♣Formation of Short Chain Volatile Organic Acids in the Automated AOM Method

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The end point in the automated AOM stability test for fats is related to the rapid production of volatile acids at the end of the induction period and usually measured by conductivity of an aqueous solution of the exit gases. It has been postulated that the reaction involves the transitory presence of a diperoxide which decomposes into two aldehydes and formic acid. The volatile acids produced by several oils were composed mainly of formic acid and significant amounts of acetic acid. In addition, acids with three or more carbon atoms, including propionic, butyric and caproic, were detected. It was found that the temperature of the water in the receiving jars was important in relation to retention of the formic acid. At temperatures above 20 C significant losses may occur. The relationships between peroxide value of the oils, the conductivity of the exit gas solutions and the organic acid content was investigated for the following fats and oils: sunflower, canola, olive, corn, peanut and soybean oil, triolein, lard and butterfat.

The oxidative stability of fats traditionally has been measured by the Active Oxygen Method (AOM) (1). In this method, a sample of the oil is heated in a tube immersed in an oil bath, and air is bubbled through the oil. At regular intervals, samples of the oil are withdrawn and the peroxide value determined. By plotting peroxide value vs time, a characteristic curve is obtained which shows a definite inflection point at which the peroxide value rapidly increases. The time to reach a peroxide value of 100 gives the AOM endpoint. Although widely used, this test has several disadvantages, including being time consuming and labor intensive. In recent years, several other tests have been developed; the most successful of these is the version based on the observation that in an oxidizing oil volatile acids are formed at the end of the induction period. The air emerging from the oil in an AOM test can be led into water and the acids titrated potentiometrically or determined conductometrically. It was demonstrated by Hadorn and Zurcher (2) that the volatile acids formed in the AOM test can be used for automated endpoint detection. Several automated systems have been described (2-4). A commercial version of this equipment is available and has been described (5).

The formation of formic acid in autoxidizing oils has been elucidated by Loury (6). He postulated that formic acid is formed by peroxidation of aldehydes. A resonance equilibrium is established between two limiting forms of an aldehyde molecule:

$$R-CH_2-\overset{+}{C}H-\overset{-}{O} \rightleftharpoons R-CH_2-CHO \rightleftharpoons R-\overset{-}{C}H-\overset{+}{C}HOH$$
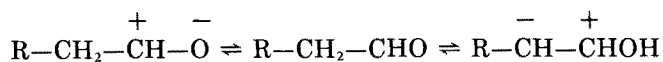
or, more exactly, between two limiting forms of the carbonyl-free radical that initiates the autoxidation chain:
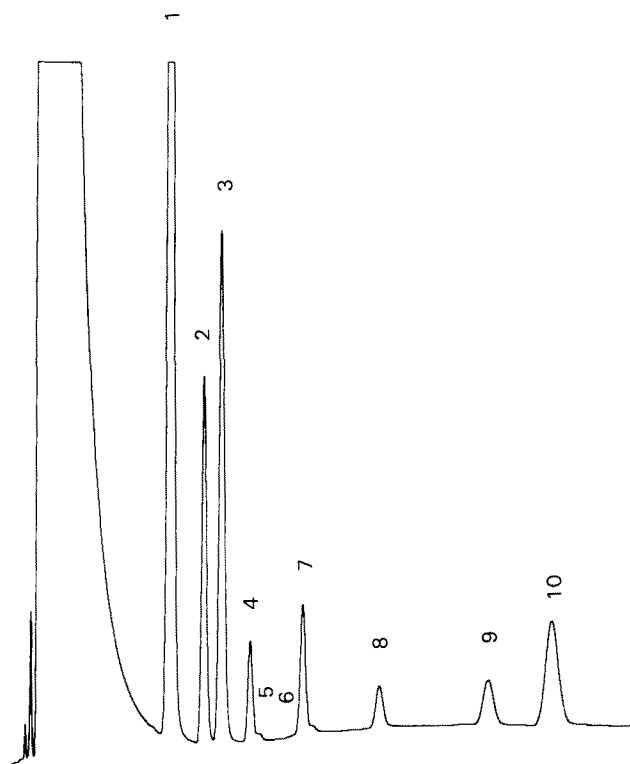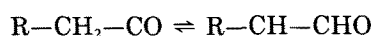
$$R-CH_2-CO \rightleftharpoons R-CH-CHO$$

