Hexanal Formation via Lipid Oxidation as a Function of Oxygen Concentration: Measurement and Kinetics

C.M. KOELSCH, T.W. DOWNES, and T.P. LABUZA

ABSTRACT

A system was developed to continuously quantify hexanal produced via lipid oxidation of oils at constant oxygen concentrations. Two kinetic models were derived from molecular mechanisms of oxidation to describe the initial (a cubic model) and accelerated (an extended model) stages of oxidation. The kinetic models illustrated the mechanistic influence of oxygen on rate constants and fit the data as well as simplistic curve fitting models. The monomolecular reaction phase showed the expected hyperbolic fit of inverse rate vs reciprocal oxygen concentration. The break point between initial and accelerated stages as function of oxygen concentration was represented by a logarithmic function.

INTRODUCTION

LIPID OXIDATION is one of the main deteriorative reactions in microbiologically stable dry and semi-moist foods (Lingnert and Eriksson, 1980). The kinetics of lipid oxidation through a free radical mechanism have been described in detail (Dugan, 1976, Frankel et al., 1981; Karel, 1986; Labuza, 1971; Nawar, 1985; Paquette et al., 1985a, b). The classical autoxidation scheme is commonly employed to illustrate the molecular mechanisms in which lipid oxidation is divided into initiation, propagation and termination steps. Kinetics of lipid oxidation through a free radical mechanism in the initiation and propagation stages have been described using both mechanistic equations and statistically based zero order and exponential curve fitting equations respectively (Labuza, 1971; Hall et al., 1985).

The time at which the initial stage of monomolecular hydroperoxide decomposition catalyzed oxidation ends and the more rapid bimolecular catalyzed phase begins is defined as the break point (Tb). Tb is generally near the threshold point of a sensorially unacetable product (Labuza, 1971). The threshold point occurs when the peroxide volatile breakdown products reach a high enough level in the headspace to cause an off odor (Nawar, 1985). This usually occurs prior to the maximum peroxide value (Labuza, 1971).

Linoelic acid, a predominant unsaturated fatty acid in many food products, can be oxidized to hexanal, octanal, and 2,4 decadienal which further decomposes into hexanal. These headspace volatiles and especially hexanal have been commonly used to gauge the extent of lipid oxidation. The odor threshold value of hexanal in cereals is approximately 0.15 ppm (Fritsch and Gale, 1976).

Numerous solutions to reduce lipid oxidation include adjusting the water activity of dry foods close to the monolayer, adding antioxidants, reducing the amount of unsaturated fat added, degassing to remove residual oxygen and gas flushing with nitrogen or vacuum packaging. A good barrier to both oxygen and water vapor is then required to maintain desirable conditions. To further minimize the reaction, exposure to high temperature and light during distribution must be reduced (Quast and Karel, 1972).

Maintaining low oxygen levels within a package is a viable approach to retard lipid oxidation (Deobald and McLemore, 1964). However, the presence of minimal (in some cases as low as 1%) oxygen in the package headspace can lead to product quality losses since the amount of oxidized fat which will produce off-odor is generally assumed to be very small yet little data exist (Tamsma et al., 1964; Labuza, 1971). Thus, to accurately assess the stability of a food product to lipid oxidation, strict control of oxygen concentration under test conditions is required to determine what level of oxygen could be tolerated, within economic limits, to give the desired shelf life.

Unbret et al. (1964) described a closed system to quantify the rate of oxygen consumption using a Warburg apparatus. In the Warburg system, the level of oxygen decreases as it is consumed by the substrate and thus the reaction rate becomes dependent upon the new oxygen level, the achieved peroxide level and amount of remaining oxidizable substrate. (especially below 5% oxygen, Frankel et al., 1981; Karel, 1986). Quast and Karel (1972) somewhat alleviated the problem of changing oxygen concentrations by periodically injecting air or the desired oxygen level at selected time intervals into the Warburg flasks. However, the oxygen concentration still fluctuated within the test cell due to time intervals between oxygen replacements. Hall et al. (1985) developed another system to allow for a high and constant oxygen concentration flow through the test cell. They did not study low oxygen levels. Amount of headspace volatiles produced from samples within the test cells was used as an index of lipid oxidation in both systems.

