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Volatile Off-flavour Compounds in Desiccated Coconut

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Aliphatic methyl ketones and secondary alcohols have been isolated from desiccated coconut and shown to be responsible for the rancid off-flavour. Two series of compounds have been isolated. The series with an odd number of carbon atoms ($C_{5,7,9,11}$) comprising pentan-2-one, pentan-2-ol, heptan-2-one, heptan-2-ol, nonan-2-one, nonan-2-ol and undecan-2-one is derived from even chain fatty acids one carbon atom longer, present in the coconut oil by a modified β-oxidation due to growth of moulds. Heptan-2-one and nonan-2-one were present in the greatest concentration in the rancid coconut. These compounds gave an odour reminiscent of rancid almonds and turpentine respectively while heptan-2-ol and nonan-2-ol gave an odour similar to rancid coconut as well as a musty, stale odour. This type of off-flavour has been called ketonic rancidity and is an oxidative variation of the hydrolytic type of rancidity. The presence and origin of the even numbered series ($C_{6,8}$), hexan-2-one, hexan-2-ol and octan-2-one is discussed. The increase in $C_{5,6,7,8,9,11}$ oxidation products in the rancid samples occurred at the expense of $C_{8,10,12}$ short chain fatty acids.

Keywords: Aliphatic methyl ketones; secondary alcohols; off-odours; rancidity; desiccated coconut; oxidation products; moulds.

1. Introduction

Coconut and coconut oil are known to become rancid relatively easily. Early work by Stokoe[1] showed that rancidity in coconut oil was due to an accumulation of an homologous series of odd numbered aliphatic methyl ketones ($C_{7,9,11}$) with heptan-2-one present in the highest concentration. This type of off-odour has been described as perfume or ketonic rancidity.[2] Running in parallel to the production of off-odours in coconut is an increase in free fatty acids.[1] These changes are caused by growth of moulds within the commodity.[3]

In this investigation a comparison was made between desiccated coconut conforming to the Sri Lankan standard specifications for desiccated coconut and consumer returns originally derived from similar coconut. The returned samples were at least 6 months old and often considerably older.

2. Experimental

2.1. Materials

Rancid desiccated coconut was obtained from customer returns. The coconut was perceptibly rancid, often discoloured and some samples appeared mouldy. Normal desiccated coconut was obtained packaged directly from the production line. All samples originated in Sri Lanka. Chemicals were obtained from BDH unless otherwise stated, and gas chromatography materials were from Supelco.

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2.2. General methods

Oil was determined in 10 g samples of normal and rancid desiccated coconut by extracting for 4 h with 150 ml ether : petroleum ether (40–60°C) (1:1 by vol.) in a Soxhlet apparatus, and the residue oven dried at 70°C to constant weight.

Water content was determined by the Dean and Stark procedure employing toluene, while water activity was determined using an electric hygrometer (Novosina IC2) at 25±0.5°C.

Iodine values were obtained using Wijs' method.[4] Free fatty acids were determined as % dodecanoic (lauric) acid by extracting 10 g desiccated coconut with 50 ml industrial spirit, boiling the suspension for 30 min and titrating with M/100 NaOH to the phenolphthalein end point.

2.3. Fatty acids

Fatty acid methyl esters were prepared using BF_3-methanol reagent (Supelco) according to the procedure of Metcalfe and Schmitz.[5] Oil (30 mg) was esterified in 5 ml reaction vessels.

Gas chromatographic (g.c.) analysis was carried out using a column (1.5 m×4 mm) packed with 15% (by wt) DEGS on 100–120 mesh diatomite CAW–DMCS. Nitrogen gas was used as the carrier (40 ml min^{-1}) with the temperature at the injector port at 250°C. The column was programmed from 60 to 200°C at 10°C min^{-1}. The FID detector was at 220°C. The acyl methyl esters were identified by co-chromatography with known standards.

2.4. Volatile off-flavours

Extraction of volatile off-flavours was carried out by vacuum distillation of the oil fraction as previously described.[6] Undecan-6-one (0.8–1.0 g) was added as an internal standard.

