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Characterization and evaluation of coconut aroma produced by *Trichoderma viride* EMCC-107 in solid state fermentation on sugarcane bagasse

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ABSTRACT

Background: Sugarcane bagasse was shown to be an adequate substrate for the growth and aroma production by *Trichoderma* species. In the present work the ability of *Trichoderma viride* EMCC-107 to produce high yield of coconut aroma in solid state fermentation (SSF) by using sugarcane bagasse as solid substrate was evaluated. The produced aroma was characterized.

Results: Total carbohydrates comprised the highest content (43.9% w/w) compared with the other constituents in sugarcane bagasse. The sensory and gas chromatography–mass spectrometric (GC–MS) analysis revealed that the highest odor intensity and maximum yield of volatiles were perceived at the 5th d of induction period. The unsaturated lactone, 6-pentyl-α-pyrone (6-PP), was the major identified volatile compound. Saturated lactones, δ-octalactone, γ-nonalactone, γ-undecalactone, γ-dodecalactone and δ-dodecalactone, were also identified in the coconut aroma produced during the induction period (12 d). A quite correlation was found between the composition and odor profile of the produced aroma. The effect of varying the concentration of sugarcane bagasse on 6-PP production and biomass growth was evaluated. The results revealed high 6-PP production at 4.5 g sugarcane bagasse whereas the biomass showed significant ($P < 0.05$) increase by increasing the concentration of sugarcane bagasse.

Conclusion: The concentration of 6-PP, the most contribution of coconut aroma, produced in present study (3.62 mg/g DM) was higher than that reported in previous studies conducted under the same fermentation conditions. The significant increase in biomass with increasing the concentration of sugarcane bagasse may be attributed to the increase in sugar content that acts as carbon and energy source.

1. Introduction

In recent years the consumers' demand for natural food additives is rising. The food and drug administration [1] stated that to qualify as 'natural', flavorings have to be produced by physical, enzymatic or microbiological processes from natural sources. The Food and Drug Administration (FDA) defines natural aroma and specifies the type of substances generally regarded as safe for use as natural flavors including any substance that is extracted, distilled, or otherwise derived from plant or animal matter. Microorganisms play an important role in the generation of natural compounds, particularly in the field of food aromas. Several reports and reviews have been published on the production of volatile compounds (aroma compounds) by microorganisms [2,3,4,5,6,7,8,9]. Although several bacteria, yeast and fungi have been reported for the production of aroma compounds, a few species of yeasts and fungi have generally been preferred, and only a few of them find industrial application due to their generally regarded as safe (GRAS) status. Nowadays, there has been an increase trend toward the more efficient use of agro-industrial wastes including sugarcane bagasse [10,11]. Wastes from the food and agricultural industries that are produced in large quantities and are rich in carbohydrates and other nutrients can serve as a substrate for the production of chemicals and enzymes by using the technique of solid state fermentation (SSF), mainly due to their low cost [12]. In Egypt, the processing of the high production of sugarcane (15.77 million tons, Ministry of Agriculture, 2011–2012) gives rise to a large amount of sugarcane bagasse whose accumulation leads to an important problem of environmental pollution. SSF has been used for the production of aroma compounds by cultivating fungi using agro industrial residues as substrates [11]. The nature of

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the solid substrate used is an important aspect. However, the solid substrate not only supplies nutrients for microbial culture, but also serves as the physical support for the growth of microbial cells. Production of coconut aroma by *Trichoderma* species in SSF have been investigated in previous studies [11,13,14]. *Trichoderm*a species are reported as good producers of the unsaturated δ-lactone 6-pentyl-α-pyrone (6-PP) [15,16]. This compound is of interest in the food industry; it was found to be the major volatile compound contributing to the coconut-like aroma in cultures of *Trichoderma viride* [17]. 6-PP is approved as a flavoring agent by Joint Expert Committee on Food Additive [16]. Fungicide properties of 6-PP were also reported [16]. Ramos et al. [13] and Penha et al. [11] stated that the biosynthesis of 6-PP by *Trichoderma* species in SSF was accompanied by the production of other compounds. However, the authors of these studies didn't identify these compounds. *T. viride* EMCC-107 was recorded only in Egypt by the Microbiological Resources, Center. This strain was used for bio-treatment of agro industrial wastes [18] but to the best of our knowledge it was not used for aroma production. Therefore, in this study the ability of *T. viride* EMCC-107 to produce high yield of coconut aroma in SSF by using sugarcane bagasse as substrate was explored. Characterization of the volatile compounds accompanied the 6-PP production and evaluation of their influence on the overall odor profile of the aroma produced during the period of fermentation was estimated. The study was extended to evaluate the effect of varying the level of sugarcane bagasse on culture growth and aroma production. Finally, the relationship between the sensory profile and composition of the produced aroma was investigated.

