Composition of Palmarosa (Cymbopogon martinii) essential oil from Madagascar

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Ladd (1984) and those of the southern and western corn rootworm found by Lampman et al. (1986) in field screening tests. Also, the floral component indole has been demonstrated to be an attractant for the western corn rootworm and the striped cucumber beetle (Andersen and Metcalf, 1986), and recent field studies have shown that 1,2-dimethoxybenzene, indole, and phenylacetaldehyde synergistically interact to enhance southern corn rootworm attraction to sticky traps (Lampman and Metcalf, 1986).

These data suggest that for the northern corn rootworm, the southern corn rootworm, and possibly the western corn rootworm floral volatiles play an important role in blossom selection. Future studies will attempt to further elucidate the effect on beetle behavior of cucurbit floral components in field trapping tests with single floral constituents and blends of these constituents.

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Composition of Palmarosa (*Cymbopogon martinii*) Essential Oil from Madagascar

Robert P. Randriamiharisoa and Emile M. Gaydou*

Twelve samples of Palmarosa essential oils from Madagascar were studied by capillary gas chromatography. The analysis using the combination of Kovats indices and gas chromatography–mass spectrometry led to the identification of 69 components. Among them, nine were determined for the first time in Palmarosa essential oil. Statistical analysis shows a high positive correlation between some monoterpenes and a high negative correlation between (*Z*,*E*)-farnesyl acetate and various monoterpenes and between geraniol and geranyl acetate.

Among the Gramineae family, some species of the genus *Cymbopogon* give by hydrodistillation essential oils of commercial interest. The essential oil of Palmarosa grass (*Cymbopogon martinii* (Roxb.) W. Wats var. martinii) is rich in geraniol and used as perfumery raw material for imparting roselike aroma in soaps and cosmetics products. The United States annual importation ranged from 10 to 20 tons during the last decade. The effect of plant spacing and application of various fertilizers on herb and essential oil yields of Palmarosa has been recently investigated (Singh et al., 1981; Pareek et al., 1983; Rao et al., 1985). Palmarosa grass produced a high oil yield at the flower open stage and early seed formation (Akhila et al., 1984; Pareek et al., 1981). From an improved calcium chloride adduct, geraniol was isolated in pure form from Palmarosa oil (Garg et al., 1975). Siddiqui et al. (1979) described a thin-layer chromatography (TLC) determination of this compound in Palmarosa oil, and for detecting the adulteration of this essential oil with gingergrass oil, Baiswara et al. (1976) described a sensitive TLC method.

Although the hydrocarbon composition of *C. martinii* has been reported first by Naves (1970) and Peyron (1973) and more recently by our laboratory (Gaydou and Randriamiharisoa, 1986), the nature and the composition of the oxygenated constituents remained inaccurate. The existence in Palmarosa oil of some oxygenated compounds such as linalool, α-terpineol, geranial, geraniol, neral nerol, geranyl acetate (Peyron, 1973), nerolidone, and α- and β-betulenols (Naves, 1970) was described, but many constituents of this fraction were unidentified.

In the course of the evaluation of the quality of Palmarosa essential oil from Madagascar, we have investigated the composition and the range of variation of the main constituents of 12 samples representing the production of this country during the years 1979–1982.

EXPERIMENTAL SECTION

Materials. The various samples of Palmarosa essential oil were obtained from freshly cut herb harvested during the years 1979–1982, by industrial steam distillation. The 12 Palmarosa oils investigated were composite samples of three producers located in northwest Madagascar (Nosy-Be, Ambanja, and Mahajanga areas). These oils were supplied by the Service du Conditionnement et du Contrôle de la Qualité des Produits of Antananarivo (Madagascar) who guarantee their authenticities.

Physical and Chemical Constants. Specific gravity and total alcohols (expressed in geraniol) were determined

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Table I. Physical and Chemical Constants of Palmarosa Essential Oils from Madagascar

	this work[a]				norms					
					Madagascar customs authorities		Grasse Essential Oil Assoc		NF	
	min	max	mean	CV	min	max	min	max	min	max
sp gravity (d_{20}^{20})	0.884	0.889	0.885	0.002	0.881	0.895	0.882	0.896	0.882	0.894
refract index[b]							1.4715	1.4780	1.472	1.478
opt rotation,[b] deg							−3	+3	−3	+3
ester index							10	70	10	40
alcohol,[c] %	85.5	90.1	87.4	1.42		>85	85	94	...	...

