Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: influence of the substrate type and the presence of precursors

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Wheat bran, cassava bagasse and sugar cane bagasse were shown to be adequate substrates for the growth and aroma production by the mould *Ceratocystis fimbriata*. Among the nutritive media tested, sugar cane bagasse complemented with a synthetic medium containing glucose (200 g l^{-1}) gave a fruity aroma while the leucine or valine-containing medium gave a strong banana aroma. Aroma production was dependent on growth and the maximum aroma intensity was detected at about the time of the maximum respirometric activity. Twenty-four compounds have been separated by GC headspace analysis and 20 were identified, among them: 1 aldehyde, 7 alcohols, 4 ketones and 8 esters. It was clearly demonstrated that the chromatographic profile of the headspace of the culture was dependent on the substrate used and on the eventual precursor added. When leucine or valine was added to the substrate, the production of total volatiles in the headspace reached values up to tenfold higher than that for ripe bananas. The Gompertz model, a logistic-like equation, was used to fit the integrated CO_2 and volatiles production data.

Micro-organisms play an important role in the generation of natural compounds, particularly in the field of food aromas. Extensive reviews dealing with flavour generation by micro-organisms have appeared in the past few years (Welsh, Murray & Williams, 1989; Janssens *et al.*, 1992; Berger, 1995). As pointed out by Bigelis (1992), filamentous fungi are interesting because they are able to produce flavouring compounds and flavour-related enzymes.

Solid substrate fermentation (SSF) is a method to cultivate microbial cells that has been extensively studied for many micro-organisms and their products (Doelle, Mitchell & Rolz, 1992), especially for moulds which are well adapted to this type of culture. SSF is also an interesting alternative for the utilization of agroindustrial solid wastes. Some recent papers have reported the production of aromas in SSF. Yamauchi *et al.* (1989) produced a fruity odour by growing a *Neurospora* strain on pregelatinized rice, while Pastore, Park & Min (1994) obtained similar results with various *Neurospora* strains from beiju, a traditional Brasilian fermented beverage. Gervais & Sarrette (1990) studied the production of coconut aroma by *Trichoderma viride* on agar and Humphrey, Pierce & Skill (1990) patented a process in which an *Aspergillus* strain, grown on cellulose fibres, produced methyl ketones from coconut oil. In koji fermentation, Ito *et al.* (1990) found that production of volatile compounds by *Aspergillus oryzae* was improved under oxygen limiting conditions. Sugawara *et al.* (1994) demonstrated the importance of the yeast *Zygosaccharomyces rouxii* in the generation of a furanone, characteristic of the aroma of miso, a traditional oriental fermented food. These studies have shown the importance of the media in the specific development of a defined aroma.

Moreover, the capacity of some *Ceratocystis* spp. to produce fruit-like aromas has already been reported (Lanza, Ko & Palmer, 1976; Senemaud, 1988; Christen, Villegas & Revah, 1994; Christen *et al.*, 1995). In this work, the ability of *Ceratocystis fimbriata* to produce aromas in SSF was explored. It involved the study of the influence of the substrates used and the addition of precursors on growth and aroma production.

MATERIALS AND METHODS

Organism and culture media

Ceratocystis fimbriata Ellis & Halst. CBS 374-83 was used. It was periodically transferred onto Potato Dextrose Agar (PDA) slants and stored at 4 °C. Three media were used: wheat bran and cassava bagasse (donated by Pr. C. Soccol,

Table 1. Culture media used for aroma production by *C. fimbriata*

Run	Substrate/Nutritive medium	Complement
1	Wheat bran	—
2	Wheat bran	Leucine
3	Wheat bran	Urea
4	Cassava bagasse	—
5	Cassava bagasse	Leucine
6	Cassava bagasse	Urea
7	Cassava bagasse	Valine
8	Sugar cane bagasse + SM*	—
9	Sugar cane bagasse + SM*	Leucine

* SM refers to the synthetic medium optimized by Christen & Raimbault (1991).

UFPR, Brazil) which can be regarded as substrates – containing carbon and nitrogen sources – and sugar cane bagasse, which is basically a support and needs to be complemented with a synthetic medium (SM) containing the carbon and nitrogen sources. Wheat bran and cassava bagasse were milled and sieved through −20+40 mesh screens to obtain particles of 0·42–0·82 mm diam. The sugar cane bagasse had been previously washed in order to eliminate residual sugars and prepared in the same manner as the wheat bran and the cassava bagasse. Each substrate was then sterilized at 103 kN m^{-2} for 15 min.

Fermentation procedure

Experiments were performed in 250 ml Erlenmeyer flasks covered with six layers of gauze, without forced aeration (surface culture). These were filled with 7·5 g Initial Dry Matter (IDM) for wheat bran, and 5·25 g IDM for cassava and sugar cane bagasses. For all experiments, initial conditions were as follows: temperature, 30°; pH, 6·0; inoculum size, 1×10^7 spores g^{-1} IDM. Initial water content was calculated according to the maximum absorption capacity of each support (wheat bran, 50% (w/w); sugar cane bagasse, 63% (w/w) and cassava bagasse, 65% (w/w)). The wheat bran and cassava bagasse were complemented with an oligoelement solution with the following composition: $Fe(NO_3)_3$ nonahydrate, 723·8 mg l^{-1}; $ZnSO_4$ heptahydrate, 439·8 mg l^{-1}; $MnSO_4$ tetrahydrate, 203 mg l^{-1}. Sugar cane bagasse was imbibed with a synthetic medium previously optimized for submerged cultures (Christen & Raimbault, 1991). This medium had the following composition: glucose, 200 g l^{-1}; urea, 7·6 g l^{-1}; $(NH_4)_2SO_4$, 18 g l^{-1}, KH_2PO_4, 4 g l^{-1}; $Ca(NO_3)_2$, 4 g l^{-1}; $MgSO_4$ heptahydrate; 3 g l^{-1}, oligoelement solution, 8 ml l^{-1}. Urea, leucine and valine were added at a concentration of 167 mmol l^{-1}. The different combinations tested are given in Table 1.

