ARTICLE

Determination of Oxidative Stability of Lipids in Solid Samples

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ABSTRACT: A gas-phase flow injection analysis (FIA) method for the direct determination of the oxidative stability of solid fat/oil samples is described. Samples are confined with a low level of oxygen in a reactor of adjustable temperature. The oxygen consumption by the sample is automatically monitored after a preset period. The temperature-dependent data exhibit Arrhenius behavior. Normally, it is difficult to directly determine the stability at ambient temperatures because of inordinately long time requirements. The close correspondence to Arrhenius behavior makes it possible to use the results obtained at higher temperature to calculate the stability of samples at lower temperature conditions, such as at ambient storage temperature. The effects of sample size, sample particle size, sample fat content and the reproducibility of the method over time were studied using synthetic and bone meal samples. The oxygen consumption was found to be linearly dependent on the amount of sample taken, inversely dependent on the particle diameter, and independent of the exact lipid content, given some minimum lipid content. The results exhibited high day-to-day reproducibility. The gas-phase FIA system developed in this work is easy to operate. Compared with the currently used method, the sample throughput rate is much faster (2–3 h for complete multitemperature characterization of a sample) and the sample requirement is much lower (~1 g). Furthermore, it eliminates the need to extract and recover the fat from the sample for further processing as is required by the other methods commonly used to measure oxidative stability.

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KEY WORDS: Active oxygen, oil/fat oxidation, oxidative stability, oxidative stability index.

Solid samples containing fat and oil span a wide variety, from milk powder to potato chips to animal feed. The lipids in such samples are gradually oxidized when exposed to air. This process mainly involves the oxidation of unsaturated fatty acids (1). Hydroperoxides are the primary products; these degrade further to secondary products such as aldehydes, ketones, and carboxylic acids (2). The formation of these chemicals is related to rancid odor, loss of nutritional value, and generation of toxic compounds (3–5) that are known to contribute to diseases and to accelerate the aging process (6–8).

There is no standard method for the direct determination of lipid oxidative stability in solid samples. Methods presently used involve multiple steps. Typically, the fat/oil present in the solid sample is extracted first. A mixed organic solvent (petroleum ether/methanol; 1:1, vol/vol) may be used to triple-extract the fat. The extract is then washed with deionized water. The washed extract is dried in a warm water bath (typically 40°C). Finally, the oxidative stability of the extracted fat is determined by some standard method such as the active oxygen method (AOM) as described in AOCS Method Cd 12-57 (9) or the oxidation stability index (OSI) as described in AOCS Method Cd 12b-92 (9). The first step requires about 5 h, and a typical AOM or OSI determination can require 20–40 h.

This approach is flawed in numerous ways. It is difficult to select a proper polarity of the extraction solvent. If the solvent is not sufficiently polar, the peroxides already formed will not be extracted. If it is too polar, the peroxides will be preferentially extracted over the fat and the analytical result will be biased. The sample must be extracted at room temperature to avoid heating and oxidizing the fat during extraction or decomposing the peroxides already present. This eliminates the use of a Soxhlet extractor. There is no guarantee that three extractions are sufficient to fully extract both the fat and the peroxides. Once extraction is complete, the solvent has to be evaporated with a rotary evaporator at low temperature to avoid oxidizing the fat. Finally, the solvent has to be fully evaporated to obtain an accurate weight of the fat.

There also is the potential that extracting fat from a solid sample may not provide an accurate picture of the actual stability of the original sample. The lipid may be preferentially located within the interior of the sample matrix and thus be less sensitive to oxidation than extraction and subsequent determination may suggest. In addition, a basic problem with the AOM and the OSI is that lipid stability is determined at a fixed temperature (98°C). Although this is far above ambient storage temperature, this temperature is used because it takes unacceptably long to make meaningful measurements at ambient temperatures. The extrapolation of such data in making decisions about the comparative stability of two different types of samples under ambient storage conditions makes the tenuous assumption that the activation energies for the lipid oxidation in the two samples are the same. It has been shown that the rate of lipid oxidation can be dependent on whether the sample is ground or whole and whether the lipid is iso-