[a] Determined from 12 samples. [b] At 20 °C. [c] Calculated as geraniol.

by NFT 75-111 and NFT 75-123 norms, respectively (Afnor, 1982).

Adsorption Chromatography. A sample of Palmarosa oil was fractionated according to the polarity of the constituents by liquid–solid chromatography. Four hundred milligrams of essential oil was placed on a glass column (400 × 8 mm i.d.) filled with 30 g of silica gel (Merck, activity II-III, 230–400 mesh). The hydrocarbons were eluted with 120 mL of *n*-pentane and the oxygenated constituents with 360 mL of diethyl ether. Both fractions were concentrated by using a Vigreux column and analyzed by GLC.

Gas–Liquid Chromatography. Analyses by GLC of the various fractions of samples of Palmarosa oils were done either on a FID-type Intersmat 12 DFL gas chromatograph or on a FID-type Girdel 30 gas chromatograph. Detector and injector temperatures were set at 230 and 220 °C, respectively. The GLC columns used were a Carbowax 20 M WCOT glass capillary column (50 m, 0.30 mm i.d., 0.15-μm phase thickness; programmed temperature 70–210 °C at 2 °C min^{-1}; inlet pressure of hydrogen used as carrier gas 0.4 bar, split ratio 35:1) and an OV-101 WCOT glass capillary column (50 m, 0.30 mm i.d., 0.15-μm phase thickness; programmed temperature 90–220 °C at 2 °C min^{-1}; inlet pressure of hydrogen used as carrier gas 1.5 bar, split ratio 70:1). Volumes of 0.2 μL were injected.

Gas–Liquid Chromatography—Mass Spectrometry. Combined GC–MS were recorded on a Girdel 30 gas chromatograph linked to a Ribermag R-10-10B mass spectrometer and coupled with a Sidar data computer. The GC column was a 0.30 mm i.d. × 50 m fused silica capillary column coated with Carbowax 20 M, 0.15-μm phase thickness. The column temperature was programmed from 70 to 210 °C at 2 °C min^{-1}, carrier gas helium, ion source 220 °C, ionization voltage 70 eV.

Retention Indices and Identification. The various constituents were identified by comparison of their Kovats retention indices (I_K) with those for authentic samples purchased if available. Since I_K varied significantly with temperature, under normal laboratory conditions the I_K could be reproduced to only ±3.5 units (Andersen and Falcone, 1969). We generally used β-caryophyllene, α-humulene, and δ-cadinene mixtures for standardization, and thus I_K can be reproduced to ±1 unit. Approximate I_K were calculated from literature data from the standards indicated above. Identification were verified by comparison of mass spectra of components with reported mass spectra. Neryl and geranyl acetates, geranyl butanoate, and geranyl isovalerate were synthesized by interesterification of the methyl esters of the acids with the corresponding alcohols catalyzed by *p*-toluenesulfonic acid.

RESULTS AND DISCUSSION

The Palmarosa essential oils from Madagascar are pale yellow fluids with a characteristic rose green odor. The oils were analyzed for some physicochemical constants, given in Table I. The analytical ranges differ among Madagascar customs authorities, Grasse Essential Oil Association (Anonymous, 1959), and NF norms (Afnor, 1982). All the samples investigated were in agreement with the norms for specific gravity and alcohol content calculated as geraniol. For the identification of components, the oil was fractionated into oxygenated and hydrocarbon components. A typical Carbowax 20 M capillary gas chromatogram of the essential oil is given in Figure 1, and a chromatogram of the hydrocarbon fraction is given in Figure 2. The components were identified by gas chromatography–mass spectrometry from indices determined on Carbowax 20 M and OV 101 columns. Literature data of retention times and mass spectra were taken from Hunter and Brogden (1964), Andersen and Falcone (1969), Moshonas and Lund (1970), Schreier et al. (1976), Jennings and Shibamoto (1980), MacLeod and Pieris (1982), Papageorgiou and Argyriadou (1981), and Tressl et al. (1983). Under the GC conditions described, estragole and γ-muurolene integrated as one peak on Carbowax 20 M; however, identification of these two components was made on the OV 101 column and by analysis of the oxygenated and the hydrocarbon fractions. The same phenomenon was observed for nerol and α-curcumene. Some sesquiterpenes such as δ- and γ-cadinenes plus cubenene, bicyclogermacrene plus β-bisabolene, and α-farnesene plus selina-4,7-diene were also integrated as one peak on Carbowax 20 M; however, partial resolution was obtained on OV 101. Identification of these components was made by deconvolution of the mixed mass spectra. Results for the 12 samples investigated are tabulated in Table II. Among the 74 products listed in Table II, 69 were identified and