For each experiment two flasks were used. They were monitored periodically for sensory evaluation. One of these was also used to assay CO_2 and volatile compounds production by headspace chromatographic analysis at different fermentation times. One experiment (run 4) was reproduced on three different occasions within a 4 month period to evaluate repeatability of the process.

Analytical procedures

The odours of the cultures were determined by evaluation with a non-trained panel consisting of six members, with no restriction in descriptive terms.

As reported previously by Desgranges *et al.* (1991) and Mitchell (1992), CO_2 production in the headspace above the culture is a good growth indicator. CO_2 concentration was measured by gas chromatography according to Christen *et al.* (1993) and expressed as % ml CO_2 100 ml^{-1} headspace. The detection limit was 0·05% CO_2.

Volatile compounds produced were characterized by injecting 2·0 ml from the headspace of the culture to a Hewlett–Packard 5890 GC equipped with a 5 metre Megabore HP-1 column and with a flame ionization detector. The temperature of the injector and detector blocks was 210°. The oven temperature program was 40° for 2 min followed by a temperature rise of 10 °C min^{-1} up to 150°. This final temperature was maintained for 2 min. The nitrogen gas flow rate was 1·5 ml min^{-1} and split ratio was 1:32. Total volatiles (TV) produced were expressed as μmol ethanol equivalent per litre of headspace (μmol l^{-1} eq. ethanol). Some compounds were also determined individually and their concentration in the headspace was expressed from standard curves as μmol l^{-1}. The headspace analysis of a ripe banana was performed as control. For this, 20 g of sliced ripe banana were equilibrated for 1 h at 30°.

Data analysis

In order to analyse the kinetic behaviour of the culture, the raw CO_2 and volatile production data, which represent an accumulation term between production and transfer from the flask to the exterior, were integrated with respect to time and fitted to the Gompertz model, previously used by Meraz *et al.* (1992) for the bacterial lactic acid production on cassava. This sigmoidal shaped model describes the evolution of a production **P** (CO_2 or TV in this case) with respect to time as follows:

$$\mathbf{P} = P_{max} \exp[-b \exp(-kt)],$$

where P_{max} (CO_{2max} or TV_{max}), is the maximum integrated value for the production (when $t \to \infty$) expressed as % (ml CO_2 100 ml^{-1} headspace) for CO_2 and μmol l^{-1} for the volatiles respectively. k (h^{-1}) represents a production rate constant and b can be used to obtain the time of maximum production rate from $t_{max} = (\ln b)/k$. Data integration and non-linear regression of the Gompertz model to obtain P_{max}, b and k values were made with the KaleidaGraph Program (Abelbeck Software, U.S.A.).

RESULTS AND DISCUSSION

Repeatability studies

From Fig. 1, it can be seen that the experimental data obtained from the system using cassava bagasse were reproducible for three independent experiments for both CO_2 production (Fig. 1*a*) and TV production (Fig. 1*c*). These raw data were then integrated and the Gompertz model was applied (Fig. 1*b*, *d*). Fitted parameters and correlation coefficient (R^2) are reported in Table 2. It can be seen that a good correlation of the time series data of the kinetic evolution may be obtained with the model for each experiment. Fitting the model to all the experimental points gives R^2 values above 0·96 (Exp. 1+2+3, Table 2). Hence, it may be concluded that the experimental set-up and the analytical methods used for both CO_2 and volatile determination were repeatable and adequate.

Growth studies

Carbon dioxide was chosen as a growth indicator and its evolution is reported in Fig. 2. For all runs, the evolution of this parameter was similar. After a short lag period (no more than 20 h), the CO_2 production drastically increased to reach