FIG. 1. Chromatogram of volatile acids produced in the automated AOM test. Identity of peaks: 1, formic acid; 2, acetic acid; 3, butanol; 4, propionic acid; 7, butyric acid; 8, valeric acid; 9, caproic acid; 10, lactic acid (internal standard).

TABLE 1

Decrease in Conductivity of a Formic Acid Solution at 20 C Under Conditions of the Automated AOM Test

Time (hr)	Conductivity (μS)
0	313
10	295
20	212
30	180
40	146
50	117
60	86

The first hybrid yields a peracid-free radical by autoxidation, and chain transfer gives the peracid:

$$R-CH_2-CO + O_2 \rightarrow R-CH_2-\overset{\overset{\textstyle O}{\|}}{C}-O$$
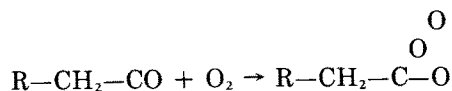
The second hybrid is able to tie up oxygen at the α carbon to yield the α-hydroperoxy aldehyde by a similar mechanism:

$$O$$
$$O$$
$$R-CH-CHO + O_2 \rightarrow R-CH-CHO$$
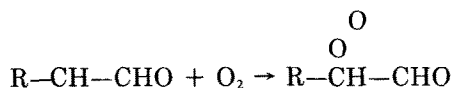
The latter results in formic acid and a new aldehyde by breakdown of the $-O-O$ and $-C-C$ bonds:

$$H$$
$$O$$
$$O$$
$$R-CH-CHO \rightarrow HCOOH + R-CHO$$
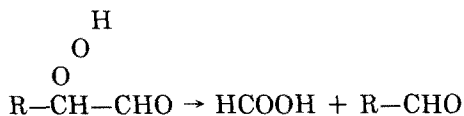
The objective of this study was to analyze the volatile acids formed from different oils under the conditions of this test and to relate this to the peroxide value in the oils and the conductivity of the solutions of exit gases.

EXPERIMENTAL

Sunflower, canola, olive, corn, peanut and soybean oils and lard were obtained from local stores. Butterfat was obtained from butter by melting, washing and filtration of the fat. Triolein was obtained from ICN Pharmaceuticals Inc., Cleveland, Ohio. Palm oil was refined, bleached and deodorized. The oils were oxidized in the automated AOM equipment described by deMan and deMan (4). In this method, the volatiles of 5 g oil heated at 100 C are collected in 100 ml distilled water. Aliquots of these solutions of volatile acids were neutralized with 0.01 N sodium hydroxide. The salt solution was evaporated to dryness in a vacuum oven at 70 C. The dried salts were reacted with 50 μl of boron trifluoride-butanol reagent at 70 C for 10 min. The butanol esters produced in this manner were taken up in hexane for analysis by GLC.

A Shimadzu GC-8AF instrument with flame ionization detector was used for the GLC analysis. The column was stainless steel, 2 mm $\times$ 2 m, packed with 10% SP1000 and 1% H_3PO_4 on Chromosorb W-AW, 100/120 mesh. Carrier gas flow rate was 12 ml/min. Column temperature was programmed from 80 to 150 C at 6 C/min. Lactic acid was used as internal standard. An example of a

chromatogram of the volatile acids in the form of their butyl esters is presented in Figure 1.