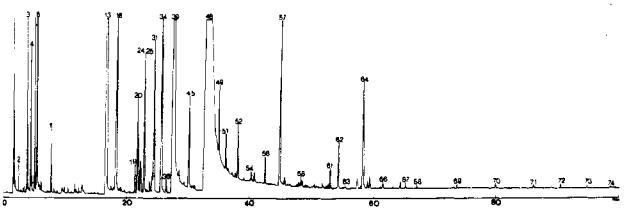

Figure 1. Typical gas chromatogram of Palmarosa essential oil from Madagascar (glass capillary column coated with Carbowax 20 M, 50 m, 0.30 mm i.d., 0.15-μm phase thickness; programmed temperature from 70 to 210 °C at 2 °C min^{-1}). See Table II for peak identification.

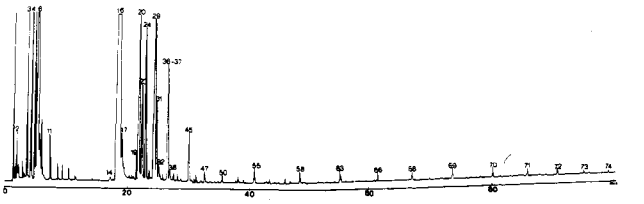

Figure 2. Gas chromatogram of the hydrocarbon fraction of Palmarosa essential oil from Madagascar. For experimental conditions, see Figure 1; for peak identification, see Table II.

Table II. Components Identified from the Palmarosa Essential Oils and Their Percentage Composition[a]