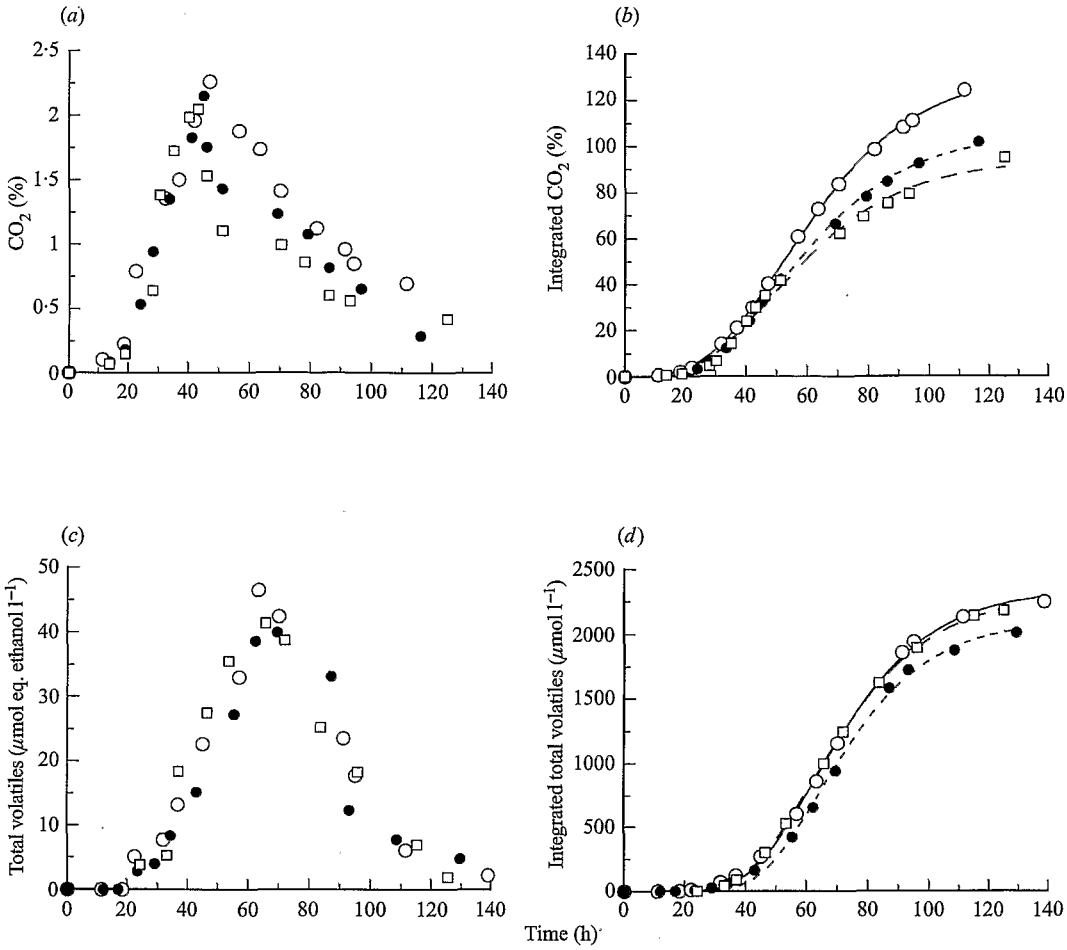

Fig. 1. Evolution of the raw data (*a*, *c*) and integrated data (*b*, *d*) for carbon dioxide and total volatile production for three independent experiments on cassava bagasse (run 4). In (*b*) and (*d*) symbols represent experimental data and curves represent the Gompertz model. ○, Exp. 1; ●, exp. 2; □, exp. 3.

Table 2. Kinetic constants for CO_2 and total volatile production of *Ceratocystis fimbriata* grown on cassava bagasse in three independent experiments

		$k(h^{-1})$	t_{max}(h)	R^2
CO_2	CO_{2max}(%)			
Exp. 1	132·6	0·041	51·4	0·998
Exp. 2	105·6	0·043	50·0	0·998
Exp. 3	94·0	0·043	48·2	0·993
Exp. 1+2+3	108·8	0·045	49·3	0·963
Total volatiles	TV_{max}(μmol l^{-1})			
Exp. 1	2344·8	0·048	62·6	0·998
Exp. 2	2100·5	0·052	64·4	0·998
Exp. 3	2300·3	0·047	61·7	0·999
Exp. 1+2+3	2265	0·048	62·8	0·992

its maximum between 40 and 60 h, and then decreased below detectable levels after the 5th day. In Fig. 2*a*, it can be seen that the maximum of CO_2 is higher for the culture on sugar cane bagasse with added synthetic medium than that on wheat bran or cassava bagasse when used alone. The addition of a nitrogen source enhanced CO_2 production for sugar cane and cassava bagasses probably due to nitrogen limitations in these unamended media (Fig. 2*c–d*). For wheat bran (Fig. 2*b*), improvement in CO_2 production was found with leucine, but not with urea, which may imply limitation of readily assimilable carbon sources.

In Table 3, modelling parameters of the integrated CO_2 data are presented. The model proved to describe the data adequately, since all the R^2 coefficients were above 0·990. The P_{max} values confirmed the influence of the substrate. For example, it can be seen that CO_2 production was lower on wheat bran than on cassava and sugar cane bagasses (runs 1, 4 and 8). The addition of a nitrogen source (particularly leucine and valine) greatly enhanced this production (more than fivefold). The production rate (k) values were comparable, all being between 0·031 and 0·064 h^{-1}, though no clear tendency can be seen in relation to the medium. There is no apparent relationship between this parameter and the amount of CO_2 produced characterized by CO_{2max}. The t_{max} values calculated are close to those observed experimentally (Fig. 2). These values are between 42·9 and 66·3 h and do not seem to be influenced by the medium used.

Aroma production

The aromas detected by olfactometry are presented in Table 4. The periods of strongest perception (t_{max}) were a few hours

