Effect of Water Activity on Secondary Products Formation in Autoxidizing Methyl Linoleate

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The role of water activity on the formation of peroxides and carbonyl compounds during lipid oxidation is important to know because there could be either beneficial or detrimental effects of water activity on lipid oxidation in stored foods. Therefore, methyl linoleate was chosen as a model lipid and was autoxidized to 1% at water activity ranging from 0.02 to 0.79 at 37°C. Oxygen uptake was monitored manometrically. The peroxide and carbonyl contents were determined upon termination of the autoxidation studies. Methyl linoleate autoxidation was characterized by three phases: i) an initial induction period of no oxygen absorption; ii) a slow rate of oxygen absorption, up to 0.15% oxidation; and iii) a relatively faster rate of oxygen absorption beyond 0.15% up to 1% oxidation. Water activity had considerable influence during the first phase. There was no induction period at or below water activity 0.22. The induction period begins at water activity 0.32 and could be extended to a limit with increase in water activity. Once the induction period was passed water activity had no influence on the rate of oxidation. However, during the second and third phases water activity becomes important in the stabilization of peroxides/hydroperoxides and decides the course of secondary reactions that follow peroxide decomposition. Higher water activity values, particularly water activity 0.67, tended to stabilize peroxides. Water activity had considerable influence on the formation of secondary products of autoxidation as evidenced by the variation in the type and quantity of carbonyl compounds at different water activity values.

KEY WORDS: Autoxidation, carbonyl compounds, methyl linoleate, peroxides, secondary products of autoxidation, water activity.

Autoxidation of lipids is known to be one of the major causes of spoilage of foods. The autoxidation reaction is influenced by various factors such as temperature, oxygen tension, presence of metal catalysts, inhibitors and water. It is now realized that it is not so much the moisture content in a food that is important as is the actual amount of water that can take part in the reactions. This is generally referred to as "water activity" and is expressed as the ratio of the vapor pressure exerted by water in a food material to that of saturated water vapor pressure of pure water at the same temperature (1).

The importance of water activity in stored dehydrated foods was noted during 1957 and 1959 by Rockland (2) and Salwin (3). However, the influence of water activity (a_w) on lipid autoxidation was first demonstrated in 1966 by Maloney *et al.* (4) by using simulated freeze-dried foods. They found water to have an antioxidant effect on oxidizing methyl linoleate. The protective effect of water was operative at an activity below the calculated Brunauer-Emmett-Teller (BET) monolayer and increased with water activity up to a value of approximately 0.50, where it leveled off. Labuza (5) noted that, generally, water had a prooxidant effect at low and high water activities and an antioxidant effect at medium water activities on lipid autoxidation in foods. Several hypotheses have been advanced to explain the protective effect of water in retarding lipid oxidation. The most important are: i) water reduces oxygen diffusion by forming a barrier for oxygen over the lipid surface (6,7); ii) water lowers the effectiveness of metal catalysts such as copper and iron (8); and iii) water forms hydrogen bonds with hydroperoxides and retards hydroperoxide decomposition (9).

Though the effect of water activity on rate of lipid autoxidation in food and in model lipid systems has been studied, data on secondary products of autoxidation are scanty. Kloepffer *et al.* (10) and Esterbauer and Schauenstein (11) observed the formation of hydroperoxides of C_5, C_8 and C_9 chainlengths in autoxidizing aqueous dispersions of methyl linoleate. These were not reported in the absence of water. Labuza *et al.* (12) found that the amounts of hexanal and heptanal produced decreased as the water activity was increased from the dry state to the monolayer region. Prabhakar and Amla (13) showed that in a walnut oil-cellulose model system oxidizing at different water activities there were qualitative and quantitative changes in the secondary products of autoxidation, namely the carbonyl compounds. The observation has been confirmed in the methyl linoleate model system (14). However, in the above studies (13,14) the lipid was autoxidized to a pre-determined period. As a consequence, the level of oxidation of samples at different water activities was never similar at the time of analysis of secondary products of autoxidation. It is important that the observed pattern of secondary reaction was a result of differences in water activities or levels of oxidation. The present work is a study of methyl linoleate autoxidation to a pre-determined level of 1% oxygen absorption at different water activities, followed by analysis for the secondary products of autoxidation.