2. Materials and methods

2.1. Microbial strain and culture media

The *T. viride* EMCC-107 purchased from the collection of Microbiological Resources Center (MIRCEN, Egypt) was used. The strain was maintained on malt extract medium at 4°C and cultured in Petri dishes at 28°C. A 5 d-old mycelium obtained under these conditions was used to inoculate the liquid media. The latter medium (50 mL), containing malt extract (20 g/L) and glucose (10 g/L), pH 5.6, was placed into 250 mL Erlenmeyer flasks and autoclaved at 121°C for 20 min before inoculation with 1 mL of mycelium grown on malt extract medium. The flasks were incubated at 28°C for 72 h on orbital rotary shaker (100 rpm). The mycelium formed was separated by decantation and rinsed twice with physiological saline solution (0.9% NaCl). It was then suspended into 50 mL of the same saline solution and 2 mL of this suspension were used to inoculate the solid cultures.

2.2. Substrate preparation

Sugarcane bagasse was obtained from a local market for the production of sugarcane juice in Egypt. The bagasse was subjected to a drying process at 60°C for 24 h and ground in a granulator mill using knives and hammer (1 mm).

2.3. Chemical composition of sugarcane bagasse

The chemical constituents of sugarcane bagasse were determined as described by AOAC [19]. Fatty acid composition of the lipid fraction was determined according to AOAC [20].

2.4. Fermentation conditions

Fermentation process was conducted for 12 days and analyzed for the production of coconut aroma at the 3, 5, 7, 9, 12th d. The solid substrate, comprising 4.5 g sugarcane bagasse was placed into 500 mL conical flask and autoclaved at 121°C for 20 min. Each flask was impregnated with 25 mL of sterile medium containing (g/L) glucose, 30.0; $(NH_4)_2SO_4$, 0.94; KH_2PO_4, 7.0; $Na_2HPO_4.7H_2O$, 2.0; $MgSO_4.7H_2O$, 1.5; $CaCl_2.2H_2O$, 0.008; $FeCl_3.6H_2O$, 0.008; and $ZnSO_4.7H_2O$, 0.0001 [14]. The flasks were inoculated with 1 mL of mycelia cell suspension and incubated at 28°C. The same conditions of SSF that revealed the high quality of coconut flavor and optimum yield of 6-PP (the most contributor of coconut aroma) was carried out but with variable levels of sugarcane bagasse (1.5, 3.0, 4.5 and 6.0 g/500 mL conical flask).

2.5. Dry matter measurement

Dry matter (DM) was determined by weight differences, 2–3 g fermented substrate was weighed and then dried to constant weight at 105°C.

2.6. Sensory evaluation

The effect of incubation time on the odor profile and intensity of the perceived odor of *T. viride* EMCC-107 culture in SSF on sugarcane bagasse was investigated. A panel of 10 members (National Research Center, Cairo, Egypt) characterized the odor of the fungal culture on the 3rd, 5th, 7th and 12th d. The odor description was assessed and the intensity was estimated on a 4 point scale (+: weak odor, ++: medium odor, +++: strong odor, ++++: very strong odor). The time of incubation period that revealed the optimum quality of coconut odor was selected to estimate the effect of varying the level of sugarcane bagasse on the intensity of the perceived odor. A nine-point hedonic scale (1 = not perceptible to 9 = strong perceptible) was used. Samples were identified with three digit code numbers and presented in a random sequence to the panelists.

