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FRUITY AROMA PRODUCTION BY *CERATOCYSTIS FIMBRIATA* IN SOLID CULTURES FROM AGRO-INDUSTRIAL WASTES

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ABSTRACT

Solid state fermentations were carried out to test the efficacy of *Ceratocystis fimbriata* to grow on different agro-industrial substrates and aroma production. Seven media were prepared using cassava bagasse, apple pomace, amaranth and soya bean. All the media supported fungal growth. While amaranth medium produced pineapple aroma, media containing cassava bagasse, apple pomace and soya bean produced a strong fruity aroma. The aroma production was growth dependent and the maximum aroma intensity was detected a few hours before or after the maximum respirometric activity. Sixteen compounds were separated by gas cromatography of the components present in the headspace and fifteen of them were identified as acid (1), alcohols (6), aldehyde (1), ketones (2) and esters (5).

Key words: *Ceratocystis fimbriata,* solid state fermentation, agro-industrial wastes, aroma

INTRODUCTION

Large amounts of solid wastes are generated during the processing of agro-industrial products, such as cassava and apple and their accumulation causes a major problem of environmental pollution. However, due to high content of starch and sugars, these residues could be used for growth of many microorganisms, including fungi, which have been utilized for centuries in the transformation of agro-industrial products (1,7, 9, 10,11, 16). A number of filamentous fungi have been tested for the production of aroma including *Ceratocystis* which produces a diversity of fruity aromas such as banana, pear, melon, apple, and lemon (2, 4, 6, 8, 12, 14, 15).

Despite of the interest in the valorization of agro-industrial wastes, not much literature is available on the production of aroma associated with the growth of molds in solid state fermentation (SSF). SSF offers several advantages on the utilization of agro-industrial substrates, such as easy handling, low cost and high productivity. In this work, we report our findings on the growth and aroma production by *Ceratocystis fimbriata* in solid state fermentation of four agro-industrial wastes.

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MATERIALS AND METHODS

Microorganism and media: *Ceratocystis fimbriata* CBS 372-83 was grown and periodically transferred on Potato Dextrose Agar (PDA) slants and stored at 4°C.

Inoculum: For inoculum, a spore suspension of *C. fimbriata* was prepared by growing it at 30°C for five days on PDA medium. Spores were collected with sterile water containing few droplets of Tween 80 and small glass beads to improve the harvest. Spore suspension contained 10^7 spores/g of initial dry matter (IDM).

Substrates preparation: Four solid substrates (see Table 1) were used in various proportions, as listed in Table 2. Soya beans were previously dried and ground in order to separate the hull. Amaranth was used in popped form. All the substrates were dried, ground, sieved through a 0.8 mm screen and autoclaved at 121°C for 15 min.

Fermentation: SSF was carried out in 250 ml Erlenmeyer flasks, covered with 6 layers of gauze, containing 15 g of dry matter. Initial water content of the media was calculated according to the maximum absorption capacity of each substrate. *C. fimbriata* was inoculated in all the media (pH 6.0) as described in Table 2 and incubated at 30°C. All the experiments were done in duplicate.

Analytical procedures: Water activity (Aw) was determined using an Aqualab CX-2 apparatus (Decagon, USA). For pH measurements, samples homogeneized with deionized water and pH was measured using a potenciometric method. CO_2 concentration was measured by gas chromatography according to Christen *et al.* (1991) and expressed as 5 ml CO_2 100 ml^{-1} headspace.

The odour of the cultures was determined by sensorial evaluation using a non-trained panel consisting of six members, with no restrictions in descriptive terms.

Volatile compounds produced were characterized by injecting 2.0 ml from the headspace of the cultures to a Hewlett-Packard 5890 GC, equipped with a 5 meter Megabore HP-1 column and with a flame ionization detector. Total volatiles produced were expressed as μ mol ethanol equivalent per litre of headspace (μ mol l-1 eq.ethanol). Some compounds were also determined individually and their concentration in the headspace was expressed from standard curves as μ mol l-1.

Table 1. Composition of the substrates. Proportions are given on dry matter basis

Substrate	Cassava bagasse	Soy bean	Apple pomace	Amaranth grain
Protein	1.6	41.1	3.5	14.5
Starch	51.3	5.6	0.0	52.0
Reducing Sugars	1.1	-	25.8	0.0
Fat	0.1	19.9	1.2	5.0
Ashes	3.1	3.8	6.1	8.7
Water saturation **	65	40	75	65

**expressed in g/100g

Table 2. Composition of the diferent solid media studied. Proportions are given on a dry matter base

Medium	Substrate*	Initial Aw	C/N
A	cassava bagasse + soya bean (8:2w/w)	0.994	29.3
B	cassava bagasse + soy bean (2:8w/w)	0.991	10.1
C	cassava bagasse + soya bean (5:5w/w)	0.993	20.5
D	cassava bagasse + soya bean + soya bean oil (4.5:4.5:1 w/w/v)	0.992	20.8
E	apple pomace + cassava bagasse + soya bean (8:1:1 w/w/v)	0.986	38.6
F	apple pomace + cassava bagasse + soya bean (3:3:4 w/w/w)	0.991	18.0
G	amaranth grain	0.996	29.4

* Salt solution as described by Christen and Raimbault (1991)

RESULTS AND DISCUSSION

Growth studies

As reported previously (5, 13), CO_2 production in the headspace above the culture was a good growth indicator. Experiments were conducted for four days under static conditions without forced aeration. All the media combinations tested, except one (medium E), were found to allow growth and aroma production (Tables 3, 4).