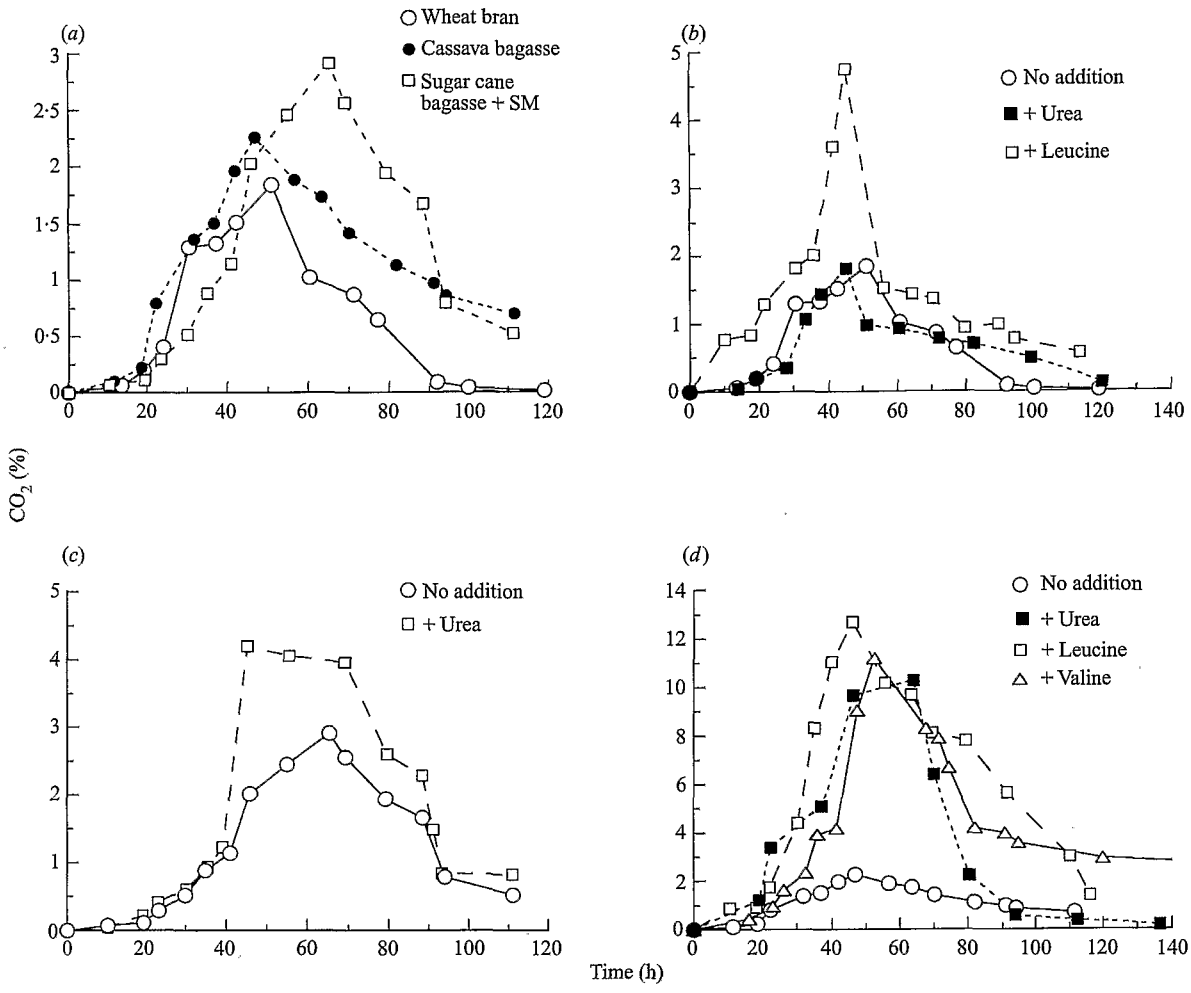

Fig. 2. Carbon dioxide evolution in the headspace of cultures of *Ceratocystis fimbriata*. (*a*) Comparison of the three substrates with no addition; (*b*) wheat bran; (*c*) sugar cane bagasse + synthetic medium; (*d*) cassava bagasse.

Table 3. Kinetic constants for the CO_2 production

Run*	CO_{2max}(%)	k(h^{-1})	t_{max}(h)	R^2
1	75·2	0·061	44·3	0·999
2	163·2	0·046	42·9	0·997
3	76·5	0·045	50·5	0·997
4	132·6	0·041	51·6	0·999
5	733·1	0·042	53·8	0·999
6	459·6	0·064	47·2	0·997
7	709·7	0·031	66·3	0·991
8	152·9	0·047	59·9	0·999
9	220·0	0·053	58·3	0·999

* As in Table 1.

Table 4. Comparison between *C. fimbriata* cultures and the ripe banana (control) for aroma and volatile compounds production

Run*	Aroma and intensity†	t_{max} (h)‡	Total volatiles max (μmol eq. ethanol l^{-1})§
1	—	—	16·1
2	Banana + + +	51	319·2
3	Apple/pear +	65	27·8
4	Banana + +	63	46·5
5	Banana + + +	58	414·3
6	Banana +	63	22·4
7	Banana + + +	56	478·5
8	Fruity + +	63	44·9
9	Banana + + +	45	213·6
Banana	Banana + + +	—	48·4

* As in Table 1.
† — none, + weak, + + medium, + + + strong.
‡ t_{max}: time of maximum perception of the aroma.
§ Experimental values.

after the time of maximum growth calculated with the Gompertz model (Table 3). When wheat bran or cassava bagasse was used without added nitrogen (runs 1 and 4), no or slight aroma was detected. In the other cases, medium or strong banana aroma was released except for wheat bran + urea (run 3), where it was rather a weak apple/pear aroma. These sensorial observations were well correlated with the maxima of total volatile compounds detected by GC (Table 5). The highest amounts were found when leucine or valine were added to the substrate (runs 2, 5, 7, 9). On the contrary, urea did not promote fruity aromas nor volatile production (runs 3, 6). It was observed that, in spite of the strong aroma of the banana, the total volatile compounds obtained in this case

Table 5. Volatile compounds identified in the headspace of 2-d cultures of *C. fimbriata* and in the headspace of a ripe banana (B)

Group	Compound	Peak no.	Run* 1	2	3	4	5	6	7	8	9	B
Aldehyde	Acetaldehyde	1	L	M	L	M	H	L	H	L	M	M
Alcohols	Ethanol	2	M	H	H	M	H	M	H	M	H	H
	2-propanol	4	—	L	L	M	M	L	L	L	—	—
	1-propanol	5	—	L	L	—	—	L	L	—	L	L
	2-methyl propanol	7	—	—	—	—	—	—	M	—	—	—
	1-butanol	8	—	—	—	—	M	—	—	—	—	L
	3-methyl butanol	11	—	M	L	L	H	L	H	—	M	L
	2-hexanol	13	L	M	L	M	M	L	H	M	L	—
	2-octanol	22	—	—	—	—	—	—	—	—	L	L
Ketones	Acetone	3	—	—	—	—	H	—	—	—	L	—
	2-hexanone	12	—	M	L	L	M	L	H	L	M	—
	2-heptanone	17	—	—	—	—	M	—	H	—	L	—
	2-octanone	21	—	—	—	—	L	—	—	—	—	—
Esters	Ethyl acetate	6	H	H	H	H	H	H	H	H	H	H
	Ethyl propionate	10	M	M	M	M	M	M	H	M	M	L
	2-methylpropyl acetate	14	—	—	—	—	—	—	L	—	L	M
	Ethyl butyrate	16	—	L	—	L	L	—	M	L	L	L
	3-methylbutyl acetate	19	—	M	—	M	H	L	H	—	H	H
	Ethyl pentanoate	20	—	—	M	—	—	—	H	—	—	—
	Ethyl hexanoate	23	—	—	—	—	L	—	—	—	L	—

* Refers to the runs listed in Table 1. H: high (concentration > 0·8 μmol eq. ethanol l^{-1}), M: medium (0·08 μmol eq. ethanol l^{-1} < concentration < 0·8 μmol eq. ethanol l^{-1}), L: low (concentration < 0·08 μmol eq. ethanol l^{-1}, —, not detected.