MATERIALS AND METHODS

Materials. The procedure for preparing carbonyl-free solvents and chemicals used were the same as reported earlier (13). Methyl linoleate was prepared by the procedure outlined by Westerfeld (15). The methyl linoleate was distilled under 1–2 mm pressure through a Vigreux fractionating column (18-cm length). The middle-cut portion, boiling between 158–162°C, was collected, flushed with nitrogen, and stored in a refrigerator as a stock material for all the experiments. The stock methyl linoleate was freed from peroxides, polar and carbonyl compounds before each experiment.

Methods. The purification procedure used was as follows. Silica gel (100 g), 60–120 mesh (BDH Chemicals Ltd., Poole, England), of 7.5% moisture, was wet packed in hexane in a 3 × 100 cm long chromatography column. Methyl linoleate (50 g) dissolved in 250 mL hexane, was applied to the column, and eluted with the same solvent. Hexane was removed from the eluent under reduced

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pressure in a rotary flash evaporator and then vacuum-distilled as outlined earlier. The methyl linoleate prepared in this manner was colorless, showed absence of peroxides and polar compounds by peroxide value (16) and thin-layer chromatography (TLC) (17) analyses, had a total carbonyl content of 78 μM/M and was 99.6% pure by gas-liquid chromatography.

Autoxidation. Autoxidation of methyl linoleate was conducted by Warburg manometry. The procedure used for calibration of Warburg flasks was the same as described by Umbreit *et al.* (18). Methyl linoleate (5.00 mL) was added to the main vessel of a 100-mL Warburg flask. The environment within the flask was maintained at a_w values between 0.02 and 0.79 (RH of 2 to 79%), with fused calcium chloride (1.5 g) for a_w of 0.02, and 1 mL of saturated salt solution (19) for the other a_w. The flasks were carefully flushed with air of desired a_w (obtained by passing air through humidification flasks containing saturated salt solutions) and attached to manometers. The flasks were transferred to a Warburg shaker waterbath maintained at 37 ± 0.1°C and operated at 120 oscillations per min. The gas vents of the flasks and the stopcocks of the manometers were closed after an equilibration period of 2 hr. The readings of the manometers were monitored periodically. When the sample absorbed nearly 10 millimoles of oxygen per mole linoleate (1% oxidation), the sample was withdrawn for peroxide (1.0 ± 0.01 g), and carbonyl (3.0 ± 0.01 g) analyses.

Evaluation of kinetic constants. The induction period (IP) was taken as the interval between the commencement of the experiment and measurable oxygen absorption (0.01–0.1 meq O_2/kg linoleate).

The procedure of Labuza *et al.* (12) was adopted for calculation of rate constants. Measurement of the slope of the plot of the square root of moles of oxygen absorbed per mole of linoleate *vs.* time gave the monomolecular rate constant (K_m). When the square root of moles of oxygen absorbed per mole of methyl linoleate (mean values of duplicate samples) was plotted against time, it showed two linear plots—one with a low slope which extended up to approximately 1.5 mM oxygen absorption per mole of linoleate (0.15% oxidation), and another with a slightly higher slope, extending over the range 1.5–10.0 mM oxygen absorption per mole of linoleate (0.15% to 1% oxidation).

Peroxide value. The AOCS method Cd 8-53 (16) was followed for analysis of peroxide value with 1 ± 0.01 g of sample.

Carbonyl compounds. The autoxidized linoleate samples (1–3 g) were dissolved in hexane (25 mL). The carbonyl compounds in the hexane solution were converted into 2,4-dinitrophenylhydrazones (DNPH) on a 2,4-dinitrophenyl hydrazine-phosphoric acid-celite (DNP) column (20). The DNP column was washed with hexane (25–250 mL) till the effluent showed no further decrease in absorption at 340 nm. The hexane effluent and washings from the DNP column were pooled and made up to a known volume with hexane (solution A). The absorbance of an aliquot of solution A was read at 340 nm in a Beckman DU spectrophotometer (Fullerton, CA); the total concentration of the carbonyl derivatives was calculated by using molar extinction coefficient of DNPH, E = 22500 (20). This represents the total carbonyl content (TCC). The DNPH in solution A was separated into dicarbonyl compounds, keto and semialdehyde esters, monocarbonyl compounds, and their classes as described earlier (14).

RESULTS AND DISCUSSION

Autoxidation behavior of methyl linoleate (Fig. 1) at different water activities was characterized by three distinct phases. Phase 1, the period during which there was no measurable oxygen absorption or the induction period (IP); phase 2, the period during which oxygen absorption was slow, up to approximately 0.15% oxidation; and phase 3, the period of accelerated oxidation, *i.e.*, from 0.15% up to approximately 1% oxidation.