Table 3. Results for growth on different solid media

Medium	CO2 max (%)	t max. (h)	Final pH	Final aw
A	12.8	63	6.3	0.997
B	10.0	21	7.5	0.991
C	8.9	21	7.1	0.997
D	7.5	21	7.2	0.996
E	0.7	40	5.1	0.970
F	6.6	21	6.9	0.991
G	7.3	22	5.5	0.989

Table 4. Aroma and volatile compounds production in the headspace of cultures of *C. fimbriata*

Medium	Aroma Intensity*	t max (h) max**	Total volatiles
A	Fruity ++++	63	124.3
B	Fruity ++	17	36.9
C	Fruity ++++	20	76.9
D	Fruity ++++	28	103.7
E	¾¾7.4		
F	Fruity ++++	31	112.0
G	Pineapple +++	39	96.6

* (none, + weak, ++ medium, +++ strong, ++++ very strong
** μ moleq.ethanol/l

In all cases, water activity was maintained at a satisfactory level for growth; in some cases the pH at the end of the fermentation was alkaline (B, C, D) which was due to the accumulation of ammonia, resulting from the hydrolysis of soya proteins (A, E, F, G), because of the liberation of organic acids. Maximum respirometric activities, represented by headspace CO_2 evolution, were noted in media B, C, D and G, where the initial concentration of protein ranged from 15 to 33%. After a small lag phase, the maximum respirometric activity reached a maximum in the first 24 hours, being detectable up to 80 hours of fermentation (Fig. 1). The level of CO_2 production was much higher in medium F than in medium E, which was probably due to the larger availability of nitrogen, caused by the addition of a larger quantity of soya (40%) than in medium (only 10%).

Aroma production

The aroma production, as detected by olfactometry, is presented in Table 4.

The aroma production was growth dependent and the maximum aroma intensity was detected a few hours before or after the maximum respirometric activity, as reflected by CO_2 production reported in Fig. 1. The maximum production of aroma in the tested media was detected at 15 hours of growth. After this time, it began to decrease, but was detectable up to three days more. In all cases, strong aroma was released, except for medium E, in which no aroma was detected, due to the high concentration of ethanol in this medium (95%). These sensorial observations correlated well with the maxima of total volatile compounds detected by gas cromatography (Table 5). The highest amounts were found in cassava bagasse with balanced proportions, apple pomace and soya (A, C, D, F, G) where the C/N molar ratio was between 18 and 30. In contrast, medium E did not produce fruity aroma. It was suggested that the pineapple aroma detected in the amaranth medium was caused by the production of esters such as ethyl butyrate, which are known to be related to the pineapple aroma (14).

Separation and identification of volatile compounds

Sixteen different compounds were detected in the gas chromatograms of the components available in the headspace from the cultures. Fifteen of these were identified by their retention time and are listed in Table 5.

The major volatile compounds found in the headspace were alcohols, esters and, in a lesser amount, ketones. The presence of the different compounds in the headspace reflected both the variations arising from each medium and the relative presence of that specific compound as determined by its vapour pressure.

The analysis on the presence of these compounds in the tested media showed that the cultures had two types of behavior: one where the esters predominated (A, B, C, D and F, representing 55 to 69% of the total volatiles) and other where the alcohols were predominant (G and E). The medium G, although predominated by the alcohols, presented production of

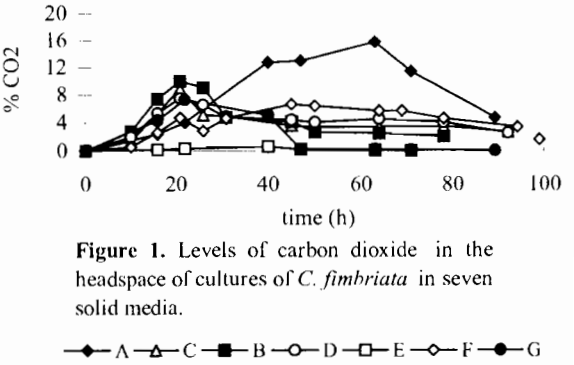

Figure 1. Levels of carbon dioxide in the headspace of cultures of *C. fimbriata* in seven solid media.

