Phytochemistry, Vol. 23, No. 12, pp. 2847–2849, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

KETONIC RANCIDITY IN COCONUT DUE TO XEROPHILIC FUNGI

JUDITH L. KINDERLERER and BRIAN KELLARD*

Department of Hotel & Catering, Sheffield City Polytechnic, Pond Street, Sheffield S1 1WB, U.K.

(*Revised received* 25 *May* 1984)

Key Word Index—*Cocos nucifera*; Palmae; coconut; *Eurotium*; *Penicillium citrinum*; ketonic rancidity; aliphatic methylketones; *sec*-alcohols.

Abstract—A type of deterioration called ketonic rancidity occurred in coconut after inoculation with four xerophilic fungi, *Eurotium amstelodami*, *E. chevalieri*, *E. herbariorum* and *Penicillium citrinum*. The fungi were incubated at low water activity and oxygen tension. A homologous series of aliphatic methyl ketones and secondary alcohols C_5–C_{11} were isolated and identified in the rancid samples after fungal growth. Evidence is presented that odd numbered methyl ketones (C_5–C_{11}) are derived from even numbered short chain fatty acids with one more carbon atoms than the ketones via a modified β-oxidation of the parent fatty acid. Heptan-2-one and heptan-2-ol are the main reaction products except in the case of *E. herbariorum* where even numbered hexan-2-one, hexan-2-ol, octan-2-one and octan-2-ol were produced. Other moulds grown on coconut under similar conditions—*Aspergillus flavus* Link and *Chrysosporium farinicola* (Burnside) Skou (*C. fastidium* Pitt)—did not cause ketonic deterioration.

INTRODUCTION

Ketonic rancidity is a type of deterioration which occurs in some oilseeds such as coconut, *Cocos nucifera* L., palm kernel oil, *Elaeis guineeusis* Jacq, and milk fat [1]. It is due to the conversion of short chain fatty acids by some moulds to a homologous series of aliphatic methyl ketones, pentan-2-one, heptan-2-one, nonan-2-one and undecan-2-one—one carbon atom less than the parent fatty acid [2]. Ketonic rancidity is sometimes called 'perfume rancidity' [3].

In a previous investigation [4] four xerophilic moulds, *Eurotium amstelodami* mangin, *E. chevalieri* mangin, *E. herbariorum* (Wiggers) Link ex Gray, and *Penicillium citrinum* Thom were isolated from rancid coconut. The aim of this investigation was to examine the production of methyl ketones in coconut after inoculation with the fungi listed above and stored under conditions of low water activity (a_w = 0.76) and oxygen tension.

RESULTS AND DISCUSSION

Figure 1 gives percent concentration (M) for methyl ketones and secondary alcohols produced after 30 days fermentation on coconut. In figures 1.1, 1.2 and 1.4 for *E. amstelodami*, *E. chevalieri* and *P. citrinum* heptan-2-one and heptan-2-ol are the main reaction products with smaller amounts of the 5, 9 and 11-carbon aliphatic methyl ketones and corresponding secondary alcohols. *E. chevalieri* differs from *E. amstelodami* in that heptan-2-ol is the main reaction product rather than heptan-2-one. In Fig. 1.3 for *Eurotium herbariorum*, octan-2-one and octan-2-ol, hexan-2-one and hexan-2-ol (even numbered homologues) are produced as well as the C_5, C_7, C_9 and C_{11} compounds. The differences in the alcohols and ketones produced by the three members of the genus *Eurotium* may allow the development of a chemical taxonomy for this genus, rather than the morphological taxonomy in current use [5]. When the unsterilized coconut was fermented under similar conditions (Fig. 1.5), a pattern of