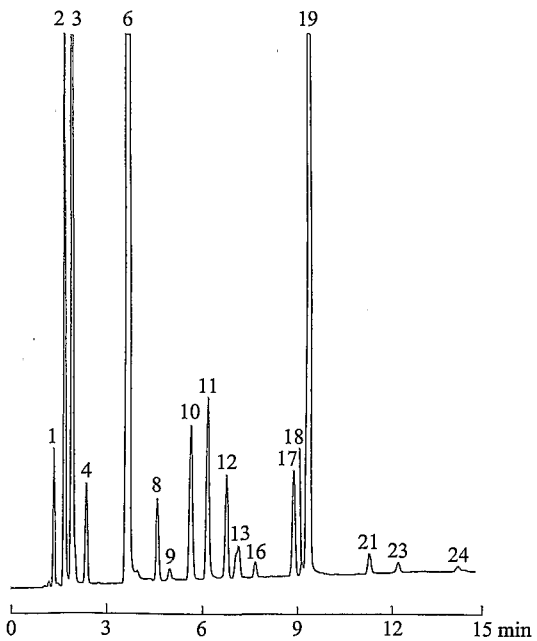

Fig. 3. Headspace chromatogram of a 2-d culture of *Ceratocystis fimbriata* grown on cassava bagasse + leucine. The numbers of the peaks correspond to those given in Table 5.

were inferior to those observed for media modified with leucine or valine.

Volatile compounds produced

Gas chromatograms of the headspace from the cultures showed a total of 24 different compounds. Of those, 20 were identified by their retention times and are listed in Table 5. As an example, Fig. 3 shows the analysis of the run made with cassava bagasse complemented with leucine (run 7).

The major volatile compounds found in the headspace were alcohols, esters and, to a lesser extent, ketones. Alcohols do not play a predominant role in flavours but are known to contribute to the overall flavour quality and are precursors of fruit-like flavouring esters. In micro-organisms, such as yeasts, all alcohols, except ethanol, are formed by the reduction of α-keto acids which are derived from amino acid metabolism (Fincham, Day & Radford, 1979; Welsh *et al.*, 1989). For this reason leucine and valine, which are synthesized from pyruvate and α-ketoisovaleric acid (Shuler & Kargi, 1992), promote the synthesis of the corresponding alcohols (2-methyl propanol and 3-methyl butanol). These, in turn, can be esterified with acetic acid to form 2-methylpropyl and 3-methylbutyl acetates. The role of pyruvate or leucine was also demonstrated for the banana (Drawert & Berger, 1981). Amino acids derived from other metabolic pathways, such as lysine, asparagine, cysteine, tryptophan, tyrosine and phenyl alanine, were found not to enhance the banana aroma (data not shown).

From Table 5, it can be seen that some compounds (1, 2, 6, 10 and 13) were detected in the headspace of all the runs. The presence of the different compounds in the headspace depends on the concentration in the solid medium, its vapour pressure and the transfer through the cover of the flask. In this sense, the headspace analysis is biased against the less volatile substances. Nevertheless, some consistent patterns were found: 2-hexanol and 2-hexanone were found in similar concentrations in runs 2–9. Also, the headspace concentration of compounds 11, 16 and 19 was increased with the addition of leucine or valine. Moreover, for the runs containing these precursors, more volatile compounds were found and in

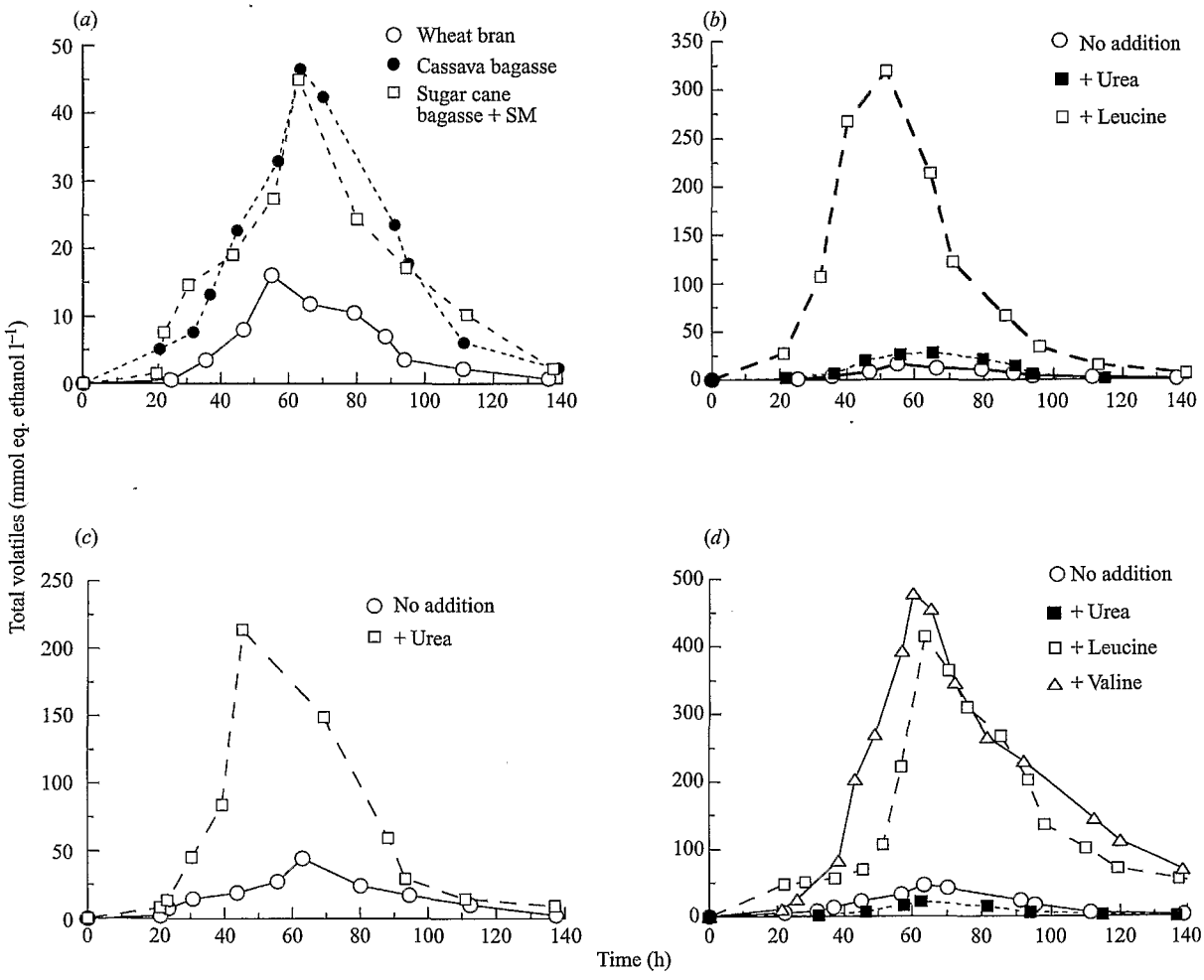

Fig. 4. Total volatile compound evolution in the headspace of cultures of *Ceratocystis fimbriata*. (*a*) Comparison of the three substrates with no addition; (*b*) wheat bran; (*c*) sugar cane bagasse + synthetic medium; (*d*) cassava bagasse.