During the progress of the oxidation, samples were removed from the oil by means of a micro-syringe with a 15-cm needle. Sample size was 1-5 μl, and peroxide value was determined by the colorimetric method of Swoboda and Lea (7). The oil sample was dissolved in 2 ml acetic acid-chloroform (3:2) and reacted with 3 drops of potassium iodide solution (1.2 g/ml), for exactly 1 min, in the dark. Five ml 2.5% cadmium sulfate and 1 ml starch indicator were added and color measured with a Spectronic 70 instrument at 555 nm in a 1-cm cell.

RESULTS AND DISCUSSION

In the automated AOM procedure, the exit gases from the sample tube are led into a jar containing distilled water. The temperature of this water may fluctuate depending on room temperature. To investigate the effect of temperature on formic acid retention, solutions of
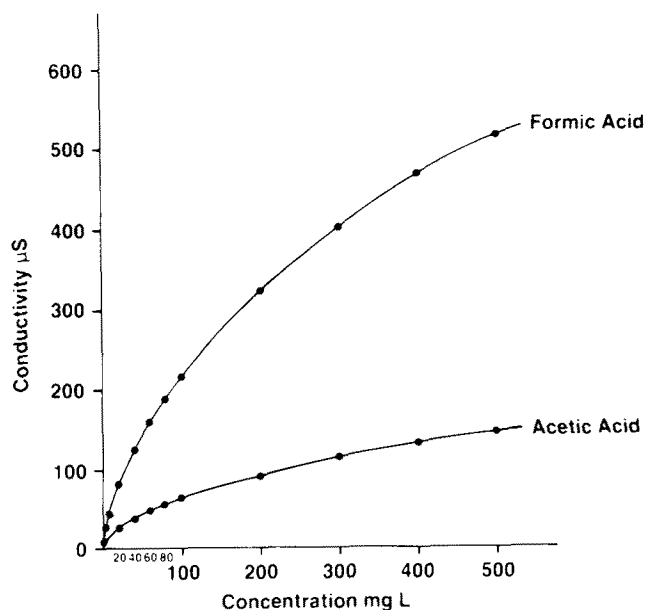

FIG. 2. Effect of formic and acetic acid concentrations on the conductivity of the exit gas solutions.

TABLE 2

Formation of Volatile Acids During Oxidation of Oils Expressed as μg/l and as % of Total Volatile Acids