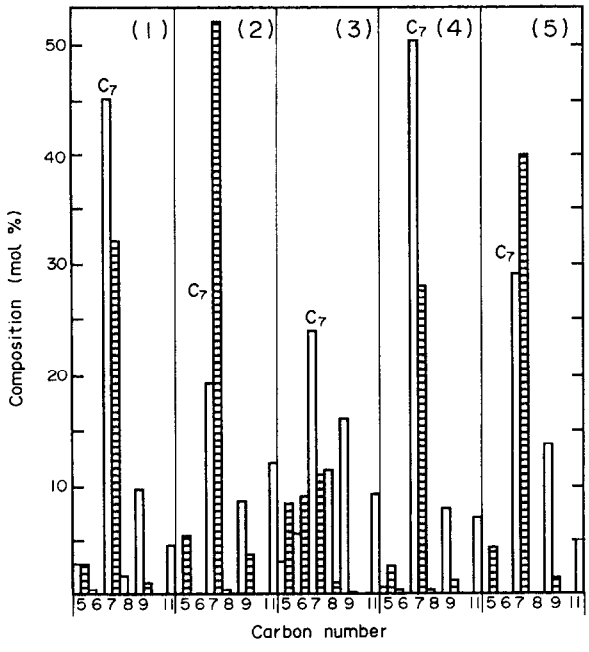

Fig. 1. Percentage composition (M) reaction products after fermentation on coconut. Key: 1.1, after 30 days with *E. amstelodami*; 1.2, after 30 days with *E. chevalieri*; 1.3, after 30 days with *E. herbariorum*; 1.4, after 30 days with *P. citrinum*; 1.5, after 12 weeks on unsterilized coconut. All fermentation at ambient temperature, a_w 0.76 and low oxygen tension. Open squares 2-aliphatic ketones, cross-hatched 2-aliphatic alcohols.

*Present address: Department of Chemistry, The University, Sheffield S3 7HF, U.K.

methyl ketones and secondary alcohols similar to *E. chevalieri* was produced. This mould was found to be the most common of the *Eurotium* species present in the original coconut.

In a previous investigation by Ward and Diener [6] in which peanuts were inoculated with *E. chevalieri*, *E. herbariorum* and *P. citrinum*, no rancidity was detected after two months storage but the authors observed a considerable increase in free fatty acids. Coconut oil, like palm kernel oil, is unusual in that there is a considerable proportion of hexanoic, octanoic, decanoic and dodecanoic acids present in the triglycerides [7]. In peanut oil, on the other hand, these acids are absent and the oil contains exclusively longer chain fatty acids [8]. The short chain fatty acids are toxic [9] and have been found to inhibit growth and oxidative phosphorylation [9, 10]. It has been stressed that it is only the undissociated acid which inhibits oxygen uptake when short chain fatty acids are the sole carbon source [11].

Methyl ketones and secondary alcohols with an odd number of carbon atoms can be derived from an even chain fatty acid by decarboxylation in a modification of the β-oxidative pathway of fatty acid oxidation [12]. A thiokinase exists in some organisms which is specific for medium chain fatty acids (four to 12 carbons) [13]. This enzyme, unlike that for longer chain fatty acids is not found in the mitochondrion where fatty acid oxidation normally occurs, but in the peroxisomes and is probably not directly involved in the complete oxidation of the fatty acids. The same is probably true for the β-oxoacyl deacylase at the branch point [12]. Similar enzymes in these moulds could shunt the acyl-CoA derivatives of the short chain fatty acids into methyl ketone formation. NADH produced by reduction of β-hydroxyacyl CoA may be used to reduce the methyl ketones to secondary alcohols.

Anaerobic conditions such as these have been shown to favour the production of alcohols rather than ketones [14]. In β-oxidation of long chain fatty acyl-CoA derivatives intermediates are not observed [15]. These moulds accumulate methyl ketones and secondary alcohols when oxidizing short chain fatty acids under these environmental conditions, although once again, the fatty acyl CoA derivatives are not observed. The complete scheme for fatty acid oxidation appears not to be used. It has been suggested that ketone production is either (a) a detoxification mechanism to inactivate the toxic fatty acids [16], or (b) a means of recycling coenzyme A [12].