In the present study, the classical definition of the induction period has been adopted, *viz.*, the period during which no measurable uptake of oxygen occurs. In addition, the autoxidation reactions were terminated at the 1% oxidation level, the level chosen by Maloney *et al.* (4) as induction period for purposes of studying the kinetics of peroxide decomposition.

The termination of autoxidation at an early stage in the present study was in view of the perceptible off-flavors in food lipids at low levels of oxidation. For the purpose of this discussion, the water activity range studied has been broadly grouped as group I, comprising a_w = 0.02, 0.11, 0.22; and group II, consisting of a_w = 0.32 to 0.79.

Influence of water activity on induction period. Group I (a_w = 0.02 to 0.22) was characterized by absence of an induction period (IP). The induction period was invariably observed in group II (Table 1, item A). The induction period was about 12 hr at a_w = 0.32 and it increased to a maximum of 20 hr at higher a_w (>0.50). These results indicate that a minimal a_w value exists, below which linoleate oxidizes without exhibiting any induction or lag period; above this minimal a_w an induction period exists, which could be prolonged to a certain extent by increasing the a_w. Beyond a certain a_w, in the present studies a_w = 0.44, the IP cannot be increased any further. The influence of water activity on prolonging the IP in methyl linoleate systems, autoxidizing at different a_w, has not been reported so far.

Influence of water activity on initial stages of autoxidation. It is interesting to note that the time required (discounting the IP) for 0.15% oxidation, at a_w values other than at a_w = 0.02 (Table 1, item B and in other runs, Table 2) varied within a narrow range. Thus, at a_w = 0.11 and 0.22, which had no IP, the time required to absorb about 1.5 mM oxygen was approximately 28 hr, *i.e.*, about the same as at a_w = 0.32 and 0.44 (which had induction periods). The monomolecular rate constants during this phase of autoxidation [$K_m \times 10^3$, expressed as $(M\ O_2/M)^{1/2}$/hr in Table 1, item D(i)] for a_w = 0.11 to 0.79 lay within a narrow range of 0.93 to 1.18. Only the dry system (a_w = 0.02) had a high rate constant of 1.65. This means that once the system crossed the IP, water activity had little effect on the rate of autoxidation. Incidentally, the level of 0.15% oxidation appears to be relevant with respect to acceptability of oils and fats, and corresponds approximately to 20 meq of peroxide O_2/kg—the acceptability limit fixed for olive oil (21). This limit for other vegetable oils has been fixed at 10 meq of peroxide O_2/kg oil (22).

The third phase of autoxidation commencing from

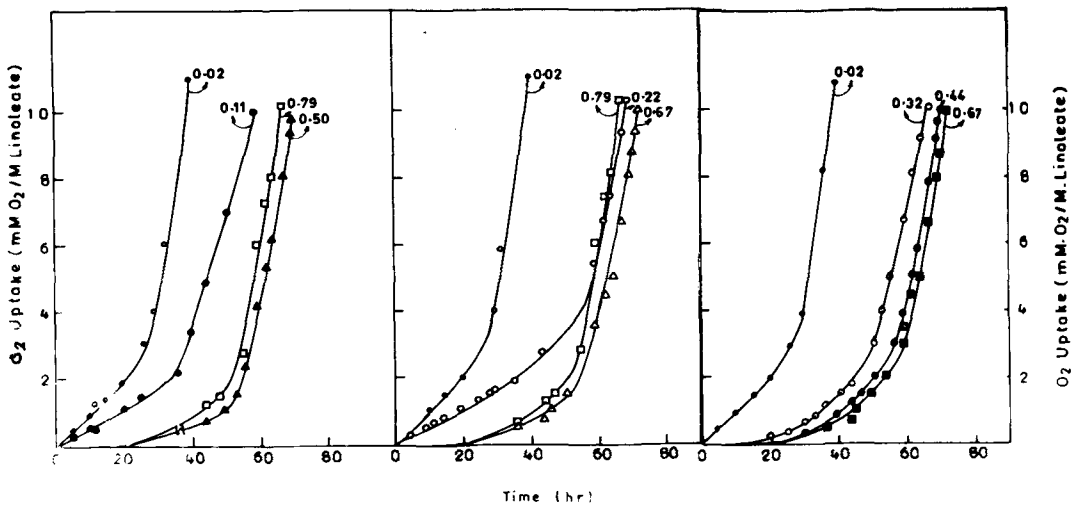

FIG. 1. Oxygen uptake of methyl linoleate autoxidized at different water activities at 37°C.