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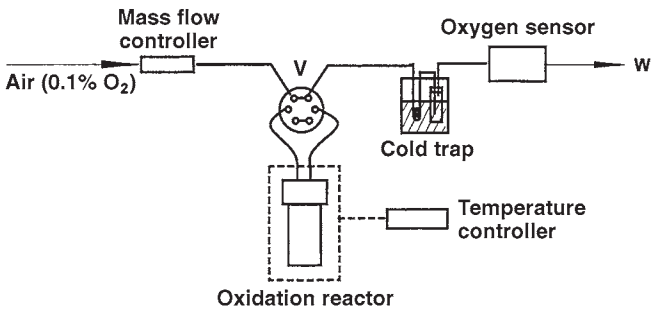
lated from the sample and then examined by itself (10). The assumption of identical activation energy between two different samples is an oversimplification due to the lack of better analytical methods.

More recently, a gas-phase flow injection analysis (FIA) system was developed in our laboratory to determine the oxidative stability of fat/oil samples (11). This is an automated stopped-flow gas-phase FIA system with an oxygen sensor and a programmable temperature reactor that measures the oxygen consumption of the same sample at various programmed temperatures. For all samples examined to date, the oxygen consumption exhibits Arrhenius behavior [in that log(oxygen consumption) is linearly proportional to reciprocal temperature], allowing the extrapolation of the results to any storage temperature. Our experiments also showed that the relative stability order of different samples is temperature dependent, and therefore the determination of stability at some fixed elevated temperature, e.g., 98°C, as carried out in the AOM or OSI, may not be meaningful. With liquid samples or sample extracts studied in the previous work [11], we found for example, peanut oil oxidizes faster than "vegetable" oil at ≤70°C but the situation is opposite at temperatures ≥125°C.

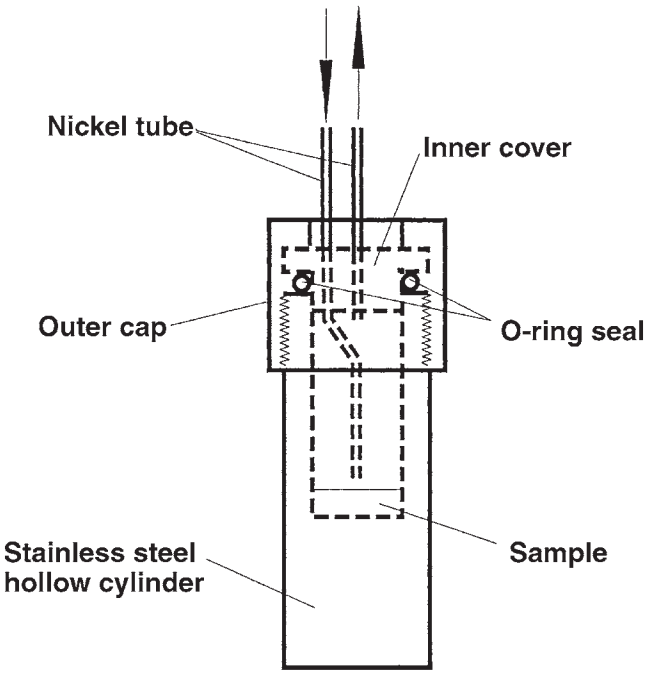
In the present paper, we report the applicability of this principle to solid samples with a newly designed reactor that can conveniently accommodate solid samples. With this reactor, the oxidative stability analysis for a solid requires only a 1-g sample, one to two orders of magnitude less than the sample requirement of conventional procedures using the traditional extraction procedure.