2.7. Extraction of aroma compounds

Sample (4.5 g) was removed from each solid state culture and placed into 250 mL flask with 50 mL distilled water. The aroma compounds were extracted from the samples with 50 mL dichloromethane. Internal standard (IS); γ-decalactone (0.5 mg in dichloromethane) was added to each sample before extraction. After extraction the mixture was dried over anhydrous sodium sulfate for 12 h and concentrated using a rotary evaporator at 40°C under reduced pressure to final volume (100 μL) under a flux of nitrogen before analysis.

2.8. Gas chromatographic (GC) analysis

GC analysis was performed by using the Hewlett–Packard model 5890 equipped with flame ionization detector (FID). A fused silica capillary column DB5 (60 m × 0.32 mm, i.d.) was used. For fatty acid analysis, the oven temperature was programmed from 50 to 240°C at a rate of 3°C/min. Helium was used as the carrier gas, at flow rate 1.1 mL/min. The injector and detector temperatures were 220 and 250°C, respectively. For analysis of the extracted coconut aroma, the oven temperature was maintained initially at 50°C for 6 min, and then programmed from 50 to 240°C at a rate of 3°C/min. The injector and detector temperatures were 220 and 240°C, respectively. The retention indices (Kovats index) of the separated volatile compounds were calculated with hydrocarbons (C8–C22, Aldrich Chemical Co.) as references. The relative concentration of each individual compound was determined by comparing the peak area of the compound in each chromatogram with that of γ-decalactone (IS), assuming all response factors were 1.

2.9. Gas chromatographic–mass spectrometric (GC–MS) analysis

The analysis was carried out using a coupled gas chromatography Hewlett–Packard (5890)/mass spectrometry Hewlett–Packard-MS (5970). The ionization voltage was 70 eV, mass range m/z 39–400 amu. The GC condition carried out as mentioned above. The isolated peaks

were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and comparison with those of authentic compounds and published data [21].

2.10. Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) by the stat graphics package, Statistical Graphics Corporation, 1993, Manugistics Inc., USA [22]. The multiple range test L.S.D. (Duncan multiple range test), with significant level at $P < 0.05$, was applied to the results to test the significant difference.

3. Results and discussion

3.1. Chemical composition of sugarcane bagasse

Production of the volatile compounds by fungi in SSF can be influenced by the type and constituents of the substrate used that act as carbon and energy sources [6,23]. In the present study the low level of moisture (11.3% w/w) in dried sugarcane bagasse may be correlated to the processing methods used during its preparation. In SSF the fermentation take place in the absence or near absence of free water, thus being close to the natural environment to which microorganisms are adapted [10]. The protein content (1.9% w/w) in dried sugarcane bagasse was as low as that detected in previous study [11]. The effect of culture parameters and choice of precursors for 6-PP biosynthesis was studied [24]. The authors concluded that a low nitrogen level was favorable for 6-PP biosynthesis. In the present study the level of lipid (0.20% w/w) was lower than that reported (1.36 and 0.7% w/w) in previous studies [11]. The fatty acid composition of the lipid fraction was palmitic, 33.64 ± 0.23%; palmitoleic 5.34 ± 1.62%; heptadecanoic, 7.78 ± 0.48%; stearic, 5.06 ± 0.23%; oleic, 23.06 ± 0.60%; linoleic, 21.04 ± 0.51% and linolenic, 4.05 ± 0.36%. It was demonstrated that it is possible to produce 6-PP from different vegetable oils containing non-hydroxylated e.g. oleic, linoleic and linolenic fatty acids [16]. The total carbohydrates comprised the highest content (43.9% w/w) compared with other constituents in sugarcane bagasse in present study. It is higher than that reported in previous literatures [11]. Sugars used as a carbon energy source by *Trichoderma* sp. for the production of 6-PP at the same efficiency as that with fatty acids [16].