higher concentrations than in the headspace of a ripe banana. The strong aroma detected in those cases may be due, in spite of the low total volatiles produced (48·4 μmol l^{-1}), to the high concentration of 3-methylbutyl acetate and to the presence of ethyl acetate, acetaldehyde and 3-methyl butanol.

Total volatile compounds production

Similar total volatile compound production was found for growth on cassava bagasse and sugar cane bagasse + SM, which were higher than that for wheat bran (Fig. 4*a*). The maxima were attained between 48 and 68 h, which coincided with maximum perception by olfactometry. In Figs 4*b–d* and Table 4, it is shown that for the three substrates the addition of leucine or valine, in addition to promoting growth, greatly enhanced the total production of volatiles. The maxima reached in these cases were between four and tenfold greater than that of the fruit.

The parameters of the model are reported in Table 6. The model was also found to be adequate to describe the integrated data of TV production, as can be seen from the R^2 coefficients. As observed for CO_2 production, the addition of leucine or valine (runs 2, 5, 7 and 9) increased TV_{max} from four to tenfold in comparison to use of the same substrate as a control (runs 1, 4 and 8). The production rate constant (k) varied between 0·039 and 0·063 h^{-1}, independently of the medium used. The t_{max} parameters coincide with those observed for the maximum perception of aroma (Table 4) and in most cases correspond to those observed experimentally (Fig. 4).

Key volatile compounds production

The evolution of the headspace concentration was evaluated for: acetaldehyde, ethanol, ethyl acetate, ethyl propionate, 3-methyl butanol and 3-methylbutyl acetate. These six compounds were chosen because they have often been reported as fungal (Kaminski, Stawicki & Wasowicz, 1974; Ito *et al.*, 1990; Sunesson *et al.*, 1995) or yeast (Piendl & Geiger, 1980) metabolites. Moreover, these compounds were produced in most of the experiments reported here (Table 3) and are known to be involved in fruity aromas in general for the first four compounds (acetaldehyde, ethanol, ethyl acetate, ethyl propionate), and more particularly, banana aroma for the other two compounds (3-methyl butanol and 3-methylbutyl acetate).

Wheat bran (runs 1–3). When grown without a nitrogen supplement, only 4 of the 6 key compounds were detected in

Table 6. Kinetic constants for the total volatile production according to the Gompertz model

Run*	TV_{max}(μmol l^{-1})	k(h^{-1})	t_{max}(h)	R^2
1	700	0·054	60·3	0·999
2	12937	0·063	47·9	0·999
3	1416	0·055	58·3	0·998
4	2421	0·045	61·5	0·997
5	20270	0·042	68·3	0·997
6	957	0·057	64·2	0·999
7	26068	0·039	67·1	0·998
8	2280	0·041	60·8	0·999
9	9123	0·059	53·5	0·999

* As in Table 1.

the headspace: acetaldehyde, ethanol, ethyl acetate and ethyl propionate with maxima values of 0·35 μmol l^{-1}, 3 μmol l^{-1}, 4·7 μmol l^{-1} and 0·6 μmol l^{-1}, respectively (Fig. 5*a*). When complemented with urea, the culture also produced 3-methyl butanol and 3-methylbutyl acetate with concentrations of 0·1 and 0·35 μmol l^{-1}, respectively (Fig. 5*b*). The first four compounds found were also present, with the N-supplement, but only at slightly higher concentrations than without urea. When leucine was added to the medium, a large increase in the concentrations of the six compounds was observed, particularly ethyl acetate (135 μmol l^{-1}) and ethanol (70 μmol l^{-1}). In this case, 3-methylbutyl acetate was present (2·1 μmol l^{-1}) and the 3-methyl butanol concentration was increased to 1 μmol l^{-1} (Fig. 5*c*). For the three experiments, maximum concentrations of these compounds were found at approx. the same times.

Cassava bagasse (runs 4–7). For this substrate, it may be observed in Fig. 6*a*, that the six compounds were produced without any N-supplement added to the medium. Although 3-methyl butanol and 3-methylbutyl acetate were found at low levels (0·25 and 0·45 μmol l^{-1}), a medium banana-like aroma was detected in the headspace. This probably indicates that in the cassava bagasse, the amino acid precursors of the alcohol and the ester are more accessible to the mould than in wheat bran. This is confirmed by the next experiment (run 5) where cassava bagasse was complemented with leucine (Fig. 6*c*). In this case, the odour detected was much stronger (Table 2) and