EXPERIMENTAL PROCEDURES

The gas-phase FIA system is shown in Scheme 1. The carrier gas flows through a mass flow controller (MFC) and through an electropneumatically operated six-port stainless-steel high-performance liquid chromatography (HPLC)-type valve V (model 7000; Rheodyne, Cotati, CA). The loop of V holds the oxidation reactor (OR), shown in Scheme 2. It is composed of a stainless-steel hollow cylinder (exterior dimensions: 155 × 33 mm, interior dimensions: 55 × 20 mm), an outer cap (o.d., 39 mm; height: 18 mm), and an inner cover insert that ensures a good seal and reduces the reactor volume. An O-ring seal is used to seal the system. The reactor is provided with 1 mm i.d. Ni inlet and outlet tubes. Two heating tapes (128 × 50 mm, 50 W at 110 V; Thermolyne, Dubuque, IA) were connected in parallel and secured around OR with glass wool thread. A platinum resistive thermometric device (RTD) was placed between the heating tape and the metal exterior of OR. The reactor temperature was programmed with a temperature controller (CN8500; Omega Engineering, Stamford, CT). The solid sample can be readily put into the reactor from the top of the stainless-steel hollow cylinder.



SCHEME 1



SCHEME 2

In Scheme 1, a cold trap is imposed between the valve V and the oxygen sensor (model 2550; Illinois Instruments, Napierville, IL). The trap consists of a 600-mL beaker filled with water in which a coil of Ni tubing (1.6 mm o.d.; 1.0 mm i.d.; 400-mm long; coiled into a 32-mm diameter coil) is immersed, followed by a "buffer" glass tube (8.6 mm i.d.; 10.2 mm o.d.; 180 mm long). The cold trap allows the oil vapor to condense and prevents premature poisoning of the oxygen sensor. The trap is periodically cleaned with acetone to remove oil vapor and thoroughly dried before use. The sensor output was acquired by a personal computer (Gateway 2000, P-75; San Diego, CA) equipped with a DAS-1601 data acquisition board (Keithley-Metrabyte, Taunton, MA). All tubing used in the gas flow system is 1-mm i.d. Ni tubes. A home-built digital timer was used to control the switching of the V and hence the residence time of the oxygen-bearing carrier gas in OR. A commercial version of the system is available from AnalTech (Lubbock, TX).

Synthetic solid samples of varying lipid content were prepared by using silica gel as the carrier matrix (100–200 mesh, ACS Reagent grade; Fisher, Fairlawn, NJ). It should be noted that many silica gel samples contain metallic impurities that appear to greatly accelerate the lipid oxidation rates. Washing

the silica gel with sodium hydroxide and then washing it copiously with water appears to be effective in deactivating the catalytic sites. The silica gel was taken in a conical flask and doped uniformly with a known amount of a solution of cottonseed oil in petroleum ether. The ether was then allowed to evaporate at room temperature with a gently flowing air stream. To avoid the catalytic site problem altogether, solid samples of varying particle size containing olive oil were prepared using glass beads (ACE Scientific Supply Co., Linden, NJ) as the carrier matrix using the same procedure.

Bone meal samples used in this study were supplied by Novus International (St. Louis, MO). One sample was sieved to produce different particle sizes (d_p: 0.25–0.60, 0.60–0.85, and >0.85 mm, hereafter referred to as small, medium, and large particles). The carrier gas containing 0.1 % O_2 (balance N_2) was purchased from TRIGAS Industrial Gases (Lubbock, TX).

Procedure. To determine oxidative stability, 1–2 g of the solid sample was loaded in to the reactor from the top. The reactor was tapped or shaken slightly to smooth and level the sample surface. The system was run with the following parameters: carrier gas (0.1% O_2, 99.9% N_2) flow rate 35 cm^3/min; reactor flush time to introduce fresh gas: 1 min minimum and reactor residence time 5 min. The system was run at least 20–30 min at the first temperature point before any data were acquired.