3.2. Dry matter (DM)

The DM of the fermented substrate showed significant ($P < 0.05$) increases by increasing the incubation period up to 7 d followed by insignificant ($P > 0.05$) increase (Fig. 1). This result is in agreement with that of De-Araujo et al. [14]. The toxic effect of 6-PP, the major volatile compound produced during fermentation, toward the fungal growth was demonstrated in previous studies [16]. The high production of 6-PP after 5 d of incubation in present study (as will be discussed below) may be transformed into other compounds. This transformation probably serves to avoid the toxic effect that this molecule has on the growth of *Trichoderma* species [25]. The effect of varying the concentration of sugarcane bagasse on the culture growth is shown in Fig. 2. It is obvious that increasing the level of sugarcane bagasse in SFF media gave rise to a significant ($P < 0.05$) increase in the biomass production. This finding may be correlated to the increase in the quantities of sugar and oil, in the solid substrate, that can be acted as a carbon and energy sources. Bonnarme et al. [16] demonstrated that oils and sugars promote biomass and 6-PP accumulation and at the same time the oil acts as a detoxifying agent against the toxicity effect of the high production of 6-PP.

3.3. Sensory evaluation

The effect of fermentation time on the characteristics of the perceived aroma and the total released volatiles produced by *T. viride* EMCC-107 in SSF on sugarcane bagasse are shown in Table 1. Strong coconut aroma was perceived after incubation for 5 d. The intensity of coconut aroma decreased after 7 d. After 9 d the odor was rather weak coconut/fruity. Whereas, after 12 d odor of the culture possessed slight sweet fruity aroma. It is obvious that, the aroma intensity was correlated with the yield of total volatiles detected by GC analysis. The effect of varying the concentration of sugarcane bagasse on the intensity of coconut aroma was investigated on the 5th d of fermentation that showed the optimum intensity of coconut aroma and highest yield of volatiles Table 1. As shown in Fig. 3, the optimum intensity of coconut aroma was perceived by using 4.5 g of sugarcane bagasse as solid substrate.

3.4. Analysis of aroma compounds

3.4.1. Effect of time of fermentation on aroma production

A total of six volatile compounds were identified in the volatiles extracted from the SSF culture of *T. viride* EMCC-107 during the incubation period (12 d). Table 2 shows the recovered amount of each compound as well as the description of its odor as reported in literatures. 6-PP, the potent aroma compound in coconut aroma, was the major compound after incubation for 5 d. It comprised more than 98% of all identified compounds as observed by GC–MS analysis. 6-PP is known as one of the major volatile compound biosynthesized by certain species of *Trichoderma* fungi which occurring commonly in soil

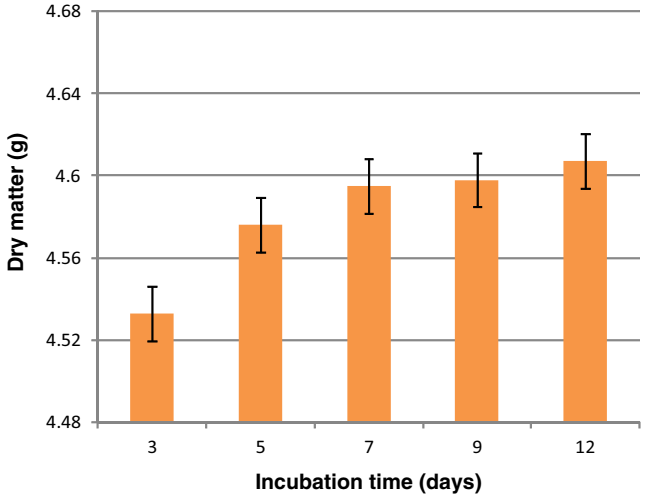

Fig. 1. Evolution of biomass during incubation period (12 d).

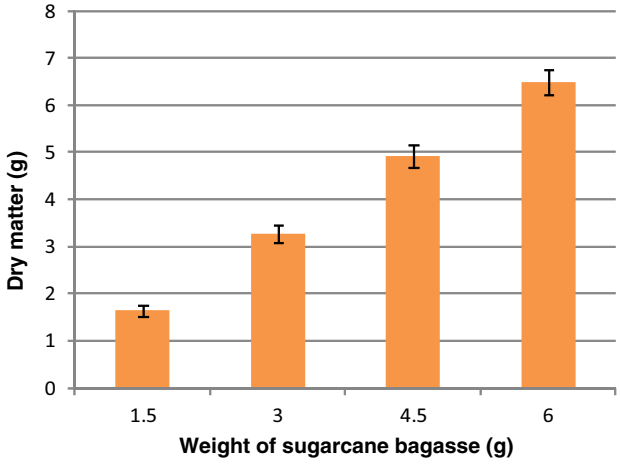

Fig. 2. Influence of sugarcane bagasse level on the biomass production.