TABLE 1

Effect of Water Activity on Induction Period and Rate of Autoxidation of Methyl Linoleate at 37°C

Phase	Water activity							
	0.02	0.11	0.22	0.32	0.44	0.50	0.67	0.79
A. Induction period (IP) (hr)	0	0	0	12	20	20	20	20
B. Time to absorb 1.5 mM O_2/M linoleate from end of IP (hr)	15	27.5	28.5	29	26.5	33	30.5	27.5
C. Time to absorb 10 mM O_2/M linoleate from end of IP (hr)	39	59	68.5	55	50.5	50	52	47
D. $K_m \times 10^3$ (M O_2/M)$^{1/2}$/hr								
i) During phase B	1.65	1.18	1.03	1.16	1.35	0.93	1.07	1.18
ii) During phase C	3.86	3.00	3.07	2.97	3.69	3.44	3.26	3.26

TABLE 2

Monomolecular Rate Constants $K_m \times 10^3$ [(M O_2/M)$^{1/2}$/hr] of Methyl Linoleate Autoxidizing at Different Water Activities at 37°C

Run no.	a_w							
	0.02	0.11	0.22	0.32	0.44	0.50	0.67	0.79
1	0.68	0.22	0.30	0.31	—	0.27	—	0.35
2	0.89	0.73	0.65	0.66	—	0.69	—	0.96
3	1.71	0.69	—	0.89	—	0.90	0.99	1.43
4	1.65	1.18	1.03	1.16	1.35	0.93	1.07	1.18

0.15% up to 1% oxidation presented no particular pattern. It was completed in 24 hr (difference in value between items B and C, Table 1) for the dry sample at $a_w = 0.02$. However, at the remaining group I a_w values of 0.11 and 0.22, the time required was slightly higher (31.5–40 hr). For group II, the time varied from 17–26 hr. Likewise, rate constants K_m [Table 1, item D(ii)] at $a_w = 0.11$, 0.22, 0.32, 0.67 and 0.79 ranged between 2.97 and 3.26; at $a_w = 0.44$ and 0.50 they were slightly higher (3.69 and 3.44); and K_m was highest at $a_w = 0.02$ (3.86). Maloney *et al.* (4) were unable to calculate the monomolecular rate constants for the methyl linoleate-cellulose freeze-dried model system because a considerable amount of oxidation had occurred before the start of the experiment. However, in later experiments, Labuza *et al.* (12) showed that the K_m at 30 and 45% RH ($K_m = 5.3$ and 4.6) were lower than the K_m for a dry system of methyl linoleate-cellulose ($K_m = 7.1$).

Influence of water activity on peroxide development. The peroxide oxygen at $a_w = 0.50$, 0.67 and 0.79 was 75%, 99% and 80%, respectively, of the total oxygen absorbed (Table 3). At $a_w < 0.44$, the peroxide oxygen varied

TABLE 3

Oxygen Uptake and Peroxide Oxygen of Methyl Linoleate Autoxidized at Different Water Activities at 37°C

Parameter	Water activity							
	0.02	0.11	0.22	0.32	0.44	0.50	0.67	0.79
1. Oxygen (O_2) uptake (μM O_2/M linoleate)	10,735	10,000	10,352	10,030	9,918	9,766	9,947	10,292
	10,733	10,003	10,350	10,032	9,920	9,763	9,945	10,289
2. Peroxide value (meq O_2/kg linoleate)[a]	91	76	78	83	77	100	134	112
3. Peroxide oxygen i) μM O_2/M linoleate[b]	6,691	5,588	5,735	6,103	5,662	7,353	9,853	8,235
ii) As % of O_2 absorbed	62.3	55.9	55.4	60.8	57.1	75.3	99.1	80.0

[a]Peroxide value (PV) by titrimetry; mean value of duplicates, variation ± 0.2%.

[b]Peroxide oxygen calculated from PV by using the equation PO = PV × 1000/13.6.

