Food Research International 42 (2009) 484–486

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Improving fruity aroma production by fungi in SSF using citric pulp

S.C. Rossi [a], L.P.S. Vandenberghe [a], B.M.P. Pereira [a], F.D. Gago [a], J.A. Rizzolo [a], A. Pandey [b], C.R. Soccol [a], A.B.P. Medeiros [a,*]

[a] *Bioprocess Engineering and Biotechnology Department, Federal University of Paraná (UFPR), Curitiba–PR, Brazil*
[b] *Biotechnology Division, CSIR, Trivandrum 695019, India*

ARTICLE INFO

*Article history:*
Received 5 November 2008
Accepted 13 January 2009

*Keywords:*
Solid-state fermentation
*Ceratocystis fimbriata*
Aroma
Citric pulp

ABSTRACT

Aromas produced by microorganisms can be recognized as natural and therefore have great economic potential. *Ceratocystis fimbriata* has the potential to produce a variety of aromas. The aim of the present work is to increase the fruity aroma production in citric pulp (CP), a waste from the citric juice production industry, using *C. fimbriata* in solid-state fermentation (SSF), testing other residues as carbon (sugarcane molasses, soya molasses) and nitrogen (soya bran or urea) sources. The studies were carried out in 250 mL Erlenmeyer flasks, pH 6.0, 75% initial moisture, 30 °C, inoculum rate $1 \times 10^7$ spores/g of CP, during 120 h. Total volatile compounds were quantified by headspace analysis in a gas chromatograph. The best production of volatile compounds was detected when the citric pulp was supplemented with 50% of soya bran, 25% of sugarcane molasses, and mineral saline solution. The production of total volatile reached 99.60 μmol/L g.



## 1. Introduction

Most food flavoring compounds are produced via chemical synthesis or extraction from natural material. However, recent market surveys have demonstrated that consumers prefer foodstuff that can be labeled "natural". Natural aromas produced by microorganisms have the advantage of being recognized as natural (Longo & Sanromán, 2006; Serra, Fuganti, & Brenna, 2005; Yilmaztekin, Erten, & Cabaroglu, 2008). They have a great economic potential for obtaining a wide range of biomolecules of interest in the food industry (Soccol, Medeiros, Vandenberghe, Soares, & Pandey, 2008). The industrial production of aromas corresponds of 25% of the food additives world market (Couto & Sanromán, 2006). The world market for flavors and fragrances is estimated in U$18.6 billions in 2008 (Soccol et al., 2008).

*Ceratocystis fimbriata* has the potential for synthesizing esters, it grows quickly and produces a variety of aromas (peach, pineapple, banana, citrus and rose), depending on the strain and culture conditions (Bluemke & Schrader, 2001; Medeiros, Pandey, Vandenberghe, Pastore, & Soccol, 2003; Medeiros, Soccol, Vandenberghe, & Woiciechowski, 2007; Pandey et al., 2000).

Solid-state fermentation (SSF) has been used for the production of aroma by cultivating yeasts and fungi in low cost substrates. It has been considered a useful tool for biomass transformation, solid waste treatment and for the production of added-value molecules such as enzymes, organic acids and biologically active secondary metabolites. One of the major advantages that SSF offers lies in the utilization of agro-industrial residues, which have no other practical applications (Graminha et al., 2008; Soccol & Vandenberghe, 2003; Soares, Christen, Pandey, & Soccol, 2000; Vandenberghe, Soccol, Pandey, & Lebeault, 2000). Citric pulp (CP) is a waste from the citric juice industries, the exportation of which revolves around a million tons per year (Abecitrus, 2008) and it costs U$10.00/ton.

The present work aimed at producing aroma compounds by *C. fimbriata* in SSF using CP as substrate and support improving fermentation conditions. Different sources of carbon, nitrogen and a saline solution were tested.

## 2. Material and methods

### 2.1. Microorganism and inoculum

*C. fimbriata* CBS 374-83 was grown and periodically transferred to Potato Dextrose Agar (PDA) medium, and stored at 4 °C. A spore suspension was prepared after a 7-day culture at 30 °C in 250 mL Erlenmeyer flasks. Spores were collected with sterile distilled water containing a few drops of Tween 80 and small glass beads. The spore suspension contained $10^7$ spores/mL, counted using the Neubauer's chamber.

### 2.2. Substrate preparation

Citric pulp was dried at 60 °C in an air circulation oven for 24 h. The dried substrates was milled and sieved to obtain particles of 0.8–2.0 mm size.

* Corresponding author. Tel.: +55 41 33613697; fax: +55 41 33613695.
*E-mail address:* adrianebpm@ufpr.br (A.B.P. Medeiros).

