# Factors Affecting the Gravimetric Determination of the Oxidative Stability of Oils

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**The variables affecting the gravimetric determination of the oxidative stability of oils were evaluated, including the storage temperature, amount of sample and surface area of the sample in contact with atmospheric oxygen, which was varied by using containers of different diameters. Based on this study, we propose the optimal working conditions for development of the oxidation step prior to gravimetric monitoring.**

KEY WORDS: Factors affecting, gravimetric stability, oxidative stability, virgin olive oils.

Resistance to oxidation is a measure of oil quality and dictates storage and usage stability. Such stability, namely the interval elapsed until the oil becomes rancid, depends both on intrinsic features (*viz.*, type of olive in olive oils, fatty acid and natural antioxidant tocopherols and polyphenols contents) and on environmental conditions (temperature, light, air exposure, type and material of the container, metal trace content, etc.).

Oil rancidity is assessed either by sensory or laboratory procedures (1,2). Because olive oils are stable for a few months under their usual storage conditions, oxidation is accelerated in laboratory tests by increasing the temperature and, occasionally, by increasing exposure to oxygen. This "forced" side of the study conditions may distort the results compared with real conditions; notwithstanding this shortcoming, the long experience in accelerated stability assays has fostered usage of this type of analysis.

There are a great variety of stability tests that can be classified as shelf-storage, oven-storage and oxygen absorption tests. There is also the active oxygen method (AOM). The applicability and disadvantages of each of these methods have been dealt with at length in the literature (3,4). The most common of such methods, the AOM (5), is rather time-consuming and costly, as it requires periodic determination of the peroxide value of heated, aerated samples to monitor the oxidation process. Hence, it fails to meet the demands of a modern analytical laboratory. These shortcomings have been partly overcome by the Rancimat instrument, marketed by Metrohm (Herisau, Switzerland) (6), which implements the method developed by Hadorn and Zurcher (7). The Rancimat performs automatic and continuous monitoring of oil oxidation, in contrast to the AOM where monitoring is discrete and manual. An additional advantage of the Rancimat method is the low cost, as it uses no chemicals. The two drawbacks of the Rancimat method are the small number of samples that can be processed in each batch (only 6) and the acquisition cost of the instrument. The former problem is crucial for olive oils, which may remain stable for over 200 h. Thus, the potential of this instrument is inadequate for olive oil laboratories under the typical harvest work load in winter. For this reason, it is necessary to develop a method at least as efficient as those currently used for this purpose, but with a higher throughput in order to avoid bottlenecks.

One of the abovementioned oxygen absorption methods is based on gravimetric monitoring of the weight increase due to the oxygen absorbed by a sample heated in an oven. This old method (8) was recently used to investigate the influence of fluorescent light irradiation and antioxidants on the effectiveness of tertiary butylhydroquinones to preserve the stability of distilled herring methyl esters (9) at moderate temperatures (30–80°C) and a daily weight frequency. The results were expressed as induction periods, which were defined as the intervals required for the samples to reach a preset percent weight increase. This method has some disadvantages: (i) the weighing frequency hinders monitoring of fast kinetics (a higher frequency would involve nocturnal weighings), and low or moderate temperatures would result in long analysis times for stable samples; (ii) discontinuous heating of the sample (which must be cooled before weighing) may give rise to irreproducible results, so the heating and cooling intervals must be accurately controlled; (iii) the method involves intensive human participation; and (iv) the working conditions (amount of sample, size and shape of the container, temperature) influence the results obtained. These limitations constrain applicability of the method to studying the effectiveness of antioxidants on some sample types. Nevertheless, the method offers two clear advantages over the Rancimat: low instrumentation costs and unlimited capacity for sample processing.

The aim of this work was to perform a systematic study of the influence of the variables affecting the gravimetric method for subsequent normalization as a prior step to the development of an alternative method for the determination of the oxidative stability of olive oils.

## EXPERIMENTAL PROCEDURES

*Materials.* A Mettler AE200 balance (Greifesee, Switzerland) with precision of ±0.1 mg and a convection oven with regulatable temperatures between 40–200 ± 1°C were used. All chemicals used were of analytical grade.