TABLE 4

Formation of Peroxides and Carbonyl Compounds in Methyl Linoleate Autoxidized at a Low Water Activity of 0.02 at 37°C

O_2 uptake (μM/M linoleate) (A)	PV		TCC	
	meq O_2/kg	As % of O_2 uptake	μM/M linoleate (B)	μM/mM O_2 uptake (B-88) × 1000/A
0	0	—	88	—
4,529	30	48.7	195	43
5,176	40	56.8	200	39
7,416	52	51.5	115	16
7,500	68	66.7	128	17
10,050	88	64.4	211	21
12,290	103	61.6	249	20

TABLE 5

Formation of Peroxides and Carbonyl Compounds in Methyl Linoleate Autoxidized at a High Water Activity of 0.67 at 37°C

O_2 uptake (μM/M linoleate) (A)	PV		TCC	
	meq O_2/kg	As % of O_2 uptake	μM/M linoleate (B)	μM/mM O_2 uptake (B-88) × 1000/A
0	0	—	88	—
4,310	52	88.7	109	25
4,310	58	98.9	84	20
5,010	68	99.9	95	19
5,250	70	98.0	67	13
7,716	102	97.2	137	18
11,030	132	88.0	140	13

in a narrow range of 55–62% of the total oxygen absorbed (49–67% at a_w = 0.02, Table 4). Thus, stability of peroxides seems to be favored with increasing water activity of the autoxidizing system. It may be noted that at a_w = 0.67, peroxide stability was the highest, accounting for as much as 88–100% (Table 5) of the total oxygen absorbed. One of the possible reasons for higher peroxide content at a_w>0.50 could be hydrogen bonding of hydroperoxides with water at high a_w (9). Martinez and Labuza (23) and Labuza *et al.* (12) observed agreement in values for oxygen uptake and peroxide content in the methyl linoleate-cellulose freeze-dried model systems up to 5% oxidation level. However, data showing agreement between oxygen uptake and peroxide content as a function of water activity in similar systems of methyl linoleate are lacking. Increased stability of hydroperoxides with increase in water activity, particularly a_w>0.50, has not been reported previously.

Influence of water activity on carbonyl compounds formation. The total carbonyl content (TCC) varied marginally at different a_w (Table 6) and accounted for less than about 1.5% of the total oxygen absorbed (Table 7, item

TABLE 6

Effect of Water Activity on Carbonyl Content of Methyl Linoleate Autoxidized at 37°C

Parameter (μM/M linoleate)	Fresh methyl linoleate	Water activity						
		0.02	0.11	0.22	0.32	0.44	0.50	0.79
A. Oxygen uptake	—	10,735	10,000	10,352	10,030	9,918	9,766	10,292
B. TCC	85	173	89	126	179	116	142	137
C. TCC at 10 mM O_2 uptake	—	82	4	40	94	31	58	51
D. Carbonyl compounds recovered from column	85	153	37	59	65	43	16	16
E. Dicarbonyl compounds (B-D) content	0	20	52	67	114	73	126	121
as % of TCC (E × 100/A-85)	—	12	58	53	64	63	89	88
F. Keto and semialdehyde esters (D-G)[a]	37	78	25	26	18	17	6	6
G. Monocarbonyl compounds[a]	48	75	12	33	47	26	10	10
H. Alkanals	40	34	<1	12	13	11	1	—

[a]Keto and semialdehyde esters and monocarbonyl compounds formed 45% and 43% of TCC at a_w = 0.02.

TABLE 7

Pattern of Distribution of Oxygen Among Peroxides and Carbonyl Compounds in Methyl Linoleate Autoxidized at Different Water Activities at 37°C

Parameters[a] (μM O_2/M linoleate)	Methyl linoleate autoxidized at a_w						
	0.02	0.11	0.22	0.32	0.44	0.50	0.79
A. Oxygen uptake	10,735	10,000	10,352	10,030	9,918	9,766	10,292
B. Peroxides' oxygen content	6,691	5,588	5,735	6,103	5,662	7,353	8,235
% of A	62.3	55.9	55.4	60.8	57.1	75.3	80.0
C. Carbonyl compounds' oxygen content[a]	97	71	97	147	95	134	127
% of A	0.9	0.7	0.9	1.5	1.0	1.4	1.3
D. Oxygen in other secondary products of oxidation by difference[b] (% of A)	36.8	43.4	43.7	37.7	41.9	23.3	18.7

[a]Carbonyl compounds' oxygen = dicarbonyl compounds + (keto and semialdehyde esters + mono carbonyl compounds/2).

[b]Oxygen in other secondary products of oxidation = {100 − % of A of (B+C)}

C). After 1% oxidation the TCC (corrected for the initial values) ranged from 4 to 82 μM for group I a_w, and 31 to 94 μM for group II a_w. The trend of formation of total carbonyl compounds at low (a_w = 0.02) and high water activity (a_w = 0.67) was similar, as can be seen from the TCC data in Tables 4-6.

