

# Fruity aroma production by *Ceratocystis fimbriata* in solid state fermentation

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## SUMMARY

Production of fruity aroma in solid state fermentation (SSF) by the fungus *Ceratocystis fimbriata* has been studied, with respect to the nature of the support/substrate, the importance of added precursors in the medium and the aeration. The aroma were characterized by the sniffing technique and GC headspace analysis, while the growth was followed by respirometry. Wheat bran, cassava and sugarcane bagasse were adequate for growth and aroma production. The nutritive media with a higher glucose concentration (200 g/l) gave a strong apple aroma, while media containing amino acids precursors, such as leucine and valine, gave strong banana aroma. Aroma production was growth dependent and the maximum aroma intensity was detected a few hours, before or after the maximum respirometric activity (0.8 to 1.6 ml CO<sub>2</sub> / h. g dry matter, after 24 h). Experiments carried out under various aeration rates (5 and 50 ml/h. g dry matter) showed that this parameter was not limiting for growth, even if the exit gas was very poor in oxygen at the lower aeration rate, giving in this case the most intense aroma. For experiments carried out without forced aeration, the same aroma were also found with higher intensity. Fourteen compounds have been separated by GC headspace and 11 of these were identified. Major compounds included acetaldehyde, ethanol, ethyl acetate, isoamyl acetate and isoamyl alcohol.

**Keywords:** Solid state fermentation, *Ceratocystis fimbriata*, fruity aroma, substrates, support, precursors, aeration, respirometry, apple aroma, banan aroma, growth dependance.

## RESUME

### **Production d'arômes fruités par *Ceratocystis fimbriata* cultivé en fermentation solide.**

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La production d'arômes fruités en fermentation solide (FMS) par un champignon filamenteux *Ceratocystis fimbriata* a été étudiée en vue d'estimer l'influence de la nature du substrat et du support, de la présence dans le milieu de culture de précurseurs d'arômes et enfin du taux d'aération des cultures sur la qualité des arômes obtenus. Les arômes produits ont été caractérisés par la technique du «sniffing» et la croissance microbienne a été suivie par respirométrie. Le son de blé, le manioc et la bagasse de canne à sucre ont été utilisés pour la culture et la production d'arômes. Les milieux de cultures à forte concentration de glucose (200 g/l) donnent un puissant arôme de poire ou de pomme, alors que les milieux riches en acides aminés tels que la leucine et la valine favorisent la production d'arôme de banane. Il a été démontré que la production d'arôme est fonction de la croissance et l'intensité maximale a été détectée quelques heures après l'activité respirométrique maximale (elle varie entre 0,8 et 1,6 ml CO<sub>2</sub>/h.g matière sèche après 24 heures). Des essais réalisés avec différents taux d'aération (5 et 50 ml/h.g matière sèche) ont montré que ce paramètre n'est pas limitant pour la croissance, même si pour des taux d'aération faibles, les effluents gazeux à la sortie du fermenteur sont pauvres en O<sub>2</sub>. Dans ce dernier cas, comme pour des fermentations réalisées sans aération forcée, les mêmes arômes ont été détectés, mais à des intensités plus fortes. Quatorze composés ont été séparés par CPG parmi lesquels onze ont pu être identifiés. Les composés majoritaires ont été les suivants: acétaldéhyde, éthanol, ethyl acétate, isoamyl acétate et isoamyl alcool.

**Mots clés:** Fermentation en milieu solide, *Ceratocystis fimbriata*, arômes fruités, substrats, support, précurseurs, aération, respirométrie, arôme de pomme, arôme de banane, croissance limitée.