RESULTS AND DISCUSSION

Performance and reproducibility. Figure 1 shows the relationship between log(oxygen consumption) and the reciprocal of the absolute temperature for a set of bone meal samples as determined by the present system. The linear r^2 values and the

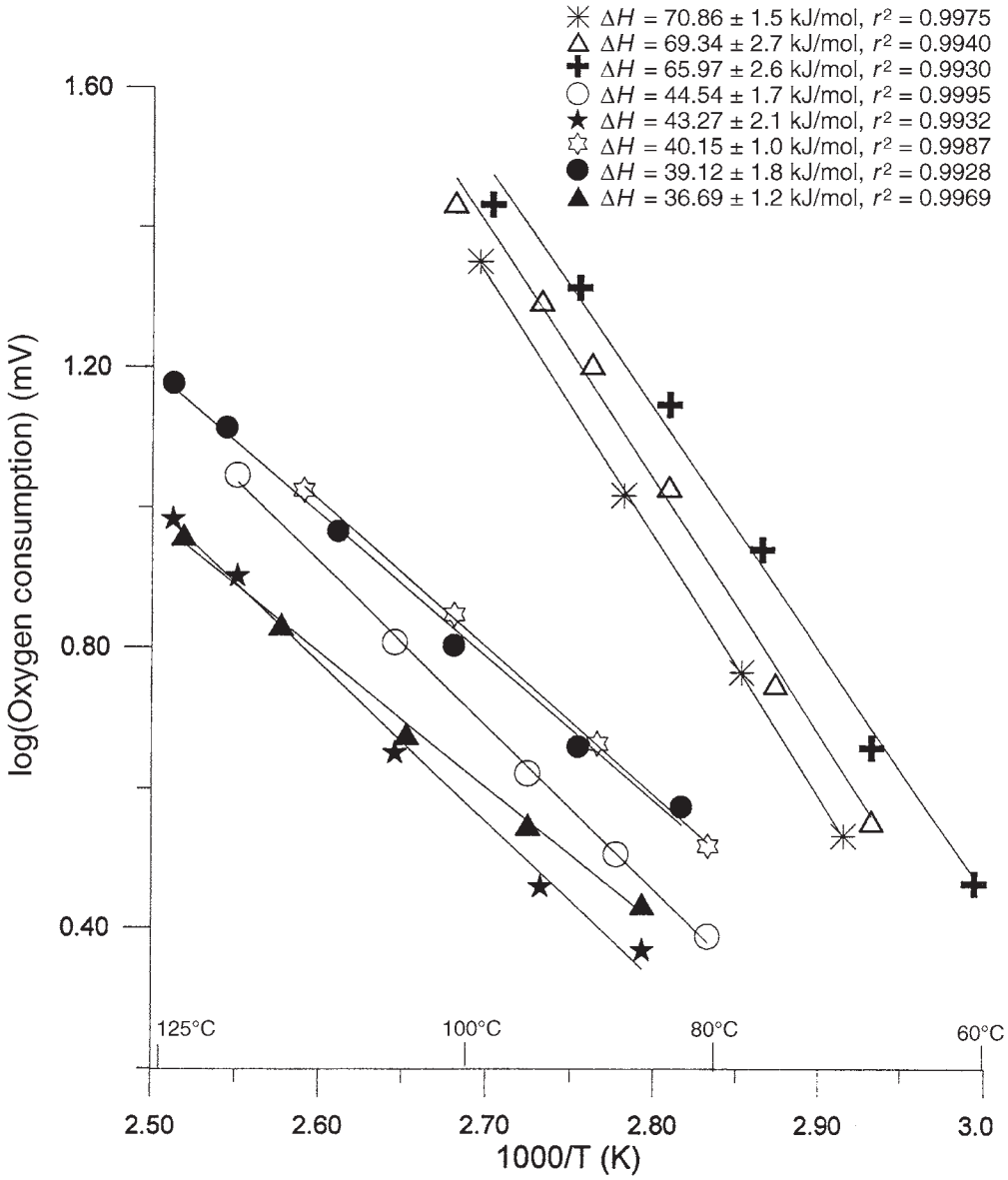


FIG. 1. Arrhenius plots for the oxidative stability of some bone meal samples.

corresponding Δ*H* values are also shown in the figure. For all samples studied, the oxygen consumption exhibited Arrhenius behavior. The samples fall into two different groups. It is interesting to note that the group of three samples that exhibit much greater oxygen consumption and greater Δ*H* values were known to have already turned rancid from their characteristic odor.