The purpose of our study was to develop a continuous system to quantify lipid oxidation at constant oxygen concentrations by continuously monitoring the rate of hexanal formation as an index of lipid oxidation. Data were then analyzed based on both curve fitting equations and kinetic models developed for the molecular mechanism of hexanal formation as a function of oxygen concentration in the headspace.

Materials & Methods

Model system

A freeze-dried model system was composed of soybean oil as oxidizable substrate (8.38 g soy oil, Hain Pure Food Company, Los Angeles, CA), distilled and deionized water (59.06 mL), Tween 20 (0.08 g), and microcrystalline cellulose (32.45 g, CMC PH 101 Food Manufacturing Corp., Philadelphia, PA.). Ingredients were mixed sequentially at high speed in a domestic blender. Initial moisture content (dry basis) was determined gravimetrically using a modification of the AOAC procedure (AOAC, 1985; Koelsch, 1989). Moisture sorption isotherm data for triplicate samples were measured gravimetrically by measuring product weight change at seven relative humidities created by salt solutions (Rockland and Nishi, 1980). Equilibrium was reached when weight change (on a dry basis) did not exceed 0.1% (Labuza, 1984). Moisture content was 2g H₂O/100g solids. This is below the calculated BET monolayer value of 3.05g H₂O/100 g solids and in a range which would increase lipid oxidation rates (Labuza, 1971).

Constant oxygen concentration apparatus:

The apparatus shown in Fig. 1 was developed to regulate oxygen, relative humidity, temperature, and light exposure and to continuously...
nitrogen
oxygen
Fig. 1—Schematic of apparatus for maintaining constant oxygen concentration with Tenax traps to continuously measure hexanal formation.

quantify headspace hexanal produced from lipid oxidation. All experiments conducted at various oxygen concentrations were done at 23 ± 1% relative humidity, 23 ± 2°C in the absence of light. Swagelok fittings (Crawford Fitting Co., Solon, OH) were used to connect the copper tubing, rotameters (Cole Palmer, Chicago, IL) and glass test cells (modified 40/50 gas washing bottle, Ace Glass Inc., Vineland, NJ).

In the apparatus, nitrogen gas was humidified to a controlled relative humidity and pure oxygen was diluted and mixed with it. An exhaust valve continuously released a controlled amount of oxygen/nitrogen mixture which allowed for sufficient volume in the gas stream for a second dilution with humidified nitrogen gas. The two dilutions provided the necessary accuracy and precision to maintain low and constant oxygen concentrations. Relative humidity (Hygrodynamics, Silver Springs, MD.) and oxygen concentration sensors (Servomex, London, England) measured the gas mixtures prior to oxygen/nitrogen flow into the oxidation test cell. Two test cells were used for continuous headspace sampling to ensure the amount of oxidizable substrate was the limiting factor at all oxygen concentrations. Prior to testing the system at each oxygen concentration, the gas flow was channeled through an equilibration cell. The oxygen concentration was considered stable if it did not fluctuate more than 1% and relative humidity 0.1% over 2 hr. Once stabilized, the product was placed in the test cell and a gas flow of 48 mL/min was channeled through this cell.

Gas flow out of the cell was filtered through a Tenax trap (0.4 g of 80/100 mesh Tenax, Ohio Valley Specialty Chemical). At predetermined time intervals, flow from the cell was switched to an alternate Tenax trap and the sample Tenax tube removed for analysis. To extract hexanal from the Tenax trap, 1.5-2.0 mL of HPLC grade 2-methylbutane (Aldrich Chemical Co., Milwaukee, WI.) was dripped through the filled capillary tube and collected by centrifuging at 750 rpm (International Equipment Co., Boston, MA.). The extractant was concentrated under nitrogen to a 0.5 mL. Recovery studies in triplicate showed a 74 ± 4% recovery of hexanal which was used as a correction factor.

Helium flow was 31 to 33 mL/min. A calibration factor ($C_0$) of hexanal was determined by use of an external standard.

