the beans, resulting in poor mold growth. Therefore, properly perforating the containers and properly packing the beans for fermentation are important.

Tempeh has a short shelf life. Steaming for a few minutes to kill the mold and to inactivate the enzymes and then freezing will extend the shelf life.

Tempehlike products can also be made (20) by fermenting whole cereal grains such as wheat, oats, barley, rice or mixtures of cereals and soybeans with *R. oligosporus*. Tempeh made from a mixture of wheat and soybeans has been shown (21-22) to have a better protein value than that made from soybeans alone, because of the complimentary effect of mixed proteins and the increased use of lysine in wheat by fermentation. Wheat tempeh and a mixed wheat-soybean tempeh are commercially available in the U.S.

Traditionally, tempeh is sliced and deep-fried, but it can be cooked by roasting, baking or sauteing just like meat. In the West, tempeh burgers and chips are popular.

REFERENCES

1. Wang, H.L., in Handbook of Processing and Ulitization of Agriculture, Vol. II, Part 2, Plant Products, edited by I.A. Wolff, CRC Press, Inc., Boca Raton, Florida, 1983, p. 91.
2. Shurtleff, W., and A. Aoyagi, Soyfood Industry Directory and Databook, The Soyfoods Center, Lafayette, California, 1983.
3. Leviton, R., Soyfoods 8:33 (1983).
4. Swain, E.W., H.L. Wang and C.W. Hesseltine, unpublished data.
5. Wang. H.L., E.W. Swain, C.W. Hesseltine and H.D. Heath, J. Food Sci. 44:1510 (1979).
6. Watanabe, T., C. Fukamachi, O. Nakayama, Y. Teramachi, K. Abe, S. Suruga and S. Mivanage, The Report of Food Research Institute, Ministry of Agriculture and Forestry, Japan, 14B (1960) (in Japanese).
7. Wilkens, W.F., L.R. Mattick and D.B. Hand, Food Technol. 21:1630 (1967).
8. Nelson, A.I., M.P. Steinberg and L.S. Wei, J. Food Sci. 41:57 (1976).
9. Fukushima, D., in Chemical Deterioration of Proteins, edited by J.R. Whitaker and Masao Fugimaki, ACS Symp. Ser. 123, Washington, D.C., 1980, p. 211.
10. Wang, H.L., and C.W. Hesseltine, Process Biochem. 17:7 (1982).
11. Appurao, A.G., and M.S. Narasingo Rao, Cereal Chem. 52:21 (1975).
12. Tsai, S.J., C.Y. Lan, C.S. Kao and S.C. Chen, J. Food Sci. 46:1734 (1981).
13. Aoki, H., Nippon Hogei Kagaku Kaishi 39:277 (1965) (in Japanese).
14. Wang, H.L., E.W. Swain, W.F. Kwolek and W.R. Fehr, Cereal Chem. 60:185 (1983).
15. Saio, K., Cereal Foods World 24:342 (1979).
16. Saio, K., M. Kamiya and T. Watanabe, Agric. Biol. Chem. 33:1301 (1969).
17. Skurray, G., J. Cunich and O. Carter, Food Chem. 6:89 (1980).
18. Hesseltine, C.W., M. Smith, B. Bradle and Ko Swan Djien, Dev. Ind. Microbiol. 4:275 (1963).
19. Wang, H.L., E.W. Swain and C.W. Hesseltine, J. Food Sci. 40:168 (1975).
20. Hesseltine, C.W., M. Smith and H.L. Wang, Dev. Ind. Microbiol. 8:179 (1967).
21. Wang, H.L., D.I. Ruttle and C.W. Hesseltine, J. Nutr. 96:109 (1968).
22. Wang, H.L. and C.W. Hesseltine, in Microbial Technology, edited by H.J. Peppler and D. Perlman, Vol. II, 1979, p. 96.

[Received June 16, 1983]

Automated AOM Test for Fat Stability[1]

J.M. deMAN and **L. deMAN**, Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT

A home-built version of the automated AOM test was used with Canola, corn, sunflower, olive and Crisco® oils, shortening and lard. The endpoint was found by measuring the conductivity of a solution of the exit gas from the reaction tube. Coefficients of variability of the samples ranged from 1.1% to 8.3%. The endpoint of the test was ca. 100 PV for Canola oil, ca. 200 PV for corn oil and 35 PV for lard. The aqueous solutions of the volatiles of three oils were used to determine the TBA value. Canola, sunflower and olive oil had TBA values ranging from 6-60 μg malonaldehyde/g at the end point. No apparent relationship was found between the TBA values of the volatiles' solutions and the PV's of the oils.

INTRODUCTION

The stability of fats is usually measured by the active oxygen method (AOM) described in the AOCS official method Cd 12-57 (1). This method suffers from the disadvantage of being time consuming, labor intensive and wasteful. Therefore, attempts have been made to come up with alternative tests and improved versions of the AOM.

Alternative tests have included procedures that involve the direct recording of oxygen absorption of fats. Systems of this type have been described by Marcuse and Remi (2) and Imaeda et al. (3). In addition, there is the well-known Schaal or oven test. These methods have been reviewed by Pardun (4). A good deal of effort has been devoted in recent years to automated versions of the AOM test. 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. Loury (5) has described the mechanism of this reaction. He postulates the transitory presence of a diperoxide; this unstable compound decomposes into two aldehydes and formic acid. Oxidation of the aldehydes can also lead to formic acid. The volatile acids consist mainly of formic acid with small quantities of acetic and propionic acid. The air emerging from the oil in the AOM test can be led into water and the acids titrated potentiometrically or determined conductometrically. A curve obtained by potentiometric titration was published by Hadorn and Zürcher (6), and this curve demonstrated that the volatile acids formed in the AOM test can be used as a basis for automated endpoint detection. During the past few years, several papers have been published dealing with the use of formic acid formation as the basis of a simplified, automated AOM test. A potentiometric titration system was described by Pardun and Kroll (7). In their system, the oil bath of the AOM method was replaced by a metal heating block. This simplification was proposed as early as 1950 by Lips (8) and subsequently by Schroeder and Draper (9). Later versions of the equipment have generally been constructed with heating blocks instead of oil baths. Hadorn and Zürcher (6) used a system involving conductometric measurement of the AOM endpoint. This principle has now been included in a commercial version of the automated AOM test (Metrohm Ltd., CH-9100, Herisau, Switzerland).