To illustrate the reproducibility of the system, repeat experimental data for three samples are shown in Figure 2. It would appear that the reproducibility of the system for the oxidative stability determination of a solid is very good.

Under our experimental conditions, the amount of sample taken occupies a relatively shallow layer in the reactor (about <7.5 mm in depth), such that the gas atop the sample has ready access to the sample surface. As a result, the extent of oxygen consumption for a bone meal sample is observed to be linearly dependent on the amount of the sample taken with a statistically zero intercept, as shown in Figure 3 (reactor temperature 135°C, residence time 5 min). Note that for this reactor (cross-sectional area 3.14 cm^2 and total volume for sample plus gas 16 cm^3), we observe that above a sample amount of ~2 g, the reproducibility decreases markedly. This is likely related to the access of the gas in the reactor to the entire sample surface. The reaction between the oxygen in the carrier and the lipid in the solid sample is a heterogeneous two-phase reaction. If the depth of the sample is too large or if it is loaded in a tight and compacted fashion, the accessibility of the gas to the sample surface will vary from run to run and affect the results. This situation is exacerbated when the particle size is small and the sample is prone to being packed tightly. When the sample amount is small, the sample occupies a relatively thin layer in the reactor, the superincumbent gas has ready access to the sample surface, and there are no problems due to limited access of the oxygen to the entirety of the sample. It is interesting to note that for liquid samples loaded into the reactor, the amount of the sample has no effect on the rate of oxygen consumption because the available surface for interaction is only the top surface and, within experimental error, has little influence on the oxygen concentration.

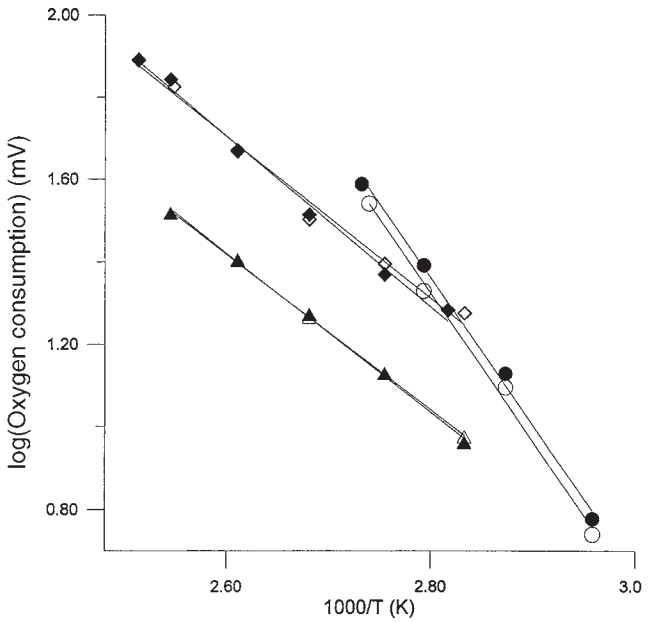


FIG. 2. Reproducibility of the experimental system for repeat runs on three samples on two different days. Open and closed symbols, respectively, represent runs on different days. Sample weights for circles and triangles, 1.0 g; for diamonds, 2.0 g.