G.c. analysis was carried out using a column (1.5 m×4 mm) packed with 10% Carbowax 20M on 80/100 mesh Chromosorb WAW. Nitrogen gas was used as the carrier at 40 ml min^{-1} and the temperature at the injector port was 250°C. The column was programmed from 70 to 165°C at 3°C min^{-1}. The FID detector was at 220°C. Retention times relative to undecan-6-one were determined and concentrations were obtained with reference to standard mixtures of homologous methyl ketones and secondary alcohols. Unknown compounds were identified by co-chromatography and combine g.c./mass spectrometry (g.c./m.s.).

Combined g.c./m.s. was carried out using a Kratos MS-25 instrument linked to a Kratos DS-55 data system, employing conditions similar to those described above. The carrier gas was helium at 30 ml min^{-1}. The jet separator was at 240°C, the ionisation current was 100 μA, source temperature 200°C, accelerating voltage 2.0 kV, resolution 600 and scan speed 1 s decade.$^{-1}$

2.5. Volatile fatty acids

A modification of the method of Remesy and Demigue[7] was used. Ethanol (5 ml) was added to desiccated coconut (1 g) and agitated for 30 min on a flask shaker. Isobutyric acid (0.97 μg) was added as an internal standard. The mixture was filtered (Whatman No. 1), neutralised with NaOH and evaporated to dryness with nitrogen gas. The residue was dissolved in 150 μl water and 50 μl H_3PO_4 (25% by vol.) added immediately before g.c. analysis.

The column (1.5 m × 4 mm) was packed with 10% (wt to vol.) GPSP-1200/1% H_3PO_4 on 80–100 mesh chromosorb WAW. Nitrogen gas was used as a carrier at 40 ml min^{-1} and the temperature at the injector port was at 175°C. The column was run isothermally at 115°C with the FID detector at 170°C. Unknown compounds were identified by relative retention times to known standards.

3. Results and discussion

It can be seen from Table 1 that the consumer returns had deteriorated organoleptically and had become rancid. In addition there was a loss of oil content in the rancid coconut samples and an increase in free fatty acids. This type of deterioration has been described in maize (corn),[8] coconut[9] and peanuts.[10] The increased water content would allow fungal growth to occur. Surprisingly the water activity, a_w, of rancid and normal coconut was similar and below the level required for

Table 1. Oil, moisture, free fatty acids content and water activity of desiccated coconut[a]

Desiccated coconut	Normal[c] s.d.	Consumer[c] return s.d.
Oil content % (by wt)	64.4±1.84	53.9±1.91
Moisture %	2.3±0.69	4.8±1.21
Free fatty acids		
% dodecanoic acid (by wt)	0.145±0.059	1.26±0.829
Water activity[b]	0.36±<0.01	0.387±0.01

[a]All results are the arithmetical mean of 8 individual samples, standard deviations are quoted.

[b]Water activity values were obtained from different coconut samples to those on which moisture determinations were made.

[c]Normal coconut samples had a pleasant, sweetish coconut-like smell whereas the consumer returns had an unpleasant rancid smell with a stale and pungent note.

microbial growth (Table 1). This implies that the commodity may have dried out since spoilage occurred.

The composition of the free fatty acids and the triacylglycerols is given in Table 2. In the rancid oil there was a decrease in octanoic, decanoic and dodecanoic acids. The apparent increase in the percentage of longer chain homologues in the rancid oil is due to the decrease in the short chain fatty acids ($C_{8,10,12}$). When the major volatile fraction is considered (Table 3) the distillate had the typical 'ketonic' odour of rancid coconut. On investigation of the volatile products two series of compounds were found. The presence of an odd numbered series ($C_{5,7,9,11}$) of methyl ketones and the corresponding secondary alcohols was demonstrated, heptan-2-one and nonan-2-one being the main end products. This odd numbered series can be derived from even numbered short chain fatty acids in a partial β-oxidation by the action of many different fungal species.[11] The pathway for the conversion of octanoic acid to heptan-2-one and heptan-2-ol is shown in Figure 1. It follows the normal β-oxidation pathway for fatty acid oxidation until the formation of β-octanoyl CoA. At this point the acid is deacylated and decarboxylated to release the methyl ketone which may undergo reduction to give heptan-2-ol utilising any free NADH in the system.[6] The decrease in the $C_{8,10}$ fatty acids can be accounted for by an increase in the $C_{7,9}$ oxidation products. The other series present was the even-numbered series ($C_{6,8}$) of ketones and secondary alcohols.