## INTRODUCTION

Microorganisms play an important role in the generation of natural flavouring compounds, particularly food aroma. A number of the extensive reviews dealing

with flavour generation by microorganisms are available (Latrasse *et al*, 1985; Welsh *et al*, 1989; Janssens *et al*, 1992). As pointed out recently by Bigelis (1992) and Christen (1995), filamentous fungi are especially interesting, because they are able to produce flavouring compounds or flavours-related enzymes. Recently, the production of aroma in SSF has been reported. Yamauchi *et al* (1989) obtained a fruity odour by growing a *Neurospora* strain on pregelatinized rice. Gervais and Sarrette (1990) studied the production of coconut aroma by *Trichoderma viride* on agar, while Humphrey *et al* (1990) patented a process, where an *Aspergillus* strain grown on cellulose fibers produced methyl ketones from coconut oil. Moreover, the capacity of some moulds from the genus *Ceratocystis* to produce fruit-like aroma has already been demonstrated (Hanssen and Sprecher, 1981; Senemaud 1988; Christen *et al*, 1994). In this work, the ability of *Ceratocystis fimbriata* to produce aroma in SSF was explored. It involved the study on the influence of the substrates/supports, the aeration flow rates and the precursors, on both growth and aroma production.

## MATERIAL AND METHODS

### ORGANISM AND CULTURE MEDIA

*Ceratocystis fimbriata* CBS 374-83 was used. It was periodically transferred onto potato dextrose agar (PDA) slants and stored at 4°C. Four substrates / supports were used: wheat bran, sugarcane bagasse, cassava and an anionic resin (Amberlite IRA-900, Rohm and Haas, USA). These were prepared according to Christen *et al* (1993). When forced aeration (packed bed) was used, the cultures were carried out in small columns placed in temperature controlled bath. Experiments without forced aeration (surface culture) were carried out in 500 ml Erlenmeyer flasks, covered with gauze or tight-sealed. The reactors were filled with 7.5 g initial dry matter (IDM) for wheat bran and Amberlite, and 5.25 g IDM for bagasse and cassava. For all experiments, initial conditions were: temperature, 30°C; pH, 6; and inoculum size  $1 \times 10^7$  spores/g IDM. Aeration rates were 50 or 5 ml/h.g IDM. Initial water content was calculated, according to the maximum adsorption capacity of each support (wheat bran, 50%; Amberlite, 58%; sugarcane bagasse, 63% and cassava, 65%). Culture conditions are given in tables 1 and 2; Nutritive medium SM is the synthetic medium optimized by Christen and Raimbault (1991), and contained 200 g/l of glucose. In all cases, an oligoelement solution, previously used by these authors, was added. Urea, leucine and valine (167 mmol/l) were used as a nitrogen source and/or precursor of the aroma.

## ANALYTICAL PROCEDURES.

The odours of the cultures were determined by sensory evaluation by a non-trained panel, consisting of six members, with no restriction in descriptive terms. Growth was characterized by respirometry and measured by gas chromatography. For packed column experiments, this allowed the calculation of the carbon dioxide production rate, the oxygen uptake rate and the respiratory quotient (RQ) (Christen *et al*, 1993). For surface cultures, O<sub>2</sub> consumption and CO<sub>2</sub> evolution were followed. Water activity and pH were also determined at the end of the fermentation. Volatiles produced during the fermentation were characterized by gas chromatography (Hewlett-Packard 5890, equipped with a Megabore HP-1 column (length, 5 m) and with a flame ionization detector) of headspace vapor from the cultures (only for experiments without forced aeration). Temperatures were: injector and detector, 210°C, oven held at 40°C during 2 min and then programmed at 10°C/min to 150°C. The nitrogen gas flow rate was 1.5 ml/min and split ratio 1:32.

Table 1. Culture conditions for experiments with forced aeration

Run	Substrate/Support	Nutritive media	Aeration rate (ml/h.g)
1	Amberlite	Potato broth	50
2	Amberlite	Potato broth	5
3	Amberlite	SM	5
4	Wheat bran	Urea	50
5	Wheat bran	-	5
6	Wheat bran	Urea	5
7	Wheat bran	Leucine	5
8	Wheat bran	Valine	5
9	Sugarcane bagasse	SM	5
10	Sugarcane bagasse	Potato broth+glucose	5

**Table 2.** Culture conditions for experiments without forced aeration  
Each experiment was made in both gauze and tight-sealed flasks.