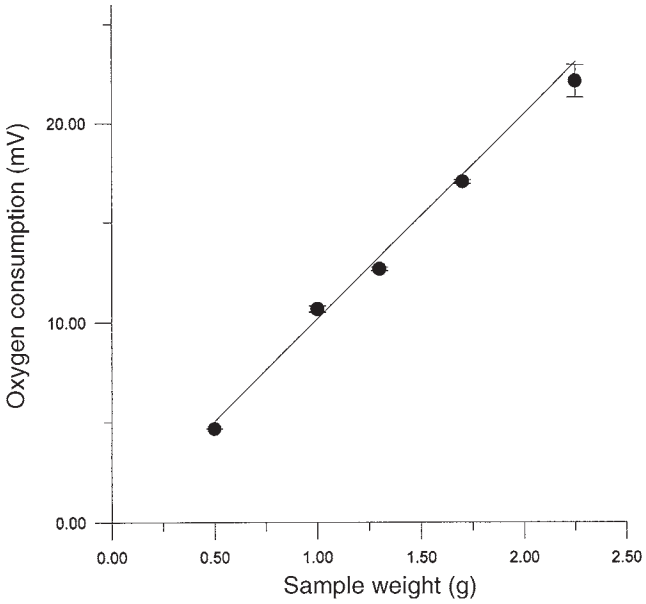


FIG. 3. The dependence of oxygen consumption on sample amount taken. Data points are means ± SD; $n = 6$.

Choice of oxygen concentration. In our previous work (11), it was established that the absolute amount of oxygen consumed depends in a first-order fashion on the oxygen concentration. As such, the quality or the nature of the kinetic or thermodynamic data obtained is not dependent on the precise value of the oxygen concentration. As long as either the O_2 concentration used is accurately known, or all experiments are conducted at the same concentration, data for different samples can be compared on a common basis. The advantages of using a low oxygen concentration are as follows: (i) long lifetime of the galvanic sensor, (ii) better sensor response linearity, and (iii) minimal change of sample composition due to oxidation, allowing reproducible multitemperature runs on the same sample aliquot.

Effect of the sample lipid content. Four silica gel samples containing 3.4, 6.3, 9.1, and 12% of cottonseed oil by weight were studied. This represents the typical range of minimum lipid content in many samples of interest. The oxygen consumption rates were measured at 130°C for a residence time of 12 min. The experimental results in Figure 4 show that, in the range studied, within reasonable limits, the lipid content of the samples has no effect on the oxygen consumption. (Of course, we do find that if the silica is not doped with the oil at all, there is no oxygen consumption.) Similar results were initially found with bone meal samples. But to avoid the influence of other confounding factors, we decided to carry out the

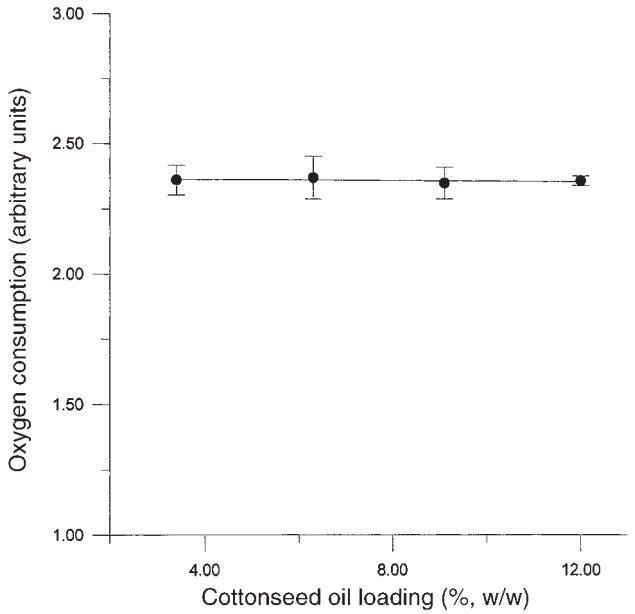


FIG. 4. Oxygen consumption is generally independent of sample lipid content, given some minimum lipid content. Data points are means ± SD; $n = 6$.