| Oil	Oxidation time (hr)	Formic conc (μg/l)	%	Acetic conc (μg/l)	%	Propionic conc (μg/l)	%	Butyric conc (μg/l)	%	Valeric conc (μg/l)	%	Caproic conc (μg/l)	%
Sunflower	33.5	293	69.0	22.9	5.4	0.6	0.1	0	0	9.2	2.2	99.0	23.3
Canola	34.0	307	74.4	80.1	19.4	14.1	3.4	0	0	1.1	0.3	10.3	2.5
Olive	41.5	162	72.0	37.0	16.5	1.7	0.8	0	0	3.7	1.6	20.5	9.1
Lard	67.0	306	79.3	46.0	11.9	4.2	0.8	0	0	11.3	2.2	38.4	7.4
Butterfat	49.5	301	57.8	80.4	15.4	18.9	3.8	40.0	7.6	28.7	5.5	5.06	9.7
Triolein	42.0	331	71.8	80.0	17.3	8.3	1.8	11.9	2.6	8.4	1.8	21.5	4.7
Peanut	52.0	393	77.7	76.5	15.1	2.6	0.5	5.1	1.0	4.6	0.9	23.9	4.7
Soybean	34.0	475	77.4	81.6	13.3	13.2	2.1	5.4	0.9	3.2	0.5	35	5.8
Corn	47.0	357	81.1	43.5	9.9	0.7	0.2	0.9	0.2	3.5	0.8	34.7	7.9
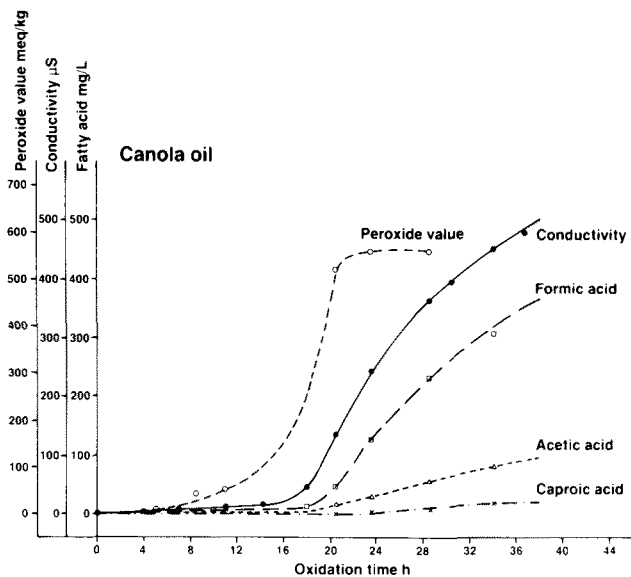
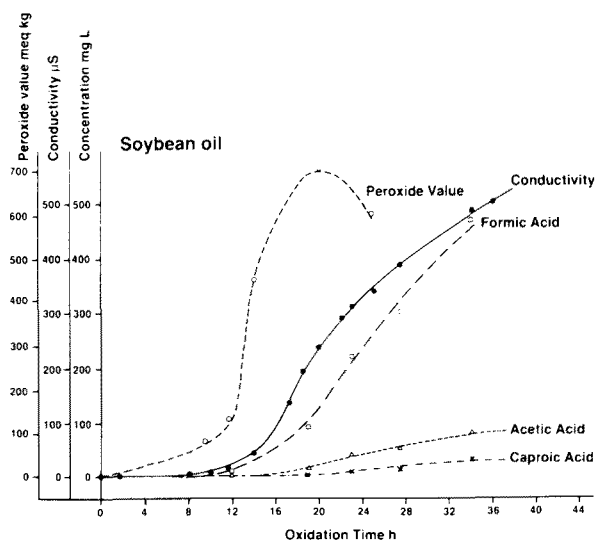FIG. 3. Canola oil peroxide value and conductivity and formic, acetic and caproic acid formation.

FIG. 4. Soybean oil peroxide value and conductivity and formic, acetic and caproic acid formation.

formic acid with a conductivity of about 300 μS were used. These solutions were kept at 10, 15, 20 and 25 C for various times while air was bubbled through at the same rate as used in the automated AOM test, 1.67 ml/s. When the solutions in the jars were kept at 10 C, there was no measurable change in conductivity after 24 hr. At a temperature of 15 C and after 30 hr, the conductivity decreased by 20 μS, after 72 hr by 50 μS. The most important temperature is 20 C because this would be close to room temperature at which the test usually would be conducted. The decrease in conductivity is indicated for a period of up to 60 hr in Table 1. The decrease during the first 10 hr is minimal and probably would have little or no effect on the outcome of the test. However, after 20 or more hr, serious losses of formic acid from the jars occur. This would be of importance for oils or fats with

high stability values. It might be advisable with such oils or fats to select a higher operating temperature so that measurement times would be shorter. A 10 C increase in temperature decreases the reaction time by a factor close to 2 (2). The problem was even more pronounced at 25 C. After 30 hr the conductivity decreased by 50%, and after 45 hr the decrease reached 100%. These results demonstrate the desirability of keeping the receiver jars at a constant temperature, preferably 15 C or lower. Following these findings, the equipment has been modified by placing the receiving jars in a tap water-cooled metal block.