Table 1
Effect of fermentation time on the aroma characteristics and production of total volatiles.

Fermentation time (d)	Aroma description	Aroma intensities	Total volatiles (mg)
3	Fermented note	++	–
5	Coconut aroma	++++	17.55[a]
7	Coconut aroma	+++	16.18[a]
9	Coconut/fruity	+	3.10[b]
12	Sweet/fruity	+	0.41[c]

+: weak, ++: medium, +++strong, ++++: very strong.
Experimental values in the same columns followed by different superscript letters are significant different ($P < 0.05$).

[17]. This compound was identified as the predominant compound with other volatile compounds in the aroma produced by *Trichoderma harzianum* in SSF [11,13] however; no study identified these other compounds. The amount of 6-PP recovered after 5 d of fermentation in the present study (3.62 mg/g DM) was higher than that reported in previous studies under the same SSF conditions. The maximum yield (2.8 mg/g DM) obtained by *T. harzianum* was achieved after 10 d of fermentation using sugarcane bagasse as support [26]. Less production of 6-PP was obtained by Ladeira et al. [27] and Penha et al. [11] (0.254 and 0.093 mg/g DM, respectively) using the same support and fermentation conditions, but with other strains of *T. harzianum*. High production of 6-PP was achieved after 5 d of fermentation (3.0 mg/g DM) by *Trichoderma* species 897 using sugarcane bagasse as support [13]. The authors estimated the recovered value of 6-PP to be equal to 940 mg/L of liquid solution adsorbed on the substrates and they reported that this value is higher than that recovered in liquid cultures. As shown in Table 2, the increase in incubation time revealed a significant decrease ($P < 0.05$) in the production of 6-PP. This finding was observed in liquid medium fermentation [28,29,30] and SSF [11,13]. It is possible that the high 6-PP concentration obtained in the present work had activated fungi metabolism to reduce this concentration as a defense mechanism trying to avoid its toxic effect [13,28] stated that after being produced, 6-PP was either adsorbed and or metabolized by fungus. The second option is more likely as 6-PP determination in the solid phase showed no traces of the molecule in fungal biomass. The authors suggested that 6-PP was transformed into other compounds as it had been observed for *Botrytis cinerea* cultures [25]. As shown in Table 2 the saturated lactones δ-octalactone, γ-nonlactone, γ-undecalactone, γ-dodecalactone and δ-dodecalactone were represented at low concentrations [16,31,32,33]. However, their presence confirmed the odor sensory analysis of the perceived aroma Table 1 during the fermentation time, particularly with the significant ($P < 0.05$) decrease of 6-PP at the end of the induction period.

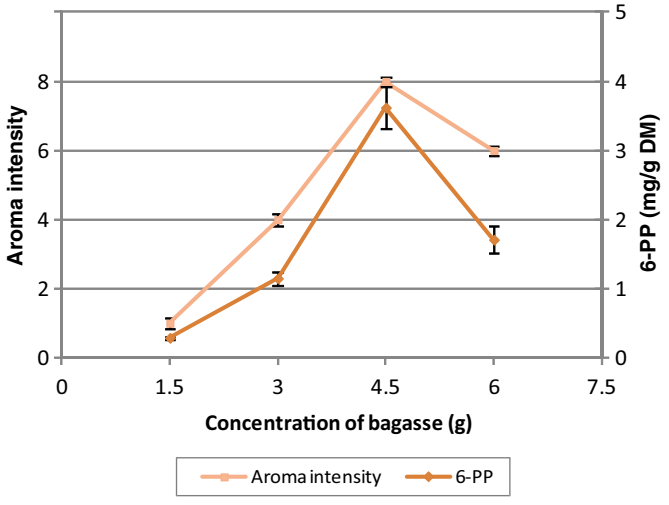

Fig. 3. Intensity of perceived coconut aroma and 6-PP production at different concentration of sugarcane bagasse.