Two types of virgin olive oil were used. One was used to study the effect of temperature [peroxide value (PV) = 15.7, free acidity 0.8% (oleic acid)] and the other for the rest of the study (PV = 9.9, free acidity 0.6%).

*Determination of the PV.* The Norm 2568/91 of the European Community (10) was used, the principle of which is as follows. The sample, dissolved into an acetic acid/chloroform mixture (3:2, vol/vol) was mixed with 1 mL of a saturated solution of potassium iodide. The released iodine was titrated with a standard solution (0.01–0.002 N) of sodium thiosulfate after manual shaking for 1 min and resting in the dark for 5 min.

*Gravimetric monitoring of the oxidation of olive oil.* Samples were poured into appropriate glass beakers after weighing and were then heated at the working temperature in the oven. They were then removed from the oven, cooled to room temperature (30 min) and weighed either every 12 h (for samples with fast oxidative kinetics) or every 24 h (for more stable samples).

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## RESULTS AND DISCUSSION

One of the main sources of interlaboratory irreproducibility lies in the selection of the assay conditions, which have a dramatic influence on prooxidant factors (temperature and oxygen exposure). In fact, the amount of sample and the size and shape of the container used determine the surface area of the sample that is exposed to air, through which oxygen interacts with the oil. This does not affect the AOM and Rancimat methods, where air is in excess. In 1958, Olcott and Einset reported (8) some data on the influence of these factors on the induction period that have been completed in the comprehensive study described below.

*Influence of temperature.* First, the influence of temperature on the oxidation rate was studied by heating 10-g oil samples at different temperatures in an oven by using identical beakers of 2.9-cm i.d. (Fig. 1). The parameter used for gravimetric monitoring was the percent weight gain, V, which is given by:

$$V = [(W_t - W_i)/W_i] \times 100 \qquad [1]$$

where $W_t$ and $W_i$ are the weights at a given time and at time zero, respectively.

As expected, an increase in temperature accelerated the oxidation process, although at temperatures above 100°C the oxidation was so fast that it resulted in nonreproducibility in the oxygen rate. For this reason, the heating temperature must not surpass 100°C if discrimination capacity is to be preserved. The four oxidation steps of the overall oxidation process (11) can be accurately distinguished in the oxidation curves shown in Figure 1. An initial induction period, in which the sample shows no appreciable changes, is followed by a more or less lengthy period of hydroperoxide formation that results in a weight increase (linear rising portion); after an interval, during which oxidation is the only occurring process, the third step involves a more markedly degrading oxidation reaction of the hydroperoxides formed in the previous step; the fourth step results in the breakage of the fatty acid chains to yield volatile species that are responsible for the off-flavor. The concurrence of the last two (or three) phenomena gives rise to a gradual decrease in the slope of the oxidation curve, which can become negative in the end.

Figure 2 shows the changes in the peroxide value and weight of a heated sample. In the first two steps (induction period and linear rising portion of the curve), the weight change curve lags behind that of the PV curve. As the peroxide decomposition starts, the PV decreases, which decreases the slope of the rising curve slightly (it only becomes negative at long times).

*Influence of the sample area/sample amount ratio.* Several experiments were carried out to investigate the influence of the ratio between sample area and sample volume.

First, the same type and amount of samples in cylindrical glass containers of different diameters were heated at 100°C in an oven. Figure 3 shows the strong influence of the oil-air surface area on the overall process. The sample held in a 13-mm diameter container remained unaltered after 100 h heating, whereas that held in a 83-mm diameter container underwent intense oxidation and lost some weight through breakage of the fatty acid chains over the same interval.

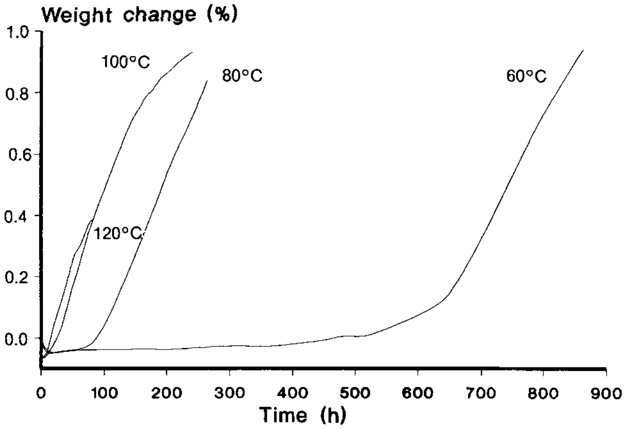

**FIG. 1.** Influence of temperature on the weight gain resulting from oxidation of olive oil. Slope of the rising portion of the curves (x $10^{-3}$): 60°C, 4.1; 80°C, 5.0; 100°C, 7.3; 120°C, 7.1.