peak[b] no.	I_K[d] CW20M	component	identifcn	relative abundance[c] min	max	mean	CV
1	1000	α-pinene	I_K	0.00	0.02	0.01	0.00
2	1035	β-pinene	I_K	0.00	0.02	0.01	0.00
3	1100	n-$C_{11}H_{24}$	I_K, MS			tr[e]	
4	1176	myrcene	I_K, MS	0.13	0.28	0.21	0.18
5	1201	α-terpinene	I_K, MS	0.04	0.18	0.12	0.01
6	1215	limonene	I_K, MS	0.15	2.16	1.53	0.22
7	1255	γ-terpinene	I_K, MS			tr	
8	1260	o-cymene	MS			tr	
9	1270	m-cymene	MS			tr	
10	1291	p-cymene	I_K, MS	0.00	0.01	0.01	0.00
11	1305	terpinolene	I_K, MS	0.01	0.23	0.10	0.03
12	1500	n-$C_{15}H_{32}$	I_K, MS	0.00	0.12	0.03	0.01
13	1543	linalool	I_K, MS	2.26	3.91	2.79	0.16
14	1562	β-cubebene	I_K, MS	0.00	0.02	0.01	0.00
15	1574	β-elemene	I_K, MS	0.00	0.01	0.01	0.00
16	1581	β-caryophyllene	I_K, MS	1.00	1.76	1.32	0.11
17	1600	n-$C_{16}H_{34}$	I_K, MS			tr	
18	1625	γ-elemene	I_K, MS			tr	
19	1635	β-helmiscapene	I_K, MS	0.05	0.11	0.10	0.01
20	1649	α-humulene	I_K, MS	0.14	2.06	0.59	0.29
21	1657	β-farnesene	I_K, MS	0.01	0.01	0.01	0.00
22	1661	estragole[f]	I_K, MS			tr	
23	1664	γ-muurolene	I_K, MS			tr	
24	1671	γ-selinene	I_K, MS	0.01	0.34	0.24	0.04
25	1675	γ-bisabolene	I_K, MS	0.00	0.04	0.01	0.00
26	1681	α-amorphene	I_K, MS			tr	
27	1685	germacrene D	I_K, MS	0.00	0.01	0.01	0.00
28	1685	oxygenated product					
29	1695	β-selinene	I_K, MS	0.00	0.05	0.04	0.01
30	1699	neryl acetate[f]	I_K, MS	0.01	0.09	0.06	0.01
31	1701	α-selinene	I_K, MS	0.13	0.36	0.21	0.03
32	1712	bicyclogermacrene	I_K, MS	0.00	0.02	0.01	0.00
33	1713	β-bisabolene	I_K, MS				
34	1719	neral	I_K, MS	0.30	0.60	0.40	0.04
35	1728	β-curcumene	I_K, MS			tr	
36	1738	δ-cadinene	I_K, MS				
37	1740	γ-cadinene	I_K, MS	0.00	0.10	0.05	0.01
38	1754	cubenene	I_K, MS				
39	1755	geranyl acetate	I_K, MS	5.09	11.80	8.25	0.89
40	1772	α-farnesene	I_K, MS	0.12	0.34	0.25	0.03
41	1777	selina-4,7-diene	I_K, MS				
42	1785	α-cadinene	I_K, MS	0.01	0.02	0.01	0.00
43	1794	nerol	I_K, MS	0.00	0.01	0.01	0.00
44	1798	α-curcumene	I_K, MS				
45	1802	germacrene B	I_K	0.00	0.02	0.01	0.00
46	1808	*cis*-calamenene	I_K, MS			tr	
47	1816	*trans*-calamenene	I_K, MS			tr	
48	1851	geraniol	I_K, MS	76.3	82.8	80.0	0.76
49	1889	geranyl butanoate[f]	I_K, MS	0.12	0.34	0.15	0.03
50	1900	n-$C_{19}H_{40}$	I_K, MS			tr	
51	1913	geranyl isovalerate[f]	I_K, MS	0.01	0.10	0.08	0.00
52	1960	calacorene	I_K, MS	0.09	0.30	0.18	0.03
53	1963	oxygenated product		0.00	0.06	0.02	0.01
54	1990	methylisoeugenol[f]	I_K, MS	0.01	0.08	0.03	0.01
55	2000	n-$C_{20}H_{42}$	I_K, MS	0.01	0.68	0.14	0.08
56	2081	caryophyllene oxide?	I_K	0.00	0.24	0.09	0.03
57	2090	oxygenated product		0.56	0.83	0.65	0.03
58	2100	n-$C_{21}H_{44}$	I_K, MS	0.01	0.04	0.03	0.00
59	2125	oxygenated product		0.01	0.08	0.04	0.01
60	2138	oxygenated product				tr	
61	2143	oxygenated product		0.00	0.04	0.02	0.00
62	2198	(*E*,*Z*)-farnesyl acetate[f]	I_K, MS	0.05	0.29	0.13	0.02
63	2200	n-$C_{22}H_{46}$	I_K, MS	0.02	0.26	0.05	0.03
64	2252	(*Z*,*E*)-farnesol[f]	I_K, MS	0.35	1.20	0.64	0.09
65	2278	(*E*,*Z*)-farnesol[f]	I_K, MS	0.00	0.10	0.04	0.01
66	2300	n-$C_{23}H_{48}$	I_K, MS	0.00	0.08	0.04	0.01
67	2344	(*E*,*E*)-farnesol[f]	I_K, MS	0.00	0.19	0.05	0.02
68	2400	n-$C_{24}H_{50}$	I_K, MS			tr	
69	2500	n-$C_{25}H_{52}$	I_K, MS			tr	
70	2600	n-$C_{26}H_{54}$	I_K, MS			tr	
71	2700	n-$C_{27}H_{56}$	I_K, MS			tr	
72	2800	n-$C_{28}H_{58}$	I_K, MS			tr	
73	2900	n-$C_{29}H_{60}$	I_K, MS			tr	
74	3000	n-$C_{30}H_{62}$	I_K, MS			tr	

[a] Percentages were calculated from the peak area of the unfractionated essential oils. FID response factors were not determined. [b] Peak numbers were given in the order of appearance in the programmed temperature GC of the unfractionated essential oil. Numbers correspond to those in Figure 1. [c] Determined from 12 samples. [d] Experimentally determined Kovats indices on the Carbowax 20 M column (see conditions in text). [e] tr = less than 0.01%. [f] Determined for the first time in Palmarosa essential oil.

