration obtained in solid-state-fermentation was, therefore, higher than that in liquid culture. Moreover, there was no evidence of growth inhibition by 6-PP in solid-state-fermentation.

Conclusion

The production of 6-PP has only been demonstrated for liquid culture recently *(11)*. The present work demonstrates that 6-PP production is possible by SSF in two stages, i.e., biomass production in a liquid culture medium, followed by biosynthesis on a solid medium impregnated with a culture medium.

The study of the aroma production showed that 6-PP was produced once the medium was inoculated. The amount of 6-PP produced, after 5 d of incubation, was greater than that reported by Prapulha et al. *(11)* during liquid culture, showing that SSF presents great potential for aroma production. Further studies are being carried out in order to study the kinetics of 6-PP production as well as the influence of environmental parameters.

Acknowledgments

Álvaro A. De Araújo thanks the Brazilian government (CNPq) and FAPESP (Fundação de Amparo à pesquisa do estado de São Paulo) for his scholarships. We thank R. F. Martini (UNICAMP, Brazil) for reviewing the manuscript.

References

1. Gross, B. and Asther, M. (1989), *Sci. Alim.* **9,** 427–454.
2. Janssens, L., de Pooter, H.L., Schamp, N.M., and Vandamme, E.J. (1992), *Process. Biochem.* **27,** 195–215.
3. Latrasse, A., Degorce-Dumas, J.R., and Leveau, J.Y. (1985), *Sci. Alim.* **5,** 1–26.
4. Welsh, F.W., Murray, W.D., and Williams, R.E. (1989), *Crit. Rev. Biotech.* **9,** 105–169.
5. Rifai, M.A. (1969), *Mycol. Papers* **116,** 1–56.
6. Collins, R.P. and Halim, A.F. (1972), *J. Agric. Food Chem.* **20,** 437–438.
7. Zeppa, G., Allegrone, G., Barbeni, M., and Guarda, P.A. (1990), *Annal. Microbiol. Enzymol.* **40,** 171–176.
8. Satry, K.S.M., Rao, C.V.P., and Manavalan, R. (1985), *Indian Parfurmer* **29,** 193–200.
9. Gervais, P., and Serrette, M. (1990), *J. Ferment. Bioeng.* **69,** 46–50.
10. Bonnarme, P., Djian, A., Latrasse, A., Féron, G., Giniès, C., Durand, A., and Le Quéré, J.-L. (1997), *J. Biotech.* **56,** 143–150.
11. Prapulha, S.G., Karanth, N.G., Engel, K.H., and Tressl, R. (1992), *Flavour Fragance J.* **7,** 231–234.