<b>Run</b>	<b>Substrate/Support</b>	<b>Nutritive media</b>
11	Wheat bran	-
12	Wheat bran	SM
13	Wheat bran	Leucine
14	Wheat bran	Urea
15	Cassava	-
16	Cassava	SM
17	Cassava	Leucine
18	Cassava	Urea
19	Sugarcane bagasse	SM + leucine

## RESULTS AND DISCUSSION

### PACKED CULTURES EXPERIMENTS WITH FORCED AERATION

At the higher aeration rate (50 ml/h.g IDM in runs 1 and 4), negligible or poor aroma was detected (Table 3). It can be assumed that the high aeration rate swept away the volatiles produced and/or oxygenated conditions reduced the synthesis of such molecules. This is why a very low aeration rate was then used (5 ml/h.g IDM). In such cases, the overall aroma detected (pear/apple and banana) were stronger. Leucine and valine, when added to the medium, served as a precursor for the development of the banana aroma (runs 7 and 8). This fruity aroma appeared very rapidly (before the first 24 h) in these cases. When no precursor was used, pear/apple aroma was also detected (runs 4, 5, 6, 9 and 10). In runs 9 and 10, two successive kinds of aroma were detected, first the pear/apple one and then at 5 days a strong peach aroma. Using this aeration rate, lower maximum CO<sub>2</sub> production rates were observed (less than 1.3 ml/h.g IDM), which indicated that, growth was limited and volatile metabolites production was favoured in poorly aerated media. In terms of support evaluation, wheat bran (supplemented or not) and supplemented bagasse, gave a better result compared with Amberlite. In all cases, water activity was maintained at a satisfactory level, but the pH at the end of fermentation was alkaline

(for wheat bran) or acidic (for bagasse). In all cases, no compounds were detected in headspace analysis of the cultures.

**Table 3. Aroma production in packed culture under forced aeration**

<b>Run</b>	<b>Aroma and intensity</b>	<b>Tmax (h) #</b>	<b>CDPR max (ml/h.g)</b>	<b>Aw final</b>	<b>pH final</b>
1	-	-	2.58	0.996	6.76
2	Banana +, then pear/apple +	67 / 91	0.95	0.973	3.05
3	Banana +	91	0.89	0.990	2.65
4	Pear/apple +	42	2.45	0.983	8.76
5	Pear/apple ++	17	1.30	0.988	9.06
6	Pear/apple ++	39	1.04	0.985	8.95
7	Banana +++	17	0.72	0.989	9.03
8	Banana +++	17	0.79	0.990	9.06
9	Pear/apple +, then peach +++	20 / 91	0.84	0.993	2.71
10	Pear/apple ++, then peach ++	68 / 116	0.70	0.998	6.24

-: none, +: weak, ++: medium, +++: strong. #: T max, time of maximum aroma perception. CDPR: CO<sub>2</sub> production rate, ml/h.g IDM.

The maximum aroma was detected, just before or after the maximum in CO<sub>2</sub> production rate was attained. Interestingly aroma production was found to be related to growth.

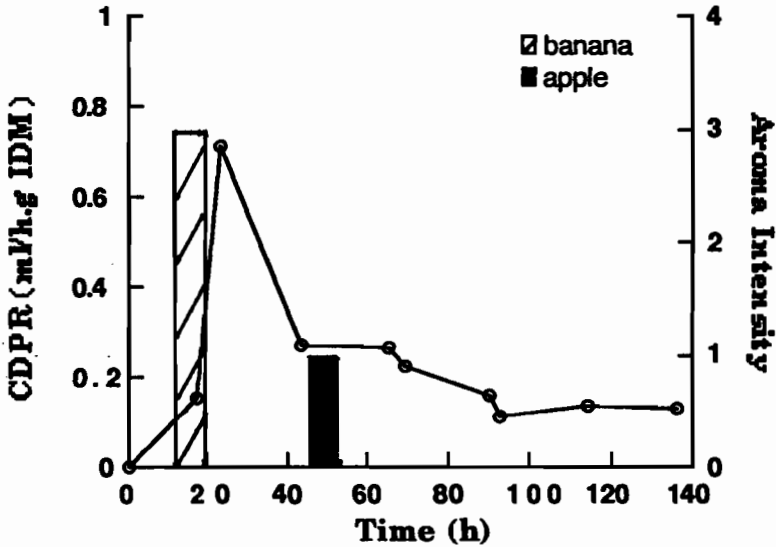


Fig. 1. CO<sub>2</sub> production rate and aroma production by *C. fimbriata* grown on wheat bran supplemented with leucine (aeration rate = 5 ml/h.g IDM).