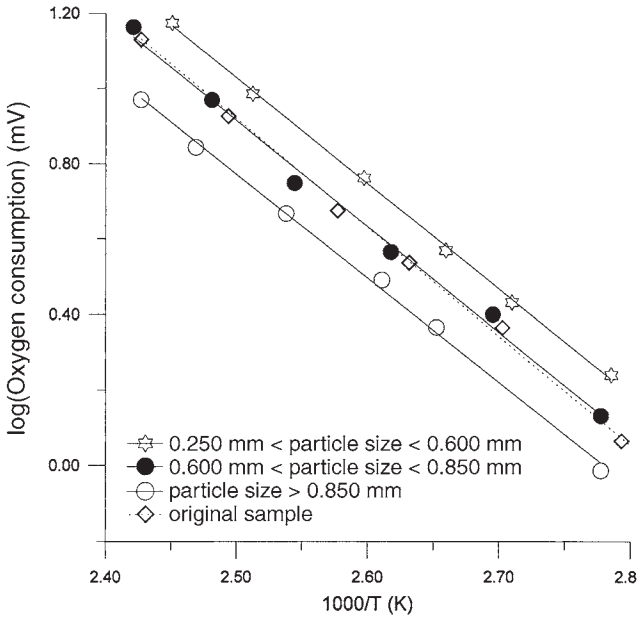


FIG. 5. Arrhenius plots for the oxidative stability of some bone meal samples as a function of particle size.

experiments with the synthetic samples where particle size could be controlled while the lipid content was varied. These observations suggest that, given some minimum lipid content sufficient to coat the surface, the rate of oxygen consumption is limited by other factors, such as accessible surface area.

Effect of the particle size of samples. The chemical reaction between oxygen and the lipids occurs on the surface of sample particles. The overall reaction rate should therefore be directly related to the total surface area of the sample taken. Given the same sample mass, particle size governs the available surface area. So, the particle size should be related to the oxidative stability of a sample. Figure 5 shows the experimental results for four bone meal samples, an original composite mixture, and three sieved fractions derived from it. As may be expected, the oxygen consumption rate is in the order 0.250–0.600 mm > 0.600–0.850 mm > 0.850 mm. The oxygen consumption rate decreases with increasing particle size. It is interesting to note that the behavior of the original, unfractionated sample is virtually the same as that of the intermediate particle size. When samples are extracted and the stability of the extracted lipid removed, any influence of particle size is also removed. In actuality, the lipid oxidation rate and stability do depend on particle size and the present instrument does indicate that. Our feeling is that samples should be studied in the form they are stored, without further grinding or processing, unless it is of interest to compare the intrinsic stabilities of two sample types, in which case one must use a similar particle size to perform meaningful comparison.

A second set of experiments was conducted with synthetic samples of uniform particle size glass beads, in four different size ranges. The lipid contents of all the samples were held constant at 2.0% by weight olive oil. The oxygen consumption rate was measured at 120°C and was found to be in the order 0.12–0.16 mm > 0.25–0.30 mm > 0.45–0.50 mm > 1.0–1.05 mm particle diameter. This represents the same pattern as the bone meal samples. If we take the mean diameter in each size class to be the geometric mean of the range extremities, the oxygen consumption rate is observed to be linearly related to the reciprocal of the mean diameter of the sample particles for both types of samples (with linear r^2 values of

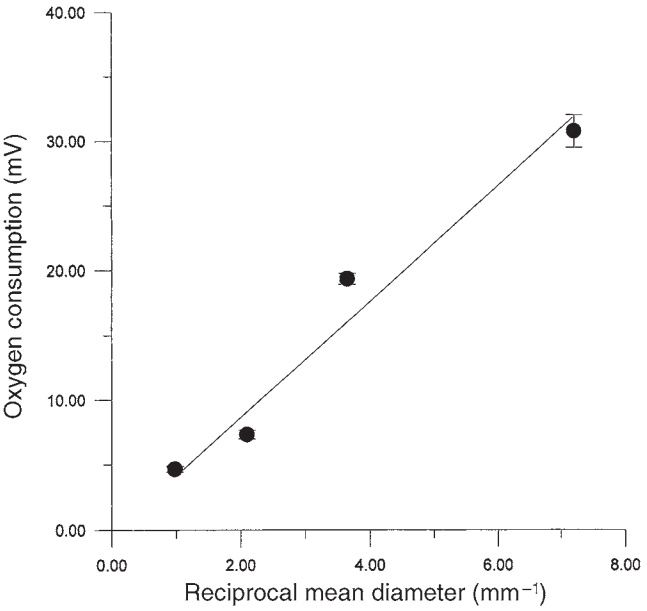


FIG. 6. A plot of the reciprocal of geometrical mean diameter of sample particles against oxygen consumption. Sample weight taken: 2.00 g; reaction temperature: 120°C; residence time: 10 min. Data points are means ± SD; $n = 6$.