Table 2
Volatile compounds produced by *T. viride* EMCC-107 grown on sugarcane bagasse during 12 d of fermentation.

Volatile compounds[b]	RI[a]	Time of fermentation (d)				Aroma description[d]
		5	7	9	12	
δ-Octalactone	1290	0.052[a]	0.013[b]	–	–	Coconut
γ-Nonlactone	1369	0.129[a]	0.323[b]	0.080[c]	–	Peach
6-Pentyl-α-pyrone	1513	3.622[a]	3.165[a]	0.400[b]	–	Coconut
γ-Undecalactone	1628	0.013[a]	0.011[a]	0.100[b]	0.062[c]	Peach
γ-Dodecalactone	1763	0.007[a]	0.006[a]	0.036[b]	–	Slight coconut/fruity
δ-Dodecalactone	1800	0.013[a]	0.004[b]	0.059[c]	0.026[d]	Sweet/fruity

Values are the average of triplicate analysis, mg/g dry matter of fermented substrate. Mean values in the same row followed by different superscript letters are significantly different ($P < 0.05$).
[a] Kovat indices.
[b] Compounds are listed according to their elution on DB5 column.
[d] Padolina et al. [31]; Bonnarme et al. [16]; Leffingwell and Alford [32]; Takeoka et al. [33].

3.4.2. Effect of sugarcane bagasse level on 6-PP production

Previous studies reported the sugarcane bagasse as the most potential solid substrate for the production of 6-PP by using *Trichoderma* species concentrations [11,14]. However, no study could be found concerning the effect of varying the level of sugarcane bagasse on the production of coconut aroma compounds, particularly 6-PP. As shown in Fig. 3 the increase in the amount of sugarcane bagasse revealed a gradual increase ($P < 0.05$) in 6-PP production up to 4.5 g (3.62 mg/g DM) followed by a significant ($P < 0.05$) decrease reaching 1.71 mg/g DM by using 6.0 g of sugarcane bagasse as solid substrate. The addition of sugarcane molasses, as carbon source, to citric pulp that used as solid substrate for production of fruity aroma by *Ceratocystis fimbriata* increased the production of the total volatiles [34]. Nevertheless, 15%, w/w of added sugarcane bagasse to citric pulp was found more effective than 25% w/w. The increase in 6-PP production may be correlated to the increase in the content of lipid and sugar that are the main constituents of sugarcane bagasse in the present study. Consistent 6-PP levels were found to be produced from glucose as well as from fatty acids (hydroxylated or non-hydroxylated) by *T. viride* TSP_2 [16]. At the same time, oil acts as a detoxifying agent against the toxicity effect of the higher production of 6-PP. As elucidated early by Vick and Zimmerman [35] 6-PP biosynthesis in *Trichoderma* species could have a first step that depends on the formation of 13-hydroperoxide from linoleic acid. The lipoxygenase reaction is followed by β-oxidation and isomerization to form 5-hydroxy-2, 4-decenoic acid. Afterwards, internal lactonization on the C_5 hydroxyl group with the carboxylic group of the same molecule gives rise to 6-PP production. The significant decrease in 6-PP production may be correlated to the fact that the high concentration of 6-PP may activate the fungi metabolism to reduce this concentration as a defense metabolism to avoid its toxic effect [11,13,28]. On the other hand 6-PP may be transformed into other compounds as mentioned before.

4. Conclusion

The production of 6-PP by *T. viride* EMCC-107 in SSF on sugarcane bagasse after 5 d of fermentation was higher (3.62 mg) than that reported in previous studies using the same substrate and fermentation conditions. The identification of the saturated γ- and δ-saturated lactones accompanied with 6-PP production confirmed the changes in aroma profile during the induction period (12 d). Variation in the level of sugarcane bagasse influenced the volatile production and culture growth. The positive correlation between the level of sugarcane bagasse and biomass production was attributed to the total sugar content that acts as carbon and energy source. In general using sugarcane bagasse as solid substrate in SSF for high 6-PP production by *T. viride* EMCC-107 has an economic and environmental benefits.