Preliminary results of analysis for volatile acids in the receiving jars had indicated that in addition to formic acid, a series of other volatile acids—acetic, propionic, butyric, valeric and caproic acid, are formed in varying
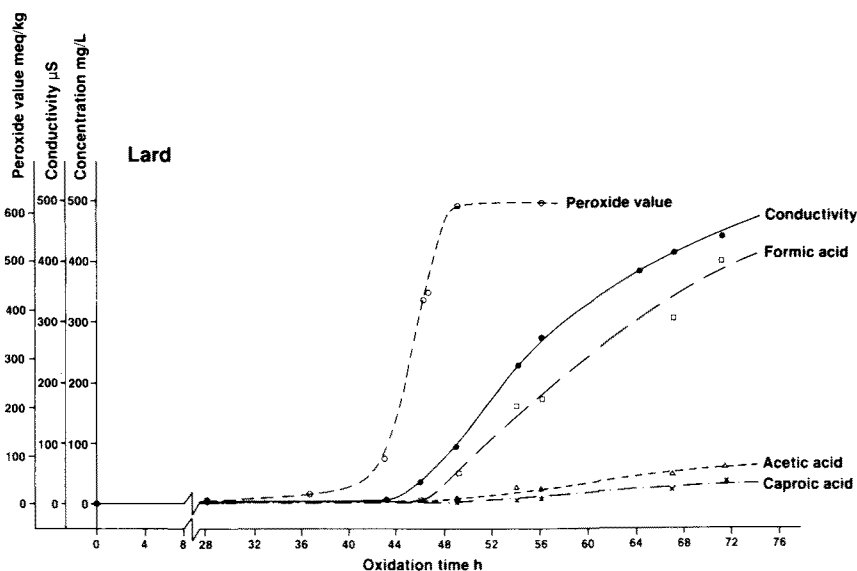

FIG. 5. Lard peroxide value and conductivity and formic, acetic and caproic acid formation.

amounts with different oils, although by far the largest proportion was formic acid. The contributions of formic and acetic acid to the increase in conductivity were measured and are shown in Figure 2. Formic acid has a much greater effect on the conductivity than has acetic acid. The contribution of the other acids to the conductivity is even less.

The oils and fats subjected to oxidation in the automated AOM equipment were analyzed for peroxide value during the course of the test and the solution of exit gases analyzed for volatile acids by gas liquid chromatography. The levels of volatile acids formed are listed in Table 2 for all of the oils at the end of the induction period. Formic, acetic and propionic acids were produced in largest quantity. Formic acid ranged from a low of 57.8% of total volatile acids in butterfat to a high of 81.1% in corn oil. Butyric acid was produced in significant amounts only in butterfat and triolein. With the exception of sunflower oil, acetic acid levels were higher than caproic acid levels. All of the oils produced some propionic acid, but only in canola and soybean oils, triolein and butterfat was the amount greater than 1% of total volatile acids. Valeric acid was produced from all of the oils, with sunflower and olive oil, triolein, lard and butterfat at levels of over 1% of total volatile acids.

The relationship between conductivity, peroxide value and volatile acid production was established for all of the oils. The information is presented for canola oil, soybean oil and lard in Figures 3, 4 and 5. The use of the micromethod for determination of the peroxide value made it possible to compare the two procedures under identical conditions. The relationship between the traditional method of determining oil stability by estimating the time required to reach a POV of 100 meq/kg and the automated method has been found to be close (3,4). Evaluation of traditional AOM time and stability obtained from conductivity measurements for all of the oils is presented in Table 3. These results confirm the close relationship between the two procedures and suggest that the automated method can be used as a valid alternative to the traditional AOM method.

TABLE 3

AOM Time as Determined by Peroxide Value and Conductivity Measurements

Oil	AOM time (POV)[a]	AOM time (conductivity)[b]
Sunflower	6.2	7.1
Canola	14.0	15.8
Olive	17.8	17.8
Corn	12.4	13.8
Peanut	21.1	21.5
Soybean	11.0	10.4
Triolein	8.1	7.4
Lard	42.7	43.2
Butterfat	2.8	2.0

[a]At peroxide value 100.
[b]At intercept of conductivity curve and time axis.

It appears from these results that the Loury hypothesis of formic acid formation (6) is applicable to all of the oils investigated. However, the presence of other acids, especially acetic and caproic, indicates the existence of some additional reaction patterns. The presence of acetic acid may indicate an attack on the β carbon of the aldehyde molecule, suggesting a more complex resonance equilibrium.

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