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ESSENTIAL OIL PRODUCED BY *CHRYSOSPORIUM XEROPHILUM* IN COCONUT

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Key Word Index—*Cocos nucifera*; Palmae; *Chrysosporium xerophilum*; fungus; substrate conversion coconut; methyl ketones; 2-heptyl esters; detoxification.

Abstract—An essential oil (0.3% v/w) was produced after fermentation of coconut for nine months by *Chrysosporium xerophilum*. Sixty per cent of the volatiles were aliphatic methyl ketones (C_5–C_{13}), esters and secondary alcohols whilst 38% were present as free medium chain length fatty acids (C_6–C_{12}). A new class of ester, 2-heptyl esters of C_8, C_{10} and C_{12} fatty acids, was identified by GC-MS and confirmed by synthesis. It is suggested that ester formation, ketone formation and alkane synthesis are mechanisms for removing medium chain fatty acids (C_6–C_{12}) which, if allowed to accumulate in the substrate, would be toxic to the fungus.

INTRODUCTION

Very often mycotoxins and volatile fungal metabolites are formed in stored oilseeds and cereals after fungal colonisation has taken place [1, 2]. Terms such as fruity, woody, earthy, mushroom or rose-like have been used to describe the odour of individual volatile compounds produced [3]. Many volatile metabolites are physiologically active and affect the growth of other organisms [4, 5]. Others may be produced in order to modify a particular functional group [6]. Reactions of this type suggest ways in which fungi can maintain homeostatic balance in the environment.

An unusual odour was reported in a solid-state fermentation of desiccated coconut with *Chrysosporium xerophilum* Pitt at low water activity and oxygen tension [7]. The aim of the present study was to identify the volatile metabolites produced after fermentation for nine months under storage conditions.

RESULTS AND DISCUSSION

The volatile compounds which were extracted and identified after fermentation with *C. xerophilum* are given in Table 1. The fatty acid composition of coconut oil is unusual in that 60% of the constituent fatty acids are medium chain length C_6–C_{14} [8]. There is also evidence that such acids are fungicidal [9, 10]. Conversion of the acids into methyl ketones one carbon atom shorter effectively removes the acid. Once formed the methyl ketone can act as a terminal hydrogen acceptor and undergo reduction to give the corresponding secondary alcohol when oxygen is restricted [11].

Most of the compounds listed in Table 1 are derived from fatty acids of intermediate chain length. Sixty per cent of the volatiles are present as methyl ketones, esters and secondary alcohols and 38% are present as free acids. A new class of ester, 2-heptyl esters of octanoic, decanoic and dodecanoic acids has been identified by GC-MS, where 2-heptyl octanoate comprises 7% of the total volatile compounds. It would appear that excess 2-heptanol produced by reduction of 2-heptanone during fermentation has been used to esterify the free fatty acids.

Considering the novelty of these esters it was felt prudent to synthesise them in order to check the identities of the natural products against authentic material. The esters were prepared by a standard method in which 2-heptanol was treated with the appropriate carboxylic acid under acidic conditions. The 2-heptyl esters thus prepared were checked for purity by TLC and gave satisfactory IR, NMR and mass spectra. The synthetic esters were shown, within experimental error, to have the same GC R_t values and mass spectra as the corresponding substances from the essential oil.

The 2-heptyl esters have an odour reminiscent of the lower boiling point fraction of a petroleum distillate. To our knowledge this is the first report of a fungal esterification of a fatty acid with 2-heptanol. 1-Octen-3-ol, 'Mushroom alcohol', has been isolated from filamentous fungi [12] and edible mushrooms [13–15]. This compound is described as the major component of a mushroom-like odour [15]. Oct-1-en-3-yl esters of formic, acetic and propionic acids have also been isolated from the edible mushroom *Agaricus bisporus* as well as other species of edible mushrooms [15]. Unlike the 2-heptyl esters which are reported in this study at concentrations of *ca* 300 mg/kg, the oct-1-en-3-yl esters were found at concentrations less than 1 mg/kg [15].