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References

[1] US Food and Drug Administration. Code of federal regulations 21CFR101.22; 2001.
[2] Janssens L, de Pooter HL, Demey L, Vandamme EJ, Schamp NM. Fruity flavours by fermentation. Med Fac Landbouww Rijksuniv Gent 1988;53:2071–7.
[3] Janssens L, De Pooter HL, Schamp NM, Vandamme EJ. Production of flavours by microorganisms. Process Biochem 1992;27:195–215. http://dx.doi.org/10.1016/0032-9592(92)80020-4.
[4] Jiang JC. Changes in volatile composition of *Kluyveromyces lactis* broth during fermentation. In: Charalambous G, editor. Food flavours, generation analysis and process influence. New York: Elsevier Science B.V; 1995. p. 1073–86.
[5] Berger RG. Aroma biotechnology. New York: Springer, Berlin Heidelberg; 1995.
[6] Christen P, Meza JC, Revah S. Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: Influence of The substrate type and the presence of precursors. Mycol Res 1997;101:911–9. http://dx.doi.org/10.1017/S0953756297003535.
[7] Bramorski A, Christen P, Ramirez M, Soccol CR, Revah S. Production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid state cultivation on tropical agro-industrial substrates. Biotechnol Lett 1998;20:359–62. http://dx.doi.org/10.1023/A:1005323229125.
[8] Soares M, Christen P, Pandey A, Soccol CR. Fruity flavor production by *Ceratocystis fimbriata* grown on coffee husk in solid-state fermentation. Process Biochem 2000; 35:857–61. http://dx.doi.org/10.1016/S0032-9592(99)00144-2.
[9] Zhang R, Wu O, Xu Y. Aroma characteristics of Moutai-flavour liquor produced with *Bacillus licheniformis* by solid-state fermentation. Lett Appl Microbiol 2013;57:11–8. http://dx.doi.org/10.1111/lam.12087.
[10] Pandy A, Soccol CR, Nigam P, Soccol VT. Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse. Bioresour Technol 2000;74:69–80. http://dx.doi.org/10.1016/S0960-8524(99)00142-X.
[11] Penha MP, Rocha-Leao MHM, Leite SGF. Sugarcane bagasse as support for the production of coconut aroma by solid state fermentation (SSF). Bioresources 2012; 7:2366–75. http://dx.doi.org/10.15376/biores.7.2.2366-2375.
[12] Longo MA, Sanromán MN. Production of food aroma compounds: Microbial and enzymatic methodologies. Food Technol Biotechnol 2006;44:335–53.
[13] Ramos AS, Fiaux SB, Leite SGF. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* in solid-state fermentation. Braz J Microbiol 2008;39:712–7. http://dx.doi.org/10.1590/S1517-83822008000400022.
[14] De Araujo AA, Pastore GM, Berger RG. Production of coconut aroma by fungi cultivation in solid-state fermentation. Appl Biochem Biotechnol 2002;98–100:747–51. http://dx.doi.org/10.1385/ABAB:98-100:1-9:747.
[15] Serrano-Carreón L, Flores C, Rodriguez B, Galindo E. *Rhizoctonia solani*, an elicitor of 6-pentyl-α-pyrone production by *Trichoderma harzianum* in a two liquid phases, extractive fermentation system. Biotechnol Lett 2004;26:1403–6. http://dx.doi.org/10.1023/B:BILE.0000045640.71840.b5.
[16] Bonnarme P, Djian A, Latrasse A, Féron G, Giniès C, Durand A, et al. Production of 6-pentyl-α-pyrone by *Trichoderma* sp. from vegetable oils. J Biotechnol 1997;56: 143–50. http://dx.doi.org/10.1016/S0168-1656(97)00108-9.
[17] Collins RP, Halim AF. Characterization of the major aroma constituent of the fungus *Trichoderma viride*. J Agric Food Chem 1972;20:437–8. http://dx.doi.org/10.1021/jf60180a010.