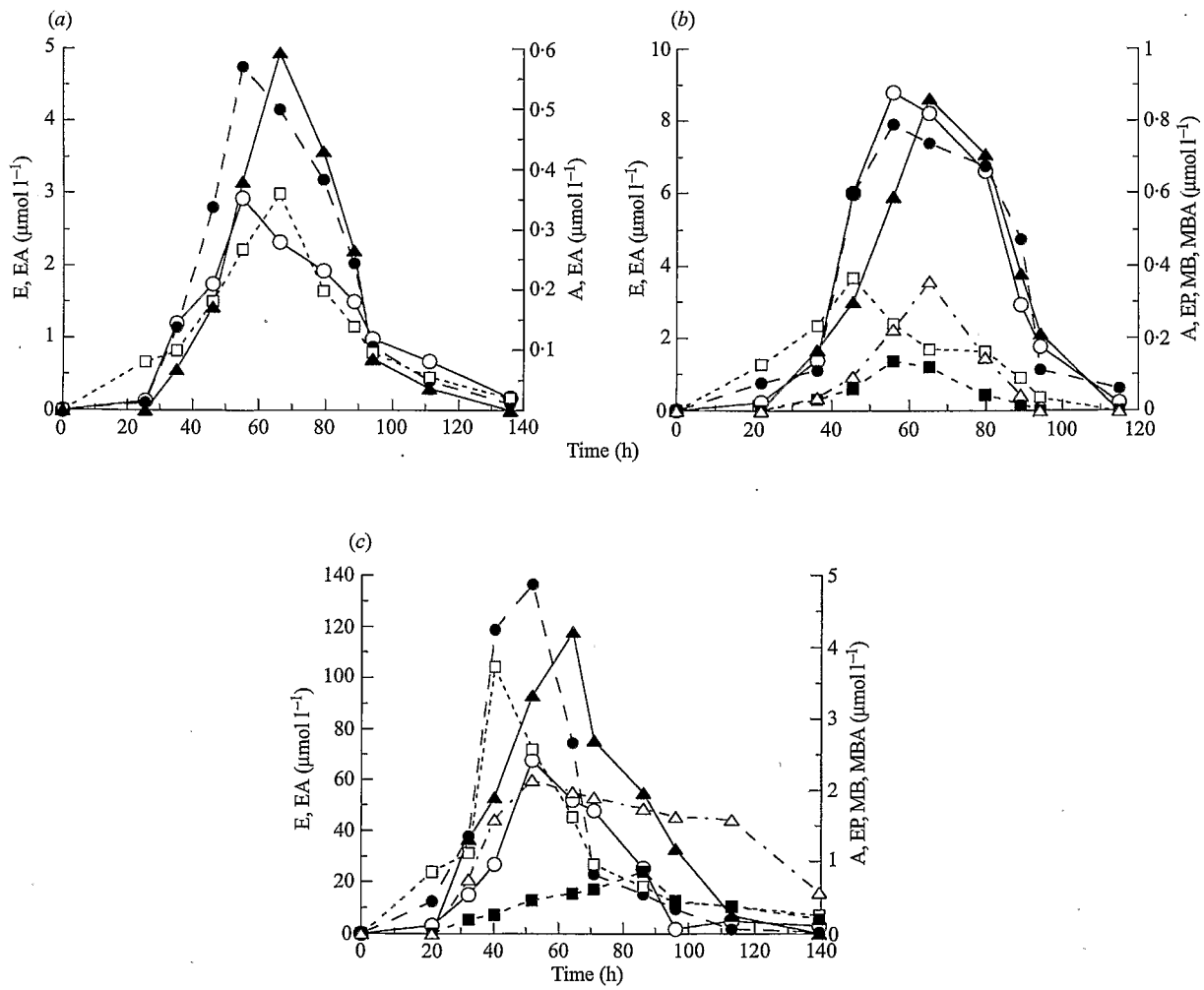

Fig. 5. Evolution of six key compounds in the headspace of cultures of *Ceratocystis fimbriata* grown on wheat bran. (*a*) No addition; (*b*) + urea; (*c*) + leucine. (○, E), Ethanol; (●, EA), ethyl acetate; (□, A), acetaldehyde; (▲, EP), ethyl propionate; (■, MB), 3-methyl butanol; (△, MBA), 3-methylbutyl acetate.