In Table 1 the molecular ratio of product to substrate (mmoles per 100 g original oil of each) is given. In almost all cases the ratio is 1 or less suggesting that toxic fatty acids are converted to the methyl ketones and alcohols without going through a complete oxidation cycle. *E. herbariorum* is different as it appears to metabolise both odd and even numbered fatty acids. The mould produces 6- and 8-carbon ketones and alcohols—hexan-2-ol, hexan-2-one, octan-2-ol and octan-2-one. Their synthesis cannot be due to α-oxidation because these are the 2-derivatives. Trace amounts of hexan-1-ol and heptan-1-ol are observed, perhaps resulting from the reduction of the corresponding aldehydes.

Not all fungi have the ability to synthesize methyl ketones and alcohols from the corresponding short chain fatty acids. *Oidium lactis*, for example, did not produce methyl ketones whereas *Penicillium palitans* did when grown under the same conditions as above on coconut oil [16]. *Aspergillus flavus* and *Chrysosporium farinicola* fail to produce methyl ketones when fermented in the same way. A number of workers have observed methyl ketone production by *P. roqueforti* in either blue cheese [17] or from a parent fatty acid with one carbon atom more than the resulting ketone [12].

The main work on the biodeterioration of oilseeds has been on lipolysis [18]. The presence of methyl ketones and alcohols producing ketonic rancidity in certain oilseeds has been ignored. Ketonic rancidity occurs when some moulds (mainly xerophiles) are grown on a substrate containing short chain fatty acids and leads to the production of ketones and secondary alcohols containing one carbon atom less than the parent fatty acid.

Table 1. Oxidation products (2-ketones + 2-alcohols mM) produced from 100 g coconut oil (products one carbon atom less than the original fatty acid)

Substrate fatty acid	*mmol substrate/100 g oil (a)	*E. amstelodami*† mmol/100 g oil (b)	b/a	*E. chevalieri*† mmol/100 g oil (b′)	b′/a	*E. herbariorum*‡ mmol/100 g oil (b″)	b″/a	*P. citrium*‡ mmol/100 g oil (b‴)	b‴/a
Hexanoic (C_6)	6.89	3.96	0.58	1.44	0.21	15.21	2.21	2.16	0.31
(C_7)						23.10			
Octanoic (C_8)	37.44	64.30	1.72	22.65	0.60	40.08	1.07	34.50	0.92
(C_9)		1.50		0.19		16.99		0.34	
Decanoic (C_{10})	48.76	9.66	0.20	3.27	0.07	24.50	0.52	4.06	0.08
(C_{11})									
Dodecanoic (C_{12})	226.63	3.51	0.02	3.49	0.02	11.00	0.05	2.99	0.01

*Data derived from ref. [8].

†Arithmetic mean of three fermentations.

‡Arithmetic mean of two fermentations.

All fermentations were incubated at ambient temp. for 30 days at a_w 0.76, low oxygen tension and pH 6.0–6.2.

In summary, the essential prerequisites for the formation of ketonic rancidity appear to be (a) the presence of appropriate moulds; (b) a low water activity; (c) a low partial pressure of oxygen; and (d) the presence of short chain fatty acids in the medium.

EXPERIMENTAL

Materials. Desiccated coconut ex Sri Lanka, obtained as a gift from Geo. Bassetts Ltd., Sheffield, was used throughout.

Fermentation. Coconut (100 g containing 65 g oil) at a_w 0.76 and pH 6.0–6.2 was inoculated with *Eurotium amstelodami* CMI 275747, *Eurotium chevalieri* CMI 279683, *Eurotium herbariorum* CMI 279684, *Penicillium citrinum* CMI 276863, *Aspergillus flavus* CMI 277248 and CMI 275104 and *Chrysosporium farinicola* CBS 625.83. The fermentations were incubated at ambient temperature for 30 days at low oxygen tension using the method described in ref. [4]. Experiments were performed in duplicate or triplicate.