The chain length of the end products depends on the pH of the coconut. An increase in pH results in an increase in the chain length of the end product.[12] This is probably due to the increase in the pK_a with increasing chain length of the fatty acids. It appears that only the dissociated acids can be metabolised further to give methyl ketones.

Table 2. Fatty acid composition (%) of oil extracted from rancid and non-rancid desiccated coconut

Component acids	Number of carbon atoms	Non-rancid	Rancid
Hexanoic	6:0	Trace	Trace
Octanoic	8:0	12.22	4.17
Decanoic	10:0	7.57	4.51
Dodecanoic	12:0	46.09	44.80
Tetradecanoic	14:0	19.18	24.80
Hexadecanoic	16:0	8.02	9.41
Octadecanoic	18:0	2.80	5.37
Octadecenoic	18:1	4.11	6.98
Octadecadienoic	18:2	Trace	Trace

Results are the arithmetical means of three determinations.

Table 3. Volatile off-flavour compounds in rancid coconut oil[a]

Off-flavour compounds	Consumer return (mg g^{-1} oil) s.d.	Rancid coconut[b] (mg g^{-1} oil) s.d.	Flavour note	Intensity[c] 1:4
Pentan-2-one	Trace	Trace	Pear drops	3
Pentan-2-ol	N.D.	0.58±0.21	Ethereal	2
Hexan-2-one	9.36±5.01	Trace	Ethereal	4
Hexan-2-ol	4.88±5.45	N.D.	Turpentine	3
Heptan-2-one	10.72±3.55	5.05±0.68	Rancid almonds	4
Heptan-2-ol	9.29±1.11	7.05±0.18	Rancid coconut	4
Octan-2-one	2.57±0.44	N.D.	Weakly ethereal	2
Nonan-2-one	19.63±0.88	2.97±0.07	Weakly turpentine	1
Nonan-2-ol	2.91±0.88	0.35±0.15	Musty, stale	2
Undecan-2-one	6.77±0.20	1.29±0.12	Weakly turpentine	1
Total	66.13	17.29		

[a]All results are the arithmetical mean of three analyses, standard deviations are quoted.

[b]Oil derived from coconut allowed to go rancid by the addition of water. Figures are derived from Kinderlerer and Kellard.[6]

[c]Flavour compounds were dissolved in maize germ oil, ketones 1:100, alcohols 1:10. The intensity figure represents 1—weak, 4—overpowering.

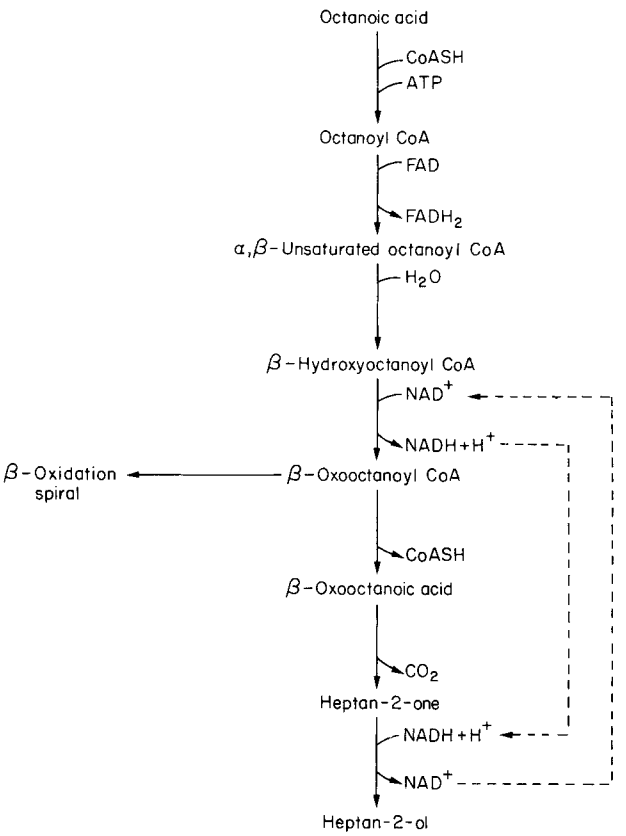

Figure 1. Modification of β-oxidation pathway of fatty acids to show the production of methyl ketones and secondary alcohols (conversion of octanoic acid to heptan-2-one and heptan-2-ol).