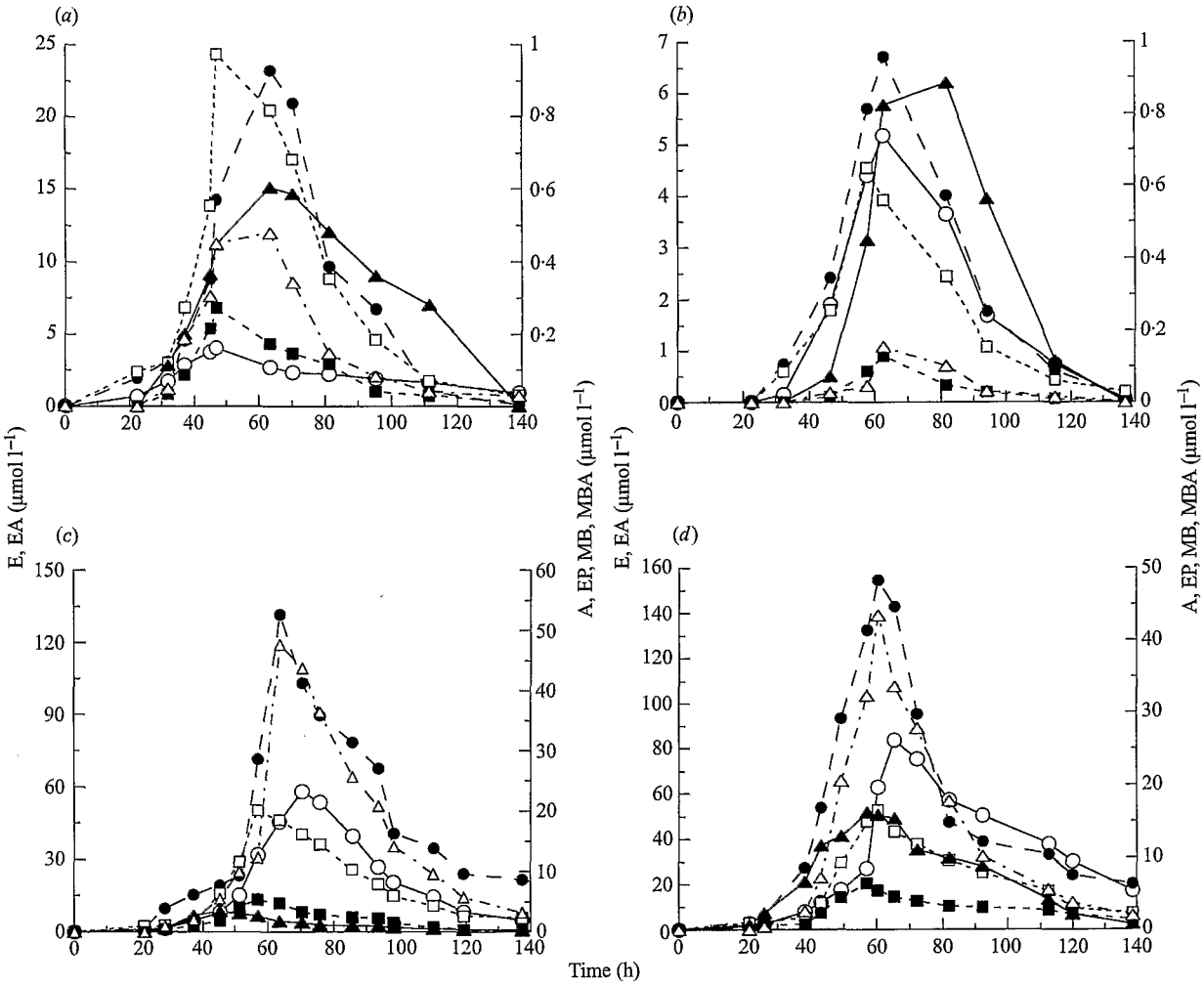

Fig. 6. Evolution of six key compounds in the headspace of cultures of *Ceratocystis fimbriata* grown on cassava bagasse. (*a*) No addition; (*b*) + urea; (*c*) + leucine; (*d*) + valine. (○, E), Ethanol; (●, EA), ethyl acetate; (□, A), acetaldehyde; (▲, EP), ethyl propionate; (■, MB), 3-methyl butanol; (△, MBA), 3-methylbutyl acetate.

the six metabolites were produced in higher amounts, attaining 132 μmol l^{-1} for ethyl acetate and 48 μmol l^{-1} for 3-methylbutyl acetate. Similar results were found when valine was added to the cassava bagasse (Fig. 6*d*). In contrast, the addition of urea to the medium did not promote the biosynthesis of these compounds (Fig. 6*b*).

Sugar cane bagasse + synthetic medium (runs 8 and 9). As in the case of wheat bran alone, no 3-methyl butanol nor its acetate ester were detected when this medium was used without any N-supplement (run 8 and Fig. 7*a*). However, the medium fruity odour detected may be due to the relatively high concentrations of ethyl acetate (26 μmol l^{-1}), ethyl propionate (1·1 μmol l^{-1} and other compounds such as ethyl butyrate (Fig. 3). When leucine was added, growth on this medium gave a strong banana-like aroma due to the higher concentrations of ethyl acetate (54 μmol l^{-1}) and 3-methylbutyl acetate (9·5 μmol l^{-1}). Maximum concentrations of the volatiles were found at similar times to those for wheat bran and cassava bagasse substrates.

Wheat bran, cassava bagasse and sugar cane bagasse complemented with a synthetic medium are adequate substrates for the growth of *Ceratocystis fimbriata*. Few volatiles were produced when each of the three substrates was used alone without supplement. Moreover, wheat bran culture exhibited no odour, cassava bagasse culture produced a weak banana odour while sugar cane bagasse + SM culture had a weak fruity odour. Aroma production for *C. fimbriata* was found to be growth associated. The production of volatile metabolites in general and of esters with fruity notes in particular was greatly enhanced (more than tenfold in some cases) by the addition of leucine or valine. Urea, when used as a supplementary nitrogen source, did not have the same effect. The production of 3-methylbutyl acetate and 3-methyl butanol was associated with the banana aroma which in turn increased in intensity as these compounds were overproduced under leucine or valine supplementation. In those cases, more volatile compounds, and these in larger amounts, were found in the culture headspace than when a banana was used as control, even though the aromas detected were similar in terms of quality and intensity. Integrated data were used to describe the accumulated production of CO_2 and TV. These data well fitted the logistic shaped Gompertz model. For both CO_2 and TV production, the parameters showed that total amounts (P_{max}) were dependent on the medium used.

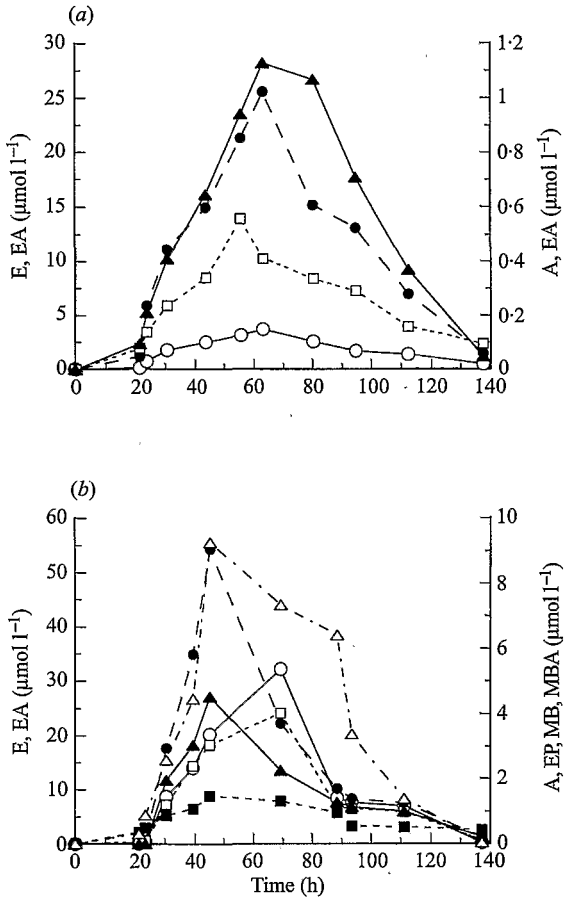

Fig. 7. Evolution of six key compounds in the headspace of cultures of *Ceratocystis fimbriata* grown on sugar cane bagasse + SM. (*a*) No addition; (*b*) + leucine. (○, E), Ethanol; (●, EA), ethyl acetate; (□, A), acetaldehyde; (▲, EP), ethyl propionate; (■, MB), 3-methyl butanol; (△, MBA), 3-methylbutyl acetate.

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