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doi:10.1016/j.foodres.2009.01.016

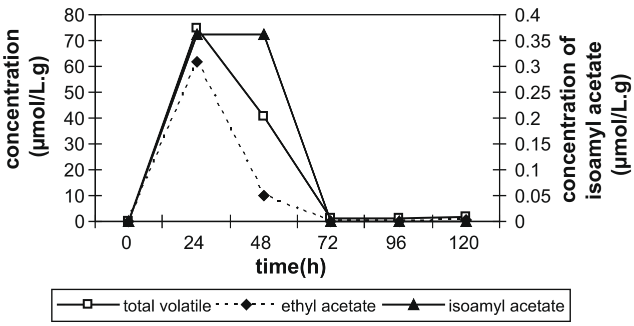


**Fig. 1.** Evolution of total volatile compounds, ethyl acetate and isoamyl acetate produced in citric pulp.

*2.3. Fermentation procedure*

Experiments were conducted in Erlenmeyer flasks, containing 15 g of substrate (dry weight basis). For all experiments, the initial conditions were: temperature, 30° C; pH 6.0; inoculum size, $1 \times 10^7$ spores/g dry matter and initial water content, 75% (w:w) (maximum absorption of the substrate). The substrates were autoclaved at 121 °C for 15 min. The effects of different sources – as carbon, nitrogen and saline solution addition – on the production of volatile compounds were investigated. Soya molasses (MS) or sugarcane molasses (MC), soya bran or urea and saline solution in different concentration ($KH_2PO_4$, $CaCl_2$ and $MgSO_4$) were used. The first study was performed only with CP. The second study tested molasses from sugarcane and soya as additional sources of carbon. The amount of molasses from sugarcane molasses and soya was added in accordance with the levels to be tested for reducing sugar content (15% and 20%). In the third study, the production of aroma was performed with the addition of urea or soya bran as nitrogen sources (2.3% and 3.8% of nitrogen). Finally, the three variables (carbon source, nitrogen source and saline solution) were tested in different concentrations and the interactions among of them were statistically analyzed with Statistica 5.0 – Statsoft (data not shown). The amount of sugarcane molasses tested was 1.6, 5, 10, 15 and 18.4 g. The addition of soya bran tested was 2.9, 3.75, 5, 7.5 and 8.35 g and the concentration of saline solution tested was $KH_2PO_4$, 0.64–7.36 g/L, $Ca(Cl)_2$, 0.16–1.84 g/L and $MgSO_4$ 0.36–6.36 g/L.

*2.4. Analytical procedures*

Aroma compounds were measured by headspace analysis of the culture in a gas chromatograph (Shimadzu model 17A) equipped with a flame ionization detector at 230 °C. The operation

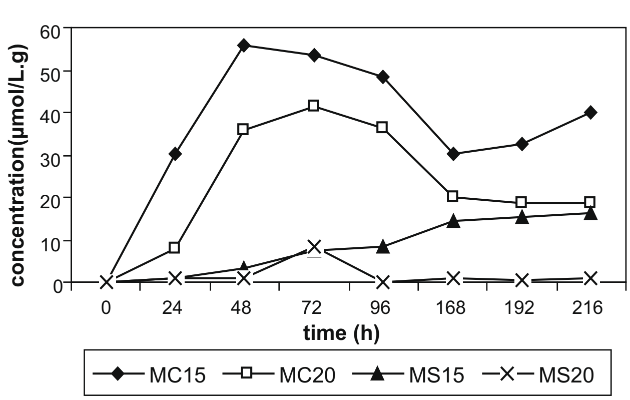


**Fig. 2.** Evolution of total volatile (TV) produced in CP with the addition of sugarcane molasses (MC) and soya molasses (MS) in concentrations of 15% and 20% of reducing sugar.

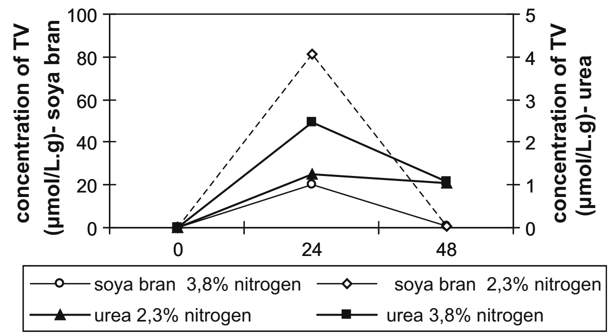


**Fig. 3.** Evolution of total volatile (TV) produced in CP with the addition of soya bran and urea in different nitrogen concentrations (2.3% and 3.8%).