Class separation of the TCC showed that dicarbonyl content was rather low (20 μM/M) at a_w = 0.02 and that it generally increased with a_w. The dicarbonyl compounds formed accounted for only 12% of the TCC at a_w = 0.02 and they increased to about 88% at a_w = 0.79. That is, at a given oxidation level, the percentage of dicarbonyl compounds (of the total carbonyl compounds) tended to increase with increase in a_w of the system.

A reversal in the trend observed for dicarbonyl content was found for keto and semialdehyde esters and the monocarbonyl compounds (Table 6, items F and G). That is, in general, systems at low a_w (group I) were characterized by low dicarbonyl content and high keto and semialdehyde esters and monocarbonyl compounds as compared to high a_w (group II) systems. This finding remains unexplained at present.

The monocarbonyl compounds showed the presence of alkanals only, unlike the earlier reports on walnut oil (13,24), and the content of alkanals was always lower than the initial values. A significant reduction in the alkanals content (Table 6, item H) was noticed at a_w>0.11. However, the alkanal class, as examined by TLC, showed a general qualitative and quantitative difference with change of a_w in the system (Table 8). It has been shown in a walnut oil-cellulose system, autoxidizing at different a_w, that the relative quantities of the four classes of monocarbonyl compounds differed at different a_w, and hence, the odor profiles of the autoxidizing walnut oil-cellulose system also varied with changes in the a_w (24). Because of low odor and taste threshold values for carbonyl compounds, even a small variation in their distribution could assume significance of off-flavor development.

TABLE 8

Pattern of Monocarbonyl Compounds Formed in Methyl Linoleate Autoxidized at Different Water Activities at 37°C

a_w	Alkanals μM/M linoleate	TLC Spot no.	$R_f \times 100$	Identity carbon number	Relative concentration of individual members of alkanal class at a_w						
					Fresh linoleate	0.02	0.11	0.22	0.32	0.44	0.50
0.02	33.7	1	0	—	21	88	36	50	60	64	56
0.11	0.4	2	19	C_3	6	—	—	—	—	—	—
0.22	11.6	3	33	C_4	12	—	13	—	—	—	—
0.32	13.0	4	40	C_5	—	—	40	—	—	—	—
0.44	10.9	5	65	C_6	56	2	—	15	12	—	—
0.50	1.0	6	76	C_7	—	5	—	—	—	—	14
Fresh		7	86	C_8	—	—	11	—	7	11	—
lino		8	95	C_9	5	5	—	35	18	—	27
leate	39.7	9	100	Solvent front	—	—	—	—	—	25	3

Ullrich and Grosch (25) have shown that hexan-1-al, oct-2-en-1-al and non-2-en-1-al are potent flavor compounds formed during autoxidation of methyl linoleate. In our studies, hexan-1-al, present at a 56% level in the initial linoleate sample (Table 8), disappeared during autoxidation at different water activities. This was presumed to be due to the participation of initially present alkanals in further autoxidation reactions as demonstrated by Schieberle and Grosch (26).

Distribution of absorbed oxygen. The data in Table 7 show that the peroxides and carbonyl compounds, together accounted for 56.3–81.3% of total oxygen absorbed (63.2% at $a_w = 0.02$, to 81.3% at $a_w = 0.79$). The carbonyl compounds accounted for only 0.7 to 1.5% of the absorbed oxygen (Table 7, item C). This agrees with observations in oxidized rubber, where only a low percentage of the total oxygen absorbed (4%) was found as carbonyl compounds (27).

It may be noted that oxygen unaccounted for, either as peroxides or as carbonyl compounds, was 18.7%–23.3% at $a_w > 0.50$ (Table 7). At lower a_w ($a_w < 0.44$), the unaccounted oxygen was rather high (36.8–43.7%) (Table 7, item D). This implies that the secondary reactions commencing with peroxide scission would, to an extent, be decided by the water activity of the system.

In conclusion, autoxidation of methyl linoleate is characterized by three distinct phases—an initial induction period, a middle phase of slow oxidation, and finally, a faster rate of autoxidation. Water activity has considerable influence during the first phase. There is no induction period at $a_w \leq 0.22$, and the IP at $a_w = 0.32$ could be prolonged to some extent by increasing the water activity. However, water activity assumes importance in stabilizing the peroxides/hydroperoxides formed during the second and third phases, and in deciding the course of secondary reactions and the products formed.

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