[18] El-Tayeb TS, Abdelhafez AA, Ali SH, Ramadan EM. Effect of acid hydrolysis and fungal biotreatment on agro-industrial wastes for obtainment of free sugars from bioethanol production. Braz J Microbiol 2012:1523–35. http://dx.doi.org/10.1590/S1517-83822012000400037.
[19] AOAC. Official methods of analysis of the Association of Official Analytical Chemists. In: Horwitz W, editor. 2000 [Publisher].
[20] AOAC. Official methods of analysis. In: Horwitz W, editor. Association of official analytical chemists, 16th ed. 1996 [Publisher].
[21] Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. USA: Allured Publishing Corporation; 1995.
[22] Statgraptics. Statistical graphics system by statistical graphics corporation. Reference manual, version 7 for DOS. Rockville Maryland, USA: Manugistics Inc.; 1993.
[23] Christen P, Bramorski A, Revah S, Soccol CR. Characterization of volatile compounds produced by *Rhizopus* strains grown on agro-industrial solid wastes. Bioresour Technol 2000;71:211–5. http://dx.doi.org/10.1016/S0960-8524(99)00084-X.
[24] Serrano-Carreón L, Hathout Y, Bensoussan M, Belin JM. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* from 18: n fatty acid methyl esters. Biotechnol Lett 1992;14:1019–24. http://dx.doi.org/10.1007/BF01021051.
[25] Poole PR, Whitaker G. Biotransformation of 6-pentyl-2-pyrone by *Botrytis cinerea* in liquid cultures. J Agric Food Chem 1997;45:249–52. http://dx.doi.org/10.1021/jf9603644.
[26] Sarhy-Bagnon V, Lozano P, Saucedo-Castañeda G, Roussos S. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* in liquid and solid state cultures. Process Biochem 2000;36:103–9. http://dx.doi.org/10.1016/S0032-9592(00)00184-9.
[27] Ladeira NC, Peixoto VJ, Penha MP, Barros EBP, Leite SGF. Optimization of 6-pentyl-α-pyrone production by solid state fermentation using sugarcane bagasse as residue. Bioresources 2010;5:2297–306.
[28] Galindo E, Flores C, Larralde-Corona P, Corkidi-Blanco G, Rocha-Valadez JA, Serrano-Carreón L. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* cultured in unbaffled and baffled shake flasks. Biochem Eng J 2004;18:1–8. http://dx.doi.org/10.1016/S1369-703X(03)00115-3.
[29] Kalyani A, Prapulla SG, Karanth NG. Study on the production of 6-pentyl-α-pyrone using two methods of fermentation. Appl Microbiol Biotechnol 2000;53:610–2. http://dx.doi.org/10.1007/s002530051665.
[30] Rocha-Valadez JA, Estrada M, Galindo E, Serrano-Carreón L. From shake flasks to stirred fermentors: Scale-up of an extractive fermentation process for 6-pentyl-α-pyrone production by *Trichoderma harzianum* using volumetric power input. Process Biochem 2006;41:1347–52. http://dx.doi.org/10.1016/j.procbio.2006.01.013.
[31] Padolina WG, Lucas LZ, Torres LG. Chemical and physical properties of coconut oil. Philipp J Coconut Stud 1987;12:4–17.
[32] Leffingwell JC, Alford ED. Volatile constituents of perique tobacco. Electron J Environ Agric Food Chem 2005;4:899–915.
[33] Takeoka GR, Flath RA, Mon TR, Teranishi R, Guentert M. Volatile constituents of apricot (*Prunus armeniaca* L.). J Agric Food Chem 1990;38:471–7. http://dx.doi.org/10.1021/jf00092a031.
[34] Rossi SC, Vanderberghe LPS, Pereira BMP, Gago FD, Rizzolo JA, Pandey A, et al. Improving fruity aroma production by fungi in SSF using citric pulp. Food Res Int 2009;42:484–6. http://dx.doi.org/10.1016/j.foodres.2009.01.016.
[35] Vick BA, Zimmerman DC. The biosynthesis of jasmonic acid: A physiological role for plant lipoxygenase. Biochem Biophys Res Commun 1983;111:470–7. http://dx.doi.org/10.1016/0006-291X(83)90330-3.