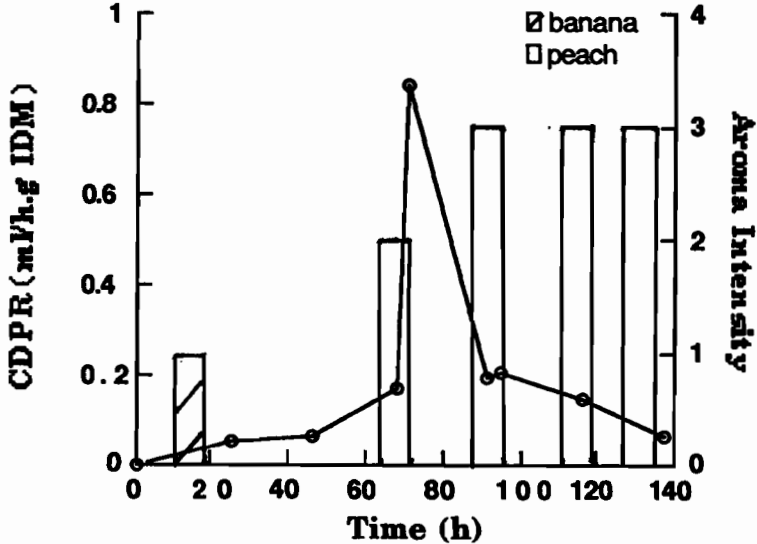


Fig. 2. CO<sub>2</sub> production rate and aroma production by *C. fimbriata* grown on sugarcane bagasse supplemented with synthetic medium (aeration rate = 5 ml/h.g IDM).

## SURFACE CULTURES EXPERIMENTS WITHOUT FORCED AERATION

In this case, 9 combinations were tested, and in each case, experiments were carried out using a gauze cover (static culture) tightly-sealed (without aeration). Sensory evaluation was only possible in the former case. In the second case, respiration was characterized by O<sub>2</sub> (%) consumption and CO<sub>2</sub> (%) accumulation in the flask. Results are presented in Table 4.

Table 4. Results of aroma production in surface cultures without forced aeration.

Run	Aroma and intensity (1)	T max (h) #	CDPR max (ml/h.g) (1)	CO <sub>2</sub> max (%) (2)
11	-	-	1.15	11.3
12	apple / pear ++	44	0.06	81.3
13	banana +++	36	0.16	31.5
14	apple / pear ++	41	1.23	22.3
15	banana ++	40	0.2	36.7
16	-	-	0.9	62.5
17	banana +++	40	0.2	29.6
18	-	-	0.45	7.3
19	banana +++	39	0.08	46.9

∴ none, +: weak, ++: medium, +++: strong. #: T max, time of maximum perception of the aroma. CDPR: CO<sub>2</sub> production rate in ml/hour/g IDM. 1 refers to gauze covered flask cultures and 2 to tight-sealed flask cultures.

All three substrates/support were found to allow growth and aroma production in aerated conditions. Aroma production was higher in forced aeration. The strongest aroma detected (banana) corresponded to that case where media were enriched with leucine (runs 13, 17, 19), while pear/apple aroma, of a lower intensity, was obtained in wheat bran culture with synthetic medium and urea (runs 12 and 14). These aroma had major intensity between the first and the second days. In tight-sealed flask cultures, growth was observed. As growth and substrate fermentation evolved, CO<sub>2</sub> was produced and the internal pressure increased. This pressure was released during sampling, which provoked the increase in CO<sub>2</sub> concentration up to 81%. These values of CO<sub>2</sub> were coupled with low O<sub>2</sub> (less than 2 % of residual oxygen), which channelled the metabolism towards the fermentative route. No Sensory evaluation

was made for tightly-sealed flask cultures, but large amounts of volatiles were produced (Fig. 3).