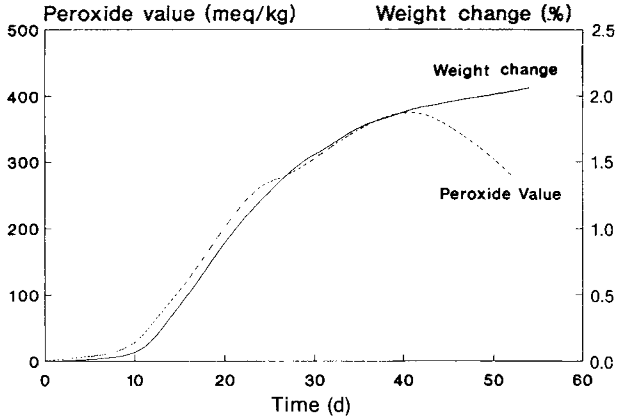

**FIG. 2.** Relationship between the change in the peroxide index and the weight of olive oil heated at 45°C in a convection oven (beaker diameter 29 mm, amount of sample 10 g).

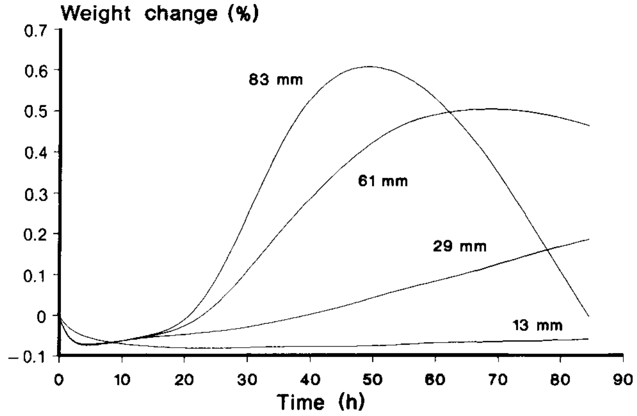

**FIG. 3.** Influence of the container surface area on the weight change of olive oil heated at 100°C. Data on curves denote container diameters. The amount of sample was 10 g in all instances. Slope of the rising portion of the curves (x $10^{-3}$): 83 mm, 3.38; 61 mm, 1.97; 29 mm, 0.42; 13 mm, 0.05.

Second, the influence of the amount of sample on the

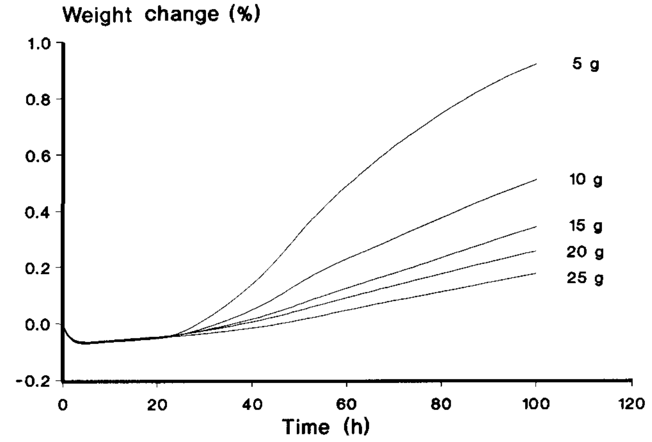

**FIG. 4. Influence of the amount of sample used on the oxidation of olive oil heated at 100°C in identical beakers of 40 mm diameter. Slope of the rising portion of the curves (x $10^{-3}$): 5 g, 2.37; 10 g, 1.27; 15 g, 0.55; 20 g, 0.44; 25 g, 0.33.**