Heptan-2-one and heptan-2-ol were the main end products in coconut fermented with *Eurotium amstelodami, Eurotium chevalieri* and *Penicillium citrinum*,[6] whereas undecan-2-one was reported in Venezuelan desiccated coconut.[13] The presence of other methyl ketones was ignored by Ludin.[13] The yield of volatile off-odours (66 mg g^{-1} oil) in this investigation of rancid coconut was greater than that found for coconut allowed to go rancid (Table 3) and considerably in excess of the threshold for the detection of rancidity (0.2 μg g^{-1}).[13] The even numbered series ($C_{6,8}$), hexan-2-one, hexan-2-ol and octan-2-one have been isolated for the first time from coconut fermented with *Eurotium herbariorum.*[6] Lin and Wilkens [14] working with fresh coconut, Allen [15] with rancid coconut oil, and Kawada and Yamazaki[16] with rancid hydrogenated coconut did not detect these compounds. They have not been found amongst the volatile constituents of mould growth.[17]

The even series are 2-derivatives and could not be obtained from α, β or ω oxidation of even numbered short chain fatty acids. The iodine values for rancid and non-rancid oils were 4.0 and 4.82 respectively suggesting that the even series could not have originated from oxidation of unsaturated fatty acids. This is confirmed in the review by Frankel[18] where they are not found as oxidation products of 16:1, 18:1, 18:2 or 18:3 unsaturated fatty acids. Of the homologous series $C_{5,6,7,8,9,11}$, heptan-2-one and heptan-2-ol gave an odour similar to rancid almonds and coconut and came close to the dominant off-odour in rancid coconut (Table 3). The other ketones contributed to the pungent overtones while the alcohols added a stale musty note. Descriptions like stale and musty are often given to off-odours in foods and may well indicate the presence of the homologous series of aliphatic secondary alcohols.

The first effect of mould growth on oil seeds is the hydrolysis of triacylglycerols to give free fatty acids.[19] Many moulds isolated from coconut have lipolytic activity.[3] Obviously the free short chain fatty acids may be further metabolised by some moulds[11] to give methyl ketones. This type of rancidity is therefore an oxidative variation of the hydrolytic type.

On investigation of the short chain volatile fatty acids ($C_{2,3,4,5}$) butyric acid (1.33 mg g^{-1}) and a trace of isovaleric acid were found only in the rancid samples, whereas a trace of acetic acid occurred in rancid and non-rancid samples. No other acids were detected in either normal or rancid samples. Butyric acid is an end product of a number of microbial metabolic pathways and will contribute to the rancid off-odour.[20]

Shredded Sri Lankan coconut is dried at 82–99°C for 15–20 min. This is equivalent to a pasteurisation process, but it has been shown that moulds are always present. Twenty-eight different species have been found by Kinderlerer[21] in desiccated coconut of which two genera of storage fungi (*Eurotium* and *Penicillium*) were found to produce a rancid off-odour when re-inoculated into sterile coconut at a_w 0.76.[3] For all oilseeds a small increase in water content will cause a large increase in the water activity[22]—providing a small safety margin for their safe storage.

4. Conclusions

Off-flavours in coconut are due to an accumulation of aliphatic methyl ketones ($C_{5,6,7,8,9,11}$). This type of off-flavour or rancidity is distinct from oxidative rancidity due to oxidation of unsaturated fatty acids and hydrolytic rancidity due to the production of free fatty acids. It is caused by the partial oxidation of fatty acids resulting from the action of moulds. Ketonic rancidity has been shown to occur in fats containing short chain fatty acids such as margarines, butter and the lauric acid fats such as coconut and palm kernel.[20] It is also found in mould-ripened cheese where the presence of methyl ketones contributes to the flavour.[20] Desiccated coconut should not, therefore, be allowed to become damp during storage.

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