Table 5. Volatile compounds identified in the headspace of 2-days cultures of *Ceratocystis fimbriata*

Group	Compound (μ mol/l)	Medium						
		A	B	C	D	E	F	G
Adehyde	Acetaldehyde	0.66	0.02	0.07	0.07	0.01	0.55	1.21
Alcohols	Ethanol	3.20	0.01	7.50	12.23	7.02	19.13	78.92
	2-propanol	0.14	0.36	0.01	0.01	0.00	0.06	0.00
	1-propanol	0.01	0.00	0.02	0.01	0.00	0.03	0.09
	1-butanol	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	Isoamilic alcohol	0.33	0.57	0.30	0.52	0.00	0.30	1.17
	2-hexanol	2.22	2.40	3.20	1.04	0.1	0.88	0.29
Ketones	Acetone	0.07	0.00	0.00	0.00	0.00	0.00	0.00
	2-hexanone	2.44	0.00	0.96	1.20	0.00	0.90	0.00
Acid	Acetic acid	0.19	0.00	0.00	0.01	0.00	0.00	0.14
Esters	Ethyl acetate	66.78	21.67	40.70	53.86	0.11	54.70	8.82
	Ethyl proprionate	9.66	1.33	4.83	4.50	0.00	4.63	0.46
	Ethyl butirate	0.30	0.16	0.27	0.28	0.12	0.31	0.60
	Butyl acetate	0.00	0.00	0.00	0.00	0.11	0.00	0.00
	Isoamyl acetate	5.78	2.43	2.22	2.44	0.00	1.88	0.00

esters too (around 10%). This was not the case in medium E, which besides showing small production of volatile compounds, produced virtually only ethanol (Fig. 2).

In all the other media, the following esters were identified through retention time comparison with a standard curve: ethyl acetate, ethyl propionate, isoamyl acetate, and ethyl butyrate. There was a clear predominance of ethyl acetate, which represented over 80% of the total esters. Among the identified alcohols, ethanol, isoamyl alcohol, 2-hexanol, 1-propanol, 2-propanol, and 1-butanol were noted. Ethanol was the leading one in the headspace of the cultures and, as a rule, was the second volatile compound most produced during the fermentations, except in medium B, due to the low concentration of cassava bagasse. Alcohols do not play a predominant role in flavours but are known to contribute to the overall flavor quality and are precursors of fruit-like flavoring esters, which are definitely present in almost all fruits (14). The presence and quantities of the different compounds in the headspace depended on their concentration in the solid medium, vapour pressure and the transfer through the cover of the flask.

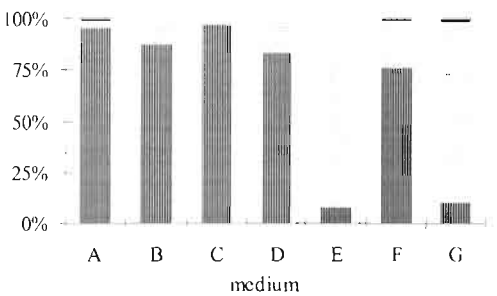

Figure 2. Representation of the volatile compounds in the headspace of *C.fimbriata* cultures grown on seven solid media.

CONCLUSION

Cassava bagasse, apple pomace, amaranth and soya bean were found to be adequate substrates for aroma production by *C. fimbriata* at different levels. A total of sixteen compounds were separated by gas cromatography and among then fifteen were identified (1 aldehyde, 6 alcohols, 5 esters, 2 ketones, 1 acid). There was clear correlation between the growth and production of the volatile compounds, since the maximum production of volatiles always occurred a few hours before or after the maximum respirometric activity. Strong fruity aroma was detected in the media in which the esters predominated (A, B, C, D, and F) representing 55 to 69% the total volatiles and specifically the pineapple aroma in the medium in which the alcohols predominated (G); in the medium in which specifically predominated the ethanol (E) no aroma was detected.

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RESUMO

Produção de aroma frutal por *Ceratocystis fimbriata* em culturas estáticas sobre resíduo sólido agro-industrial

Este estudo explorou a versatilidade de *Ceratocystis fimbriata* de crescer e produzir aromas naturais sobre substratos de resíduos agro-industriais. Bagaço de mandioca, bagaço de maçã, amaranto e soja em diferentes proporções compuseram os sete meios utilizados, mostrando ser substratos adequados para o crescimento e produção de aroma por este fungo em fermentação no estado sólido. Todos os meios contendo bagaço de mandioca, bagaço de maçã e soja em sua composição proporcionaram um forte aroma frutal, enquanto, o meio de amaranto produziu um agradável aroma de abacaxi. A produção de aroma foi dependente do crescimento, visto que a máxima intensidade do aroma foi detectado poucas horas antes ou depois da atividade respiratória máxima. Foram detectados dezesseis compostos pela cromatografia de gás no headspace das culturas, e quinze deles foram identificados: 1 ácido, 6 alcoois, 1 aldeído, 2 cetonas e 5 ésteres.

Palavras-chave: *Ceratocystis fimbriata,* fermentação no estado sólido, resíduos agro-industriais, aroma.

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