Our data shows that three different types of reaction have occurred in order to inactivate the carboxyl group of the aliphatic fatty acids. First, esterification has occurred with alcohols such as ethanol, pentanol, 3-methyl-1-butanol, heptanol, hexanol and 2-heptanol which are

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Table 1. Volatile compounds isolated from coconut after a nine month anaerobic fermentation with three different isolates of *Chrysosporium xerophilum*

		RR_t	Area (%)	Area (%) of individual classes
1. Alkanes				
Undecane		0.15	0.1	0.1
2. Alkenes				
1-Nonene		0.01	0.1	
1-Decene		0.11	0.1	0.1
3a. Primary alcohols:				
Ethanol		0.08	tr	
3-Methyl-1-butanol		0.33	0.1	0.3
Heptanol		0.72	0.2	
Nonanol		1.07	0.1	
3b. Secondary alcohols				
2-Pentanol		0.21	0.2	
2-Hexanol		0.38	0.1	
2-Heptanol		0.48	8.3	
2-Octanol		0.65	0.3	
2-Nonanol		0.84	4.3	
2-Undecanol		1.21	3.4	
2-Tridecanol		1.37	0.5	17.5
4. Esters				
Heptyl acetate		0.59	0.1	
Nonyl acetate		0.96	0.6	
Heptyl octanoate		1.47	0.1	
Ethyl hexanoate		0.41	tr	
Ethyl octanoate		0.70	1.0	
Ethyl decanoate		1.04	tr	
Ethyl dodecanoate		1.44	2.3	
Ethyl tetradecanoate		1.83	0.3	
3-Methylbutyl octanoate		1.09	0.6	
Pentyl decanoate		1.52	0.1	
Pentyl dodecanoate		1.89	0.5	5.6
5. Esters of secondary alcohols				
2-Hexyl octanoate		1.15	0.2	
2-Heptyl octanoate		1.34	7.3	
2-Heptyl decanoate		1.67	0.2	
2-Heptyl dodecanoate		1.99	2.4	12.0
6. Alkanones (methyl ketones)				
2-Heptanone		0.29	3.5	
2-Octanone		0.44	0.3	
2-Nonanone		0.84	8.5	
2-Undecanone		1.00	11.5	
2-Tridecanone		1.37	1.6	25.3
7. Alkanoic acids (carboxylic acids)				
Octanoic		1.77	13.4	
Decanoic		2.11	5.9	
Dodecanoic		2.44	18.5	37.7
8. Summary:				
Derivatised acids:				
Esters	(5)		12.0	
	(4)		5.6	
Methyl ketones	(6)		25.3	
Sec. alcohols	(3b)		17.1	
			60.0%	
Free fatty acids	(7)		37.7%	

Retention times (RR_t) are relative to 2-undecanone.

tr = Trace.

Fermentations were carried out at 25° at an initial a_w of 0.90. Results are expressed as % composition of oil.

produced during metabolism. The formation of simple esters by fungi may be a mechanism for removing both acids and alcohols, which, if allowed to accumulate in the medium would be toxic to the fungus [6]. In fact, Nandi demonstrated that esters had relatively little effect on the rate of linear growth of some storage fungi [16]. Secondly, decarboxylation (partial β-oxidation) of fatty acids of intermediate chain length gives methyl ketones which are one carbon atom shorter than the parent fatty acid. Lastly, carboxylic functions are inactivated by the production of alkanes and alkenes. These hydrocarbons could be derived from unsaturated fatty acids such as oleic and linoleic acids.

There is evidence that fatty acids are transported into the fungal hyphae in the undissociated form [17]. Reactions which derivatise the carboxyl group will inhibit transport into the cell and thus prevent any fungicidal or toxic effects these acids may have had. Ketone production, ester formation and alkane synthesis represent substrate conversion of fatty acids rather than *de novo* synthesis of volatile fungal metabolites.

EXPERIMENTAL

Fungal isolates. Two isolates of *C. xerophilum* (FRR 3162, 3317) were isolated from Sri Lankan coconut [7] and a third isolate (FRR 530–the type culture) was a gift from Dr John Pitt. All isolates were maintained on malt yeast 50% glucose (My50G) [18]. Spore suspensions were prepared by the methods of ref. [18].