Extraction of methyl ketones and alcohols. Coconut (100 g) was extracted with dry Et_2O (200 ml × 4) by soaking the coconut with solvent for 30 min, filtering (Whatman No. 1 filter paper) and evaporating the combined filtrates to dryness under red. pres. The oil was weighed (about 43 g), undecan-6-one added as an internal standard (0.8–1.0 g), and vacuum distilled (0.2 mm Hg at 70°) for 30 min onto a cold finger filled with liquid N_2 [1]. The distillate was washed off with Et_2O, dried in N_2 and stored at 4°.

GC. The column (1.5 m × 4 mm) was packed with 10% (w/w) Carbowax 20 M on acid washed Chromosorb W. N_2 gas was used as the carrier (40 ml/min) with the temperature at the injector point of 250°. The column was programmed from 70° to 165° at 3° per min. The FID detector was at 220°. R_ts relative to undecan-6-one were determined and concns were obtained with reference to standard mixtures of homologous methyl ketones and secondary alcohols. Unknown compounds were identified by co-chromatography and combined GC/MS.

GC/MS. A Kratos MS—25 instrument linked to a Kratos DS—55 data system was used under similar conditions to those desribed above. The carrier gas was He at 30 ml/min. The jet separator was at 240°, the ionization current was 100 μA, source temperature 200°, accelerating voltage 2.0 kV, resolution 600 and scan speed 1 sec/decade.

Acknowledgements—We would like to thank Bruce Middleton (Department of Biochemistry, Nottingham University) for discussions of fatty acid metabolism, Peter Ashton and Ian Johnstone (Department of Chemistry, Sheffield University) for running mass spectra, Deirdre Wilson for technical assistance and David Busfield (Viota Foods) for his support. B. K. would also like to thank Sheffield L.E.A. and Viota Foods for financial support.

REFERENCES

1. Forss, D. A. (1973) *Prog. Chem. Fats Other Lipids* **13**, 177.
2. Forney, F. W. and Markovetz, A. J. (1971) *J. Lipid Res.* **12**, 283.
3. Pearson, D. (1976) *The Chemical Analysis of Foods*, p. 496. Churchill Livingston, London.
4. Kinderlerer, J. L. (1984) *Food Microbiol.* **1**, 23.
5. Raper, K. B. and Fennell, D. I. (1965) *The Genus Aspergillus.* Williams & Wilkins, Baltimore.
6. Ward, H. S. and Diener, U. L. (1961) *Phytopathology* **51**, 244.
7. Young, F. V. K. (1983) *J. Am. Oil Chem. Soc.* **60**, 374.
8. Sober, H. A. (1968) *The Handbook of Biochemistry.* CRC Cleveland, E-20.
9. Teh, J. S. (1974) *Appl. Microbiol.* **28**, 840.
10. Lawrence, R. C. (1966) *J. Gen. Microbiol.* **44**, 393.
11. Rolinson, G. N. (1954) *Appl. Bacteriol.* **17**, 190.
12. Lawrence, R. C. and Hawke, J. C. (1968) *J. Gen. Microbiol.* **51**, 289.
13. Gurr, M. I. and James, A. T. (1980) *Lipid Biochemistry: An Introduction*, p. 75. Chapman & Hall, London.
14. Franke, W., Platzech, A. and Eichhorn, G. (1962) *Arch. Mikrobiol.* **41**, 154.
15. Greville, G. D. and Tubbs, P. K. (1968) *Essays Biochem.* **4**, 155.
16. Stokoe, W. M. (1928) *Biochem. J.* **22**, 80.
17. King, R. D. and Clegg, G. H. (1979) *J. Sci. Food Agric.* **30**, 197.
18. Eggins, H. O. W. and Coursey, D. G. (1968) *Int. Biodeterioration Bull.* **4**, 29.