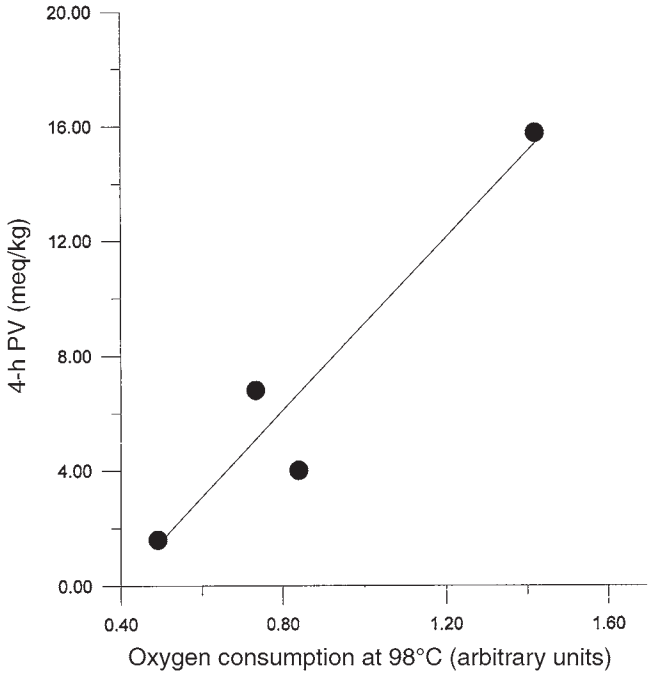


FIG. 7. A plot of oxygen consumption predicted against peroxide value (PV) by abbreviated active oxygen method (4 h at 98°C).

0.9650 and 0.9567, respectively, for the glass bead and bone meal samples, 86°C data for the bone samples are considered); this is shown in Figure 6 for the glass bead samples. For the same mass taken, this means that the oxygen consumption rate is proportional to the total surface area of the sample.

Oxygen consumption and 4-h AOM. In practice, the stability of samples is often determined by an abbreviated version of the AOM in which the peroxide value (PV) is determined after 4 h of oxidation under the conditions of the traditional AOM. The present method produces a different type of information (in two-dimensional data space) compared to traditional or abbreviated AOM procedures and as such, the results cannot be directly compared. This has been discussed in detail elsewhere (11). However, since the intent of both methods is to produce some measure of oxidative stability, a common basis of comparison may involve the comparison of the 4-h PV obtained by the abbreviated AOM with our data, the latter being reduced to one dimension, to simply the oxygen consumption at 98°C. For four samples, the 4-h PV was determined by a certified commercial laboratory and these results are shown in Figure 7 plotted against the oxygen consumption predicted for the same samples at 98°C from our data. While the correspondence is not perfect, the general trend is the same. This has important practical implications in that the data from this analyzer can be related to traditional data obtained with the older techniques. It also suggests that the new method will be useful in the comparison of relative efficacies of the various antioxidants available to the industry.

In summary, the oxidation of lipids necessarily involves the consumption of oxygen. Compared with AOM, which uses the formation of peroxides, an intermediate product, as a quantitative index of oxidative stability of samples, the extent of oxygen consumption is a more reasonable parameter for the determination of oxidative stability. The experimental system described here exhibits very good reproducibility under optimized conditions. A typical multitemperature oxidative stability analysis with the gas-phase FIA system requires 2.5–3 h. This is much faster than traditional methods. The ability to predict the relative stability of samples at some temperature different from the experimental conditions by taking advantage of Arrhenius behavior is very useful in practice. The experimental system is simple instrumentally and requires no chemicals other than a relatively small flow of an inexpensive carrier gas.

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