**Table 1**
Volatile compounds produced by *Ceratocystis fimbriata* CBS 374-83 in SSF in citric pulp.

| Peak number | RT (min) | Compounds |
|---|---|---|
| 1 | 2.046 | Acetaldehyde |
| 2 | 2.16 | Ethanol |
| 3 | 2.4 | A[a] |
| 4 | 2.87 | Ethyl acetate |
| 5 | 4.54 | Propyl acetate |
| 6 | 5.47 | Ethyl isobutyrate |
| 7 | 5.81 | 2-Hexanone |
| 8 | 6.38 | 2-Hexanol |
| 9 | 7.96 | Isoamyl acetate |

RT = Retention time in minutes.
[a] A: Unidentified.

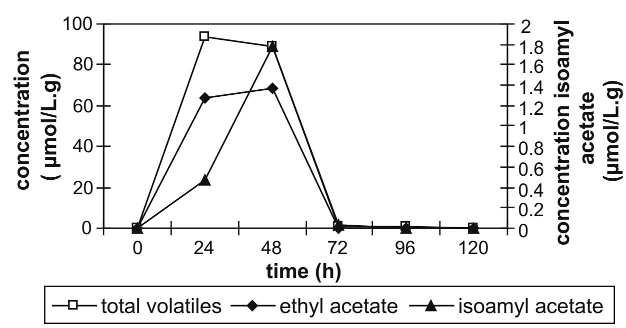


**Fig. 4.** Evolution of total volatile compounds, ethyl acetate and isoamyl acetate in CP after the addition of nutrients and saline solution (50% of soya bran, 25% of sugarcane molasses and saline solution).

conditions were: a 30 m $\times$ 0.32 mm HP-5 capillary column, column temperature of 40 °C–150 °C at a rate of 20 °C/min, injector temperature at 230 °C. Total and individual volatiles were expressed as μmol/L of headspace, as ethanol equivalent. The standard compounds were used to identify microbial aroma compounds.

## 3. Results and discussion

In the first study with citric pulp as substrate, the maximum total volatile (TV) concentration was obtained after 24 h of fermentation (Fig. 1). The results obtained with the addition of a supplementary carbon source showed that sugarcane molasses was better than soya molasses (Fig. 2). The total volatile production reached 66 μmol/L g with 15% of reducing sugar concentration. The addition of soya molasses did not contribute to improve the production of total volatile. Soya bran was better than urea as a nitrogen source (Fig. 3). Nine volatile compounds were detected in all experiments: acetaldehyde, ethanol, ethyl acetate, propyl acetate, ethyl isobutyrate, 2-hexanone, 2-hexanol, isoamyl acetate and one still unidentified compound (Table 1).

The best condition was reached when CP was supplemented with 50% of soya bran (3.8% of nitrogen), 25% sugarcane molasses (11.3% of reducing sugars) and saline solution (2 g/L of $KH_2PO_4$, 0.5 g/L of $CaCl_2$ and 1 g/L of $MgSO_4$) (Fig. 4). The total volatile concentration was around 100 μmol/L g. Previously, the best production was reached with 25% of soya bran (2.3% of nitrogen), but without the addition of sugarcane molasses and saline solution in 15 g of CP (Fig. 3). Comparing the production of total volatiles and isoamyl acetate before and after addition of nutrients, the concentrations of total volatile increased from 80 for 100 μmol/L g of substrate and the isoamyl acetate increased from 0.3 for 1.66 μmol/L g of substrate. Isoamyl acetate confers a banana aroma characteristic and has a high aggregate value for the food industry (Romero, Calvo, Alba, & Daneshfar, 2007). In the present study, the concentration of isoamyl acetate increases 5.5 times (Figs. 1 and 4). Comparing these results with those presented by Medeiros et al. (2003) and Soares et al. (2000), the production of total volatile compounds and isoamyl acetate in citric pulp reached greater values in a shorter time of fermentation.

## 4. Conclusion

Citric pulp is an adequate substrate for aroma production by *C. fimbriata*. Sugarcane molasses, soya bran and mineral salts flavored aroma produce a considerable amount of isoamyl acetate accumulation. The best condition was reached when CP was supplemented with 50% of soya bran (3.8% of nitrogen), 25% of sugarcane molasses (11.3% of reducing sugar), and saline solution (2 g/L of $KH_2PO_4$, 0.5 g/L of $CaCl_2$ and 1 g/L of $MgSO_4$). The total volatile concentration was 99.60 μmol/L g. This fact opens new promising perspectives for this process.

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