Derivation of kinetic models

Mathematical models based on the molecular mechanisms of oxidation for production of hexanal, were derived for both initial monomolecular hydroperoxide decomposition period and the more rapid bimolecular stage of oxidation. In general, the rate at which the oxygen reacts with unsaturated lipid, as a function of oxygen pressure is:

$$\frac{dO_2}{dt} = \frac{[ROH]^2[RH][O_2]}{k_1[RH]+k_2[O_2]}$$

where $[O_2]$ is the oxygen concentration, $[RH]$ the substrate concentration, $k_1$ and $k_2$ are constants, $R_i$ is the rate of initiation and is equal to $k_{init}[ROOH]$ for the initial monomolecular stage of peroxide $[ROOH]$, decomposition. As each oxygen molecular reacts in the propagation steps, one peroxide molecule, $[ROOH]$, forms, thus:

$$\frac{d[ROOH]}{dt} = \frac{[ROH]^2[RH][O_2]}{k_1[RH]+k_2[O_2]}$$

As shown by Labuza (1971) at high levels of oxygen in the headspace, $k_1[RH] << k_2[O_2]$ and thus the rate is independent of oxygen concentration so that:

$$\frac{d[ROOH]}{dt} = k_4[ROOH]^{1/2}$$

where $k_4$ is the overall rate constant for the monomolecular period. This takes into account all the rate constant terms and should be independent of oxygen level in this upper range. At low levels of the extent of oxidation, i.e. when the substrate concentration does not change significantly, rate of formation of peroxides as a function of time is thus found by integration of Eq. (3) to give (Maloney, 1966; Labuza, 1971):

$$[ROOH]^{1/2} = \frac{k_4}{2} t = k_m t$$

In this case $k_m$ is now the rate constant in units of (concentration)$^{1/2}$. 

Volume 56, No. 3, 1991—JOURNAL OF FOOD SCIENCE—817
ODXIDATION AS FUNCTION OF O₂ CONC...
Fig. 2—Hexanal formation as function of time for oxidation of soybean oil at 23°C and four levels of constant oxygen concentration.

Fig. 3—Cube root of hexanal formed vs time (Eq. 6) for initial stage of monomolecular hydroperoxide decomposition during oxidation of soybean oil at 23°C for different oxygen concentrations. Statistical fit results given in Table 1.

Fig. 4—Hexanal concentration vs normalized time (T−Tr,J for rapid bimolecular hydroperoxide decomposition period during oxidation of soybean oil at 23°C for different oxygen concentrations. Solid lines represent fit of data to Eq. (9) based on the mechanism. Statistical fit results given in Table 2.

Table 1—Rate constants for hexanal formation during monomolecular stage of lipid oxidation

<table>
<thead>
<tr>
<th>%O₂</th>
<th>Cubic kinetic model (Eq. 6)</th>
<th>Zero order (Eq. 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k₆ x 10³ ppm/hr</td>
<td>k₅ x 10³ ppm/hr</td>
</tr>
<tr>
<td>%</td>
<td>95% confidence limits</td>
<td>95% confidence limits</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>r²</td>
</tr>
<tr>
<td>1.2</td>
<td>0.77 ± 0.27</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>4.5</td>
<td>2.68 ± 11.13</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>10.0</td>
<td>2.26 ± 0.09</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>15.4</td>
<td>3.76 ± 22.30</td>
<td>0.82 ± 0.02</td>
</tr>
</tbody>
</table>

* Standard error

Table 2—Rate constants for hexanal formation during accelerated stage of lipid oxidation

<table>
<thead>
<tr>
<th>%O₂</th>
<th>Kinetic model (Eq. 9)</th>
<th>Exponential (Eq. 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k₇ x 10³ hr⁻¹</td>
<td>k₈ x 10³ hr⁻¹</td>
</tr>
<tr>
<td></td>
<td>95% confidence limits</td>
<td>95% confidence limits</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>r²</td>
</tr>
<tr>
<td>1.2</td>
<td>12.11 ± 3.95</td>
<td>7.89 ± 3.96</td>
</tr>
<tr>
<td>4.5</td>
<td>24.23 ± 9.12</td>
<td>8.74 ± 9.12</td>
</tr>
<tr>
<td>10.0</td>
<td>13.73 ± 0.07</td>
<td>10.74 ± 0.07</td>
</tr>
<tr>
<td>15.4</td>
<td>10.89 ± 0.87</td>
<td>17.77 ± 0.87</td>
</tr>
</tbody>
</table>

* Standard error

RESULTS

THE CONSTANT oxygen concentration apparatus performed as designed to maintain low oxygen concentration at constant relative humidity, light, and temperature conditions for continuously quantifying hexanal formation. The concentration of hexanal (ppm, w/w) in soybean oil as a function of time for 1.2, 4.5, 10.0 and 15.4 % oxygen is presented in Fig. 2. Figure 3 and 4 show representative plots for equations (5) and (9) based on kinetic models for monomolecular and bimolecular hydroperoxide decomposition regions at the four constant oxygen levels tested. The constants calculated for each of the kinetic expressions are presented in Tables 1 and 2 for the initial and accelerated phases respectively.