Table III. Correlation Matrix[a] between the Principal Constituents[b] of Palmarosa Essential Oil

	limonene	α-terpinene	nerol	geraniol	(E,Z)-farnesyl acetate	β-helmiscapene	β-caryophyllene	linalool	calacorene	α-humulene	δ- + γ-cadinene + cubenene	geranyl acetate
myrcene	0.92	0.84	−0.33	−0.10	−0.80	−0.47	0.38	0.78	−0.74	−0.86	−0.89	0.39
limonene		−0.95	−0.27	−0.12	−0.90	−0.12	0.32	0.68	−0.73	−0.88	−0.80	0.44
α-terpinene			−0.33	0.07	−0.86	0.17	0.17	0.48	−0.61	−0.80	−0.73	0.27
nerol				−0.81	−0.08	−0.62	−0.69	−0.06	−0.31	−0.03	0.07	0.66
geraniol					0.38	0.14	0.22	−0.45	0.63	0.39	0.25	−0.92
(E,Z)-farnesyl acetate						−0.01	−0.09	−0.59	0.86	0.89	0.72	−0.68
β-helmiscapene							0.90	0.51	−0.12	−0.26	−0.37	−0.09
β-caryophyllene								0.53	0.09	−0.07	−0.19	−0.22
linalool									−0.62	−0.62	−0.74	0.54
calacorene										0.94	0.78	−0.86
α-humulene											0.84	−0.69
δ- + γ-cadinene + cubenene												−0.47

[a] Determined from 12 samples. [b] The higher content of which are >0.1%. Only components having a correlation coefficient >0.80 with another are given.

9 have been determined for the first time in Palmarosa essential oils: estragole, neryl acetate; geranyl butyrate and isovalerate; methyl isoeugenol; (*E,Z*)-farnesyl acetate, (*Z,E*)-, (*E,Z*)-, (*E,E*)-farnesols. Among these components, 48 have a content higher than 0.01%. The components the content of which are higher than 1% are as follows: limonene (1.53%); linalool (2.79%); β-caryophyllene (1.32%); geranyl acetate (8.25%); geraniol (80%).

The oxygenated components of Palmarosa essential oils represent about 90–95%. Except for geraniol (76–83%), linalool (2–4%), and geranyl acetate (5–12%), the other components were found at 0.1% level or lower. Only one carbonyl component was found in Palmarosa: neral (0.4%). Five oxygenated compounds were not identified as shown in Table II. Of these, two may correspond to α- and β-betulenol determined by Naves (1970).

Statistical analysis of the results for components found at the 0.1% level was investigated. The matrix of correlation between components having a correlation coefficient higher than 0.8 is given in Table III. Among the monoterpenes, some of them were highly correlated (myrcene–α-terpinene, 0.84; myrcene–limonene, 0.92; α-terpinene–limonene, −0.95). The correlation coefficient between nerol and linalool was lower (−0.45), but a high correlation coefficient was observed for geraniol and nerol (−0.81) and geraniol and geranyl acetate (−0.92). Among the sesquiterpenes β-helmiscapene was highly correlated to β-caryophyllene (0.90) and α-humulene to calacorene (0.94). Among the esters, (*Z,E*)-farnesyl acetate was negatively correlated to monoterpenes such as myrcene (−0.80), limonene (−0.90), and α-terpinene (−0.86) and positively correlated to α-humulene (0.89). The number of samples investigated was too small to detect any change in composition in relation with the 4 years studied.

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Registry No. α-Pinene, 80-56-8; β-pinene, 127-91-3; myrcene, 123-35-3; α-terpinene, 99-86-5; limonene, 138-86-3; γ-terpinene, 99-85-4; *o*-cymene, 527-84-4; *m*-cymene, 535-77-3; *p*-cymene, 99-87-6; terpinolene, 586-62-9; linalool, 78-70-6; β-cubebene, 13744-15-5; β-elemene, 515-13-9; β-caryophyllene, 87-44-5; γ-elemene, 29873-99-2; β-helmiscapene, 66141-12-6; α-humulene, 6753-98-6; β-farnesene, 18794-84-8; estragole, 140-67-0; γ-muurolene, 30021-74-0; δ-selinene, 473-14-3; γ-bisabolene, 495-62-5; α-amorphene, 20085-19-2; germacrene D, 23986-74-5; β-selinene, 17066-67-0; neryl acetate, 141-12-8; α-selinene, 473-13-2; bicyclogermacrene, 24703-35-3; β-bisabolene, 495-61-4; neral, 106-26-3; β-curcumene, 28976-67-2; δ-cadinene, 483-76-1; γ-cadinene, 39029-41-9; cubenene, 29837-12-5; geranyl acetate, 105-87-3; α-farnesene, 502-61-4; selina-4,7-diene, 105834-89-7; α-cadinene, 24406-05-1; nerol, 106-25-2; α-curcumene, 644-30-4; germacrene B, 15423-57-1; *cis*-calamenene, 483-77-2; *trans*-calamenene, 40772-39-2; geraniol, 106-24-1; geranyl butanoate, 106-29-6; calacorene, 38599-17-6; methyl isoeugenol, 93-16-3; caryophyllene oxide, 1139-30-6; (*E,Z*)-farnesyl acetate, 24163-98-2; (*E,E*)-farnesol, 106-28-5; geranyl isovalerate, 109-20-6; (*Z,E*)-farnesol, 3790-71-4; (*E,Z*)-farnesol, 3879-60-5.