## SEPARATION AND IDENTIFICATION OF GC DETECTED COMPOUNDS

Fig. 3 gives headspace chromatograms of gauze covered aerated and tight-sealed cultures of run 19. Fourteen compounds were detected. Eleven compounds were identified through retention time comparison with a standard and could be classified according to their relative quantity (peak area). Ethanol, ethyl acetate, ethyl propionate and isoamyl acetate in major; acetaldehyde, isoamyl alcohol and isobutyl acetate in intermediate and 1-propanol, 2-propanol, 1-butanol and amyl acetate in small amounts are formed. Among them, isoamyl acetate and isoamyl alcohol are known to be the major compounds in the banana aroma, while acetaldehyde, ethanol and ethyl acetate are always present in fruit aroma. Other minor compounds, like ethyl propionate and isobutyl acetate are also reported to be present in fruit aroma. Some differences can be observed between gauze covered and tightly-sealed cultures. Acetaldehyde peak is bigger, ethyl propionate peak is smaller and unknown peak #13 is absent in the latter. Unfortunately, it is not possible to evaluate directly the impact of these differences on the aroma.

## CONCLUSION

Wheat bran, cassava and sugarcane bagasse were found to be adequate substrates/support for aroma production by *C. fimbriata*. Amino acids, such as valine or leucine, direct banana-like aroma. Other aroma (peach, apple) were also detected in the absence of any precursor. The corresponding compounds of banana aroma (isoamyl alcohol and isoamyl acetate) were detected in the headspace of the culture at relatively high amounts. A total of 14 compounds were separated by GC, and, among them, 11 were identified (1 aldehyde, 5 alcohols and 5 esters). Work is currently continued to identify the unknown peaks and to quantify the identified compounds. Very low aeration (5 ml/h.g IDM) or passive diffusion favoured the production of strong aroma. Results were highly improved in these conditions, in comparison with those obtained by Christen *et al* (1994) at higher aeration rates. In the tightly-sealed flasks, the fungus was able to ferment the carbohydrates present in the medium (glucose in the case of bagasse, starch in the case of wheat bran and cassava). The fact that very low or no aeration is required opens interesting technological perspectives for the production of fruity aroma by *C. fimbriata*.

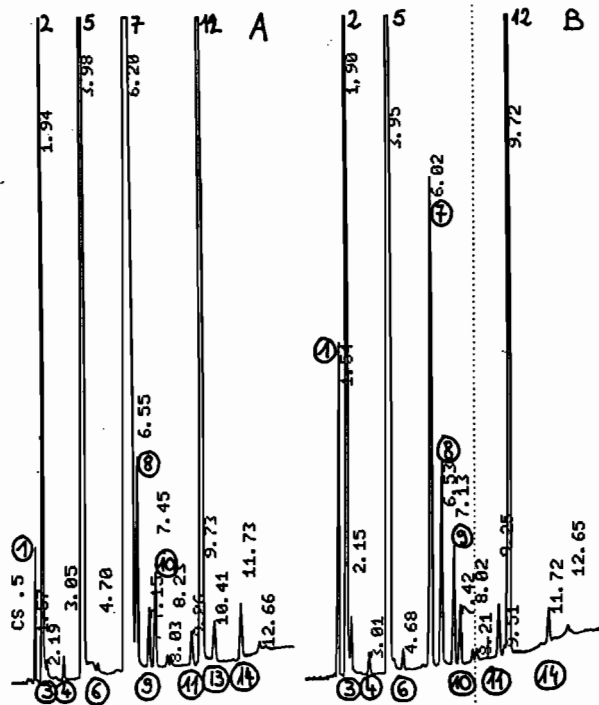


Fig. 3. Headspace chromatograms of 48 h cultures of gauze covered (A) and tight-sealed (B) cultures of *C. fimbriata* grown on bagasse + synthetic medium (glucose = 200 g/l). 1: acetaldehyde, 2: ethanol, 3: 2-propanol, 4: 1-propanol, 5: ethyl acetate, 6: 1-butanol, 7: ethyl propionate, 8: isoamyl alcohol, 9: unknown, 10: isobutyl acetate, 11: unknown, 12: isoamyl acetate, 13: unknown, 14: amyl acetate.

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