Solid state fermentation of coconut. Desiccated coconut (medium grade) ex Sri Lanka was obtained as a gift from Geo. Bassett & Co. Ltd, Sheffield. The coconut was sterilized by gamma irradiation (10 kGy) using a ^{60}Co source. Coconut (100 g) was put into sterile 500 ml Kilner jars to which 5 ml of spore suspension and 5 ml H_2O was added to give an initial a_w of 0.89. The jars were sealed with neoprene rings and well shaken prior to incubation at 25° for nine months in the dark. Duplicate fermentations were performed for each fungal isolate.

Extraction of essential oils. Fermented coconut (100 g) was dist. for 4 hr with H_2O (250 ml) in an essential oil still (designed by Mr Martin Humphrey, Bush Boake & Allen). The oil was stored at 4° under N_2 gas prior to analysis.

GC analysis. Essential oil was analysed using the conditions described in ref. [8]. GC/MS analyses of the oils were made using a 50 m FSOT FFAP column. The temp. was prog. between 60 and 200° at 3° C/min. Analyses were confirmed by co-chromatography with known standards [8]. RR_t values were determined relative to 2-undecanone.

Preparation and analysis of 2-heptyl esters. To 2-heptanol (3.65 g, 31 mmol) and the carboxylic acid (62 mmol) was added 0.1 g of concd H_2SO_4. The mix. was heated at 120° for 3 hr. 2-Heptyl octanoate: The cooled reaction mixt. was added to satd $NaHCO_3$ soln (50 ml) and the ester extracted with 40/60° petrol (100 ml). The organic fraction was washed with $NaHCO_3$ soln (2 × 50 ml), then dried ($MgSO_4$), rotary evapd and purified. 2-Heptyl decanoate: The cooled reaction mixt. was filtered to remove solid decanoic acid then purified. 2-Heptyl dodecanoate: The cooled reaction mixt. was purified directly. All esters were purified by CC on silica gel, using petrol as eluant. Purity was checked by the occurrence of only one spot for each ester after TLC (silica gel/petrol). Average yield: 50%.

2-Heptyl octanoate. IR: 1730 cm^{-1} (COOR'); ^1H NMR (60 MHz, $CDCl_3$): δ 1.2 (*m*, 27H), 2.3 (*t*, 2H, CH_2COOR'), 4.9 (*m*, 1H, CO_2CH); EIMS (GC/MS) 70 eV: *m/z* 242 ($[M]^+$, 0.2%), 227 ($[M-Me]^+$, 0.5), 145 ($[C_7H_{15}COOH_2]^+$, 28), 144 ($[C_7H_{15}COOH]^+$, 18), 127 ($[C_7H_{15}CO]^+$, 100), 98 ($[M-RCOOH]^+$, 47), 57 ($[C_4H_9]^+$, 82).

2-Heptyl decanoate. IR: 1730 cm^{-1} (COOR'); ^1H NMR (60 MHz, $CDCl_3$): δ 1.2 (*m*, 31H), 2.3 (*t*, 2H, CH_2COOR'), 4.9 (*m*, 1H, COOCH); EIMS (GC-MS) 70 eV: *m/z* 255 ($[M-Me]^+$, 0.1%), 173 ($[C_9H_{19}COOH_2]^+$, 39), 172 ($[C_9H_{19}COOH]^+$, 34), 155 ($[C_9H_{19}CO]^+$, 100), 98 ($[M-RCOOH]^+$, 83%), 57 ($[C_4H_9]^+$, 94).

2-Heptyl dodecanoate. IR: 1725 cm^{-1} (COOR'); ^1H NMR (60 MHz, $CDCl_3$): δ 1.2 (*m*, 35H), 2.3 (*t*, 2H, CH_2COOR'), 4.8 (*m*, 1H, COOCH); EIMS (GC-MS) 70 eV: *m/z* 283 ($[M-Me]^+$, 0.04%), 201 ($[C_{11}H_{23}COOH_2]^+$, 44), 200 ($[C_{11}H_{23}COOH]^+$, 45), 183 ($[C_{11}H_{23}CO]^+$, 100), 98 ($[M-RCOOH]^+$, 81), 57 ($[C_4H_9]^+$, 85).

GC-MS analysis on a 25 m FSOT SE-30 column (50–230° at 20°/min), showed that each of the three esters matched, in R_t and MS, each of the three products in the essential oil.

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