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Oxidation of Tryptophan in the Presence of Oxidizing Methyl Linoleate

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Tryptophan (Trp) oxidation to kynurenine (kyn) and *N*-formylkynurenine (NFK) was studied in the presence of oxidizing methyl linoleate. The rate of Trp oxidation was found to depend on pH, ionic strength, and the presence or absence of metals and metal chelators. The presence of copper (5 ppm) increased the rate of oxidation, and EDTA decreased it. When Trp was present in a tripeptide (Gly-L-Trp-Gly) the rate of Trp oxidation was greater than that found in like samples where Trp was free instead of bound. Trp in control samples (no methyl linoleate) also oxidized relatively rapidly, but the rates were always less than those observed in samples containing Trp and methyl linoleate. Approximately 1–2% of the Trp in either free or bound form in a tripeptide (no added catalyst or inhibitor) was converted to kyn and NFK in just 72 h at room temperature.

Oxidation in foods has been a problem for centuries. It leads to changes in nutritional and functional properties and may even result in development of toxic substances (Fontana and Toniolo, 1976; Yong and Karel, 1979). Although lipids are considered the most oxidation-prone constituents of foods, some amino acids in free or combined forms are also susceptible to oxidation. The amino acids cystine, methionine, histidine, tyrosine, and tryptophan (Trp) are all susceptible to oxidation, and the conditions need not be severe (Yong et al., 1980). Protein-bound forms of these amino acids can also be oxidized under conditions that sometimes prevail in stored food. With regard to protein-bound Trp, storage for 4 weeks at 37 °C in the presence of oxidizing methyl linoleate resulted in a 25–30% increase in the chemically determined loss of Trp as compared to that encountered in control samples (Nielsen et al., 1985a,b). In another study, the kynurenine (a major degradation product of oxidized Trp) content of dolphin flesh increased from 0.9 to 3.5 μg/g during exposure of the fish to sunlight for 8 h (Takahashi, 1984). Thus, Trp can be oxidized in either free or bound form, and study of Trp in less complex forms has the advantage of facilitating separation and quantification of major degradation products.

Oxidation of proteins and amino acid residues occurs by a free-radical mechanism. Free radicals can be initiated by ionizing or ultraviolet radiation, by visible light, or by free-radical transfer from other molecules (Fontana and Toniolo, 1976; Schaich, 1980). Peroxidizing methyl linoleate is very efficient at transferring its free radicals to other substances including proteins and amino acids (Gunstone and Norris, 1983). Similar behavior would be expected from linoleate in foods, when it is bound in triacylglycerols and phospholipids.

Free tryptophan (Trp) in food and simple systems oxidizes readily in the presence of light or peroxidizing lipids, participates in the Maillard reaction, and is destroyed under the usual conditions for acid hydrolysis (Yong and Karel, 1979). The degradation products of Trp oxidation are similar regardless of whether the degradation is caused by peroxidizing lipids, ionizing radiation, or photooxidation (Yong and Karrel, 1979). Protein-bound Trp is less susceptible to oxidation than protein-bound lysine or methionine (Nielsen et al., 1985a).

Loss of Trp through oxidation is important because it is an essential amino acid; the resulting products can contribute to off-flavors in irradiated foods, can lead to the yellowing of wool in sunlight, can contribute to the development of yellow and brown cataracts in the lens of the human eye, and can reduce the activities of some enzymes; and at least two of the products (kynurenine and *N*-formylkynurenine) are suspected promoters of urinary bladder carcinogenesis in mice (Bryan, 1971; Friedman and Finley, 1971; Matsushima et al., 1982; Nielsen and Hurrell, 1984; Wolf, 1984; Yong and Karel, 1979).

Oxidation of proteins and amino acids is affected by many environmental factors such as pH, temperature, water activity, and the presence of catalysts or inhibitors (Erickson, 1982; Mitchell and Henick, 1962). Therefore the extent of oxidation and the rate at which it occurs is not easily predicted.

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