As shown in Table 1, the more kinetically appropriate cubic model and zero order models provided statistically comparable fits. The r² values ranged from 0.82 to 0.95 and 0.88 to 0.99 for the cubic and zero order models respectively. The standard error was of the same order of magnitude for both models. The cubic model of eq. (6) represents a mechanistic basis by which oxidation can be followed and predicted through the monomolecular oxidation phase, and as seen, is operable over the whole oxygen concentration range studied. Although a simple statistical model may fit better (higher r²), from a kinetics standpoint is better to use a mechanistic model, since the components of that model have some theoretical basis and can be tested under different conditions such as for oxygen concentration dependence, as we have done, or temperature dependence based on the Arrhenius relationship.

The reciprocal of the rate constant for hexanal production (k₇) from the derived kinetic model for the initial monomolecular stage of oxidation is plotted against the reciprocal of oxygen concentration in Fig. 5 as a demonstration of Eq. (13b). As seen, the function fits over the whole oxygen concentration range studied.
LIPID OXIDATION AS FUNCTION OF O₂ CONC.

Figure 5—Hyperbolic plot of inverse of overall rate constant, $k_o$, for formation of hexanal during monomolecular initial oxidation stage vs inverse oxygen concentration showing fit of Eq. (13b) and dependence of rate constant on oxygen concentration.

Figure 6—Hyperbolic plot of inverse of overall rate constant, $k_r$, for formation of hexanal during rapid bimolecular stage of oxidation vs inverse oxygen concentration showing lack of fit of Eq. (18) which theoretically should give a straight line.

Table 2 shows results for the accelerated stage of oxidation. Neither the kinetically based model or exponential equation provided a good fit for the data. The large confidence intervals associated with both the kinetic and exponential constants suggested need for more data. Theoretically $k_r$ should be independent of oxygen level but at 10% oxygen, the value was lower than the other oxygen levels. Again with only four test levels of oxygen, and with the lower number of points available in the rapid oxidation period, the differences of the values of $k_r$ may be not significant. An additional reason for this difference could be that the rate constants cannot be determined separately by the non-linear regression technique, so any error in estimation of $k_r$ the oxygen dependent term would impact on the value of $k_r$.

Figure 6 shows the reciprocal of $k_r$ plotted versus the reciprocal of the oxygen concentration. The results did not fit the expected straight line for the hyperbolic function of Eq. (18). Above some critical level of oxygen, $1/k_r$ should be constant unless there was no critical level, otherwise it should increase, as was seen for the oxygen dependent constant in the monomolecular period. In this case between 10% and 1% there was an inverse function, exactly opposite that which the equation predicted, i.e. $k_r$ decreased as oxygen concentration increased. Reasons for lack of expected fit could be that breakdown of peroxides, into secondary products like hexanal as well as further reactions of hexanal and peroxides became significant. Since $k_r$ was not constant, there was a suggestion that this had occurred. In addition, as noted above, the lower number of data points at any oxygen level meant that the non-linear fitting routine may converge on a set of numbers that fits the line, as does the non-mechanistic exponential equation, however those sets of numbers (the rate constants) have no physical meaning. Published reports in food science kinetics have many examples of such improper fits (Stamp, 1990). More data in the 1 to 10% oxygen range is needed.

The breakpoint between the two stages of oxidation was found by setting the equations for each stage equal to each other and solving for break time. Again if the above equations are followed, the level of hexanal at the breakpoint, should be independent of oxygen level. An empirical logarithmic relation as shown in Fig. 7 was found with break point hexanal level increasing as oxygen level decreased. The break point determined from the kinetic models more closely fit the logarithmic plot, $r^2 = 0.93$, than the curve fitting equations with $r^2 = 0.76$. Thus the kinetic models were more appropriate to predict break points at any oxygen concentration between 1.2 and 15.4%. Since the break point should be close to the end of shelf life of a food, this would be useful as a prediction model for any other oxygen level. These results also indicated that oxidation is very complex, and to predict stability of foods more data at many oxygen levels will be needed.

REFERENCES


Continued on page 834