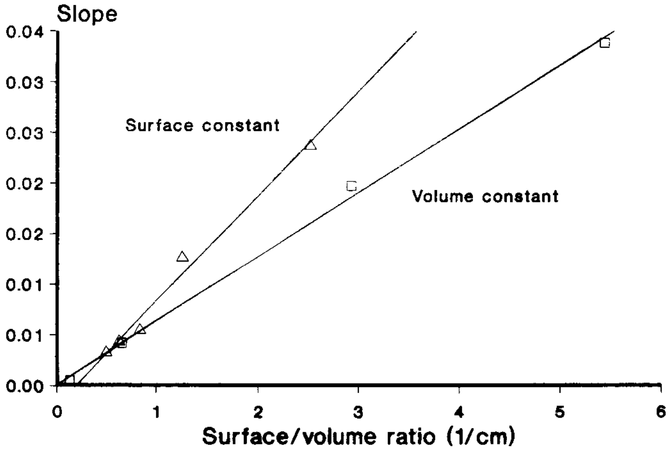

**FIG. 5. Variation of the slope of the oxidation curve of olive oil according to whether the volume (10 g of sample) or the surface area (12.6 $cm^2$) is kept constant.**

oxidation at 100°C in an oven was studied by using the container of 40 mm of diameter (Fig. 4). On comparing curves in Figures 3 and 4 one can conclude that variation of the surface area at a constant volume is more influential on the oxidation process than the volume at a constant surface area. However, the variation range of the studied parameter (*i.e.*, the surface area/volume ratio) was narrower in the latter experiment. Thus, when the slope was plotted against the surface/volume ratio ($cm^{-1}$) for both experiments (Fig. 5), it was clear that the influence of the amount of sample was more marked.

Third, the effect of changing the surface area and sample volume was studied. Beakers of different diameters were used in which the height of oil was kept constant in this experiment (11 mm) and the oven temperature was 100°C. In this experiment, the distance to be travelled by the oxygen in the bulk sample to react with the suitable molecules was always the same; thus, the surface area/volume ratio was kept constant as well. The results plotted in Figure 6 show that the oxidation process in this case always occurs in a similar way.

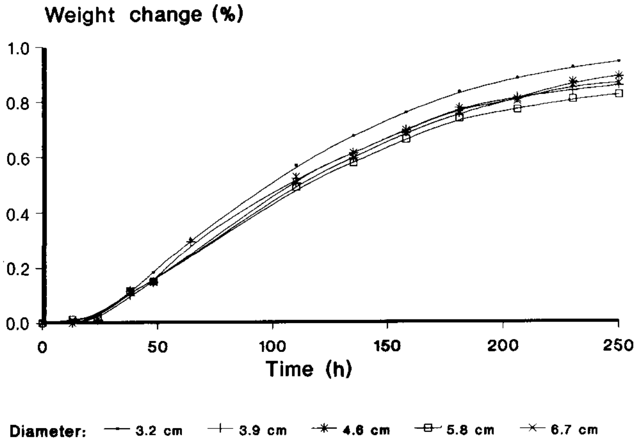

**FIG. 6. Influence of the beaker diameter on the oxidation of olive oil at a constant height (11 mm) of sample.**

The results obtained in this work show the strong dependence of the oxidation of olive oil on the studied parameters. From these results the following working conditions are proposed as optimal for development of the oxidation step prior to gravimetric monitoring: (i) Use of small containers with test-tube diameters to minimize the space required for each sample, thus increasing productivity. (ii) A small amount of sample must be used to keep a large surface/volume ratio, but very small amounts of sample would give rise to small changes in the weight to be monitored which, in turn, would result in increased relative weighing errors. A compromise must be adopted. (iii) A working temperature of 100°C is suggested to achieve an adequate oxidation rate and to decrease the analysis time (in turn, these conditions would provide inadequate information on the oxidation curve of unstable samples). (iv) To obtain more information, one must use a higher weighing frequency, which requires strict timing of the process (both are arduous tasks). A straightforward way of avoiding this time-consuming, tedious work is to implement the method by using a robotic station. This is currently being investigated at our laboratory.

## ACKNOWLEDGMENT

Comisión Interministerial de Ciencia y Tecnología (CICyT) is thanked for financial support (Grant No. PTR 89-0113).

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[Received June 25, 1992; accepted December 10, 1992]