[1] Presented at the 73rd AOCS Annual Meeting, Toronto, 1982.

This paper deals with the application of a home-built version of the automated AOM test to a number of oils and fats with emphasis on the reproducibility of results and the relationship between the peroxide value and the recorded endpoint of the reaction.

EXPERIMENTAL PROCEDURES

The air supply is provided by a diaphragm pump (Dyna-pump) with a capacity of 3.7 L/min. The air is washed at the suction side of the pump by bubbling it through a solution of 20 g/L potassium dichromate and 10 g/L sulfuric acid, filtered through glass wool and dried over Drierite. The flow of air to the individual tubes is regulated by flow meters. For routine applications, these can be replaced by calibrated capillary tubes as described in AOCS method Cd 12-57.

The sample containers are Pyrex test tubes with side arm 20 × 150 mm, and the sample size is 5 g of oil or fat. The tube is closed by a rubber stopper holding the air inlet tube, which reaches to about 3 mm from the bottom of the sample tube. The air containing the volatiles exits through the side tube and is bubbled through distilled water in a glass jar that also holds a conductivity electrode. The sample tubes are placed in a heating block (Thermolyne Dri Bath, Sybron Corp.) maintained at 97.8 C. The temperature within the block varies within ± 0.2 C. The assembly consists of 6 complete units so that 6 tests can be performed at the same time. The recording of 6 curves can be done using a 6-point recorder or a switching mechanism. The 6 electrodes were attached to a 6-channel automatic switch model SAS 1 (Bach Simpson Ltd., London, Ont.). The switch was set for successive 5-min periods, resulting in each electrode recording for 5 min out of 30 min in regular sequence. The conductivity meter was a Radiometer model CDM 3 set at 500 micro Siemens. The recorder was a single pen strip chart recorder operated at 10 mV and a chart speed of 33 mm/hr.

The cleaning of the glassware is of special importance. The usual procedure of using chromic acid has been replaced by using a special detergent (10). The glassware is rinsed with petroleum ether to remove oil, then washed in hot water and detergent using a brush. It is then boiled for one hour in a 5% solution of Extran 300 (BDH Chemicals, Toronto, Ontario), rinsed with tap water followed by three distilled water rinses and dried in an oven. Extran 300 is suggested by the manufacturer as a safe alternative to chromic acid. An additional advantage of eliminating the use of chromic acid is that residual chromium ions, which might catalyze oxidation, are excluded.

The oils used in this study were refined and bleached Canola oil from western Canadian refineries; Canola, corn, sunflower, olive and Crisco® oil, shortening and lard from local retail stores and lard without antioxidants from a local plant. All of the samples had initial peroxide values of less than 1. Breakdown products of fat oxidation contain carbonyls that were estimated by the T.B.A. value as μg of malonaldehyde/g of fat. The method of Tarladgis et al. (10) was used to determine the T.B.A. value of the water containing the volatiles. The peroxide value was determined using AOCS method Cd 8-53 (1).

RESULTS AND DISCUSSION

Figure 1 shows a typical recorder tracing of six samples of olive oil and Figure 2 of Crisco® oil in the automated AOM equipment. The endpoint is determined by extrapolating the upward portion of the curve to the time axis. The point where these lines intersect is taken as the endpoint. Tests were run on a number of oils and fats and the results are

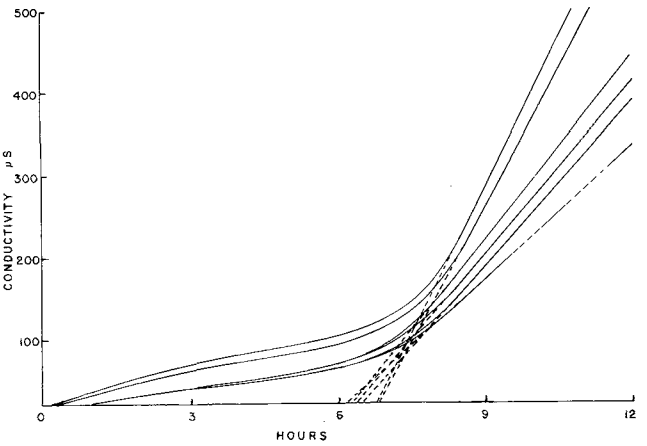

FIG. 1. Typical record of a stability test of 6 identical samples of olive oil in the automated AOM test.

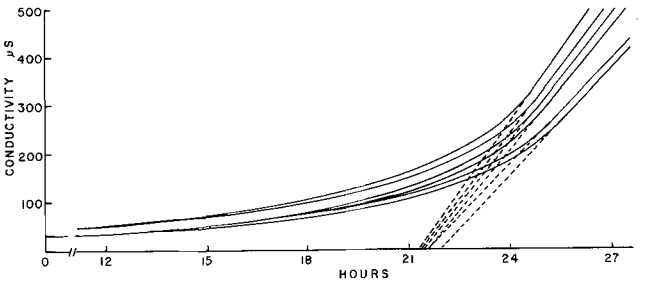

FIG. 2. Typical record of a stability test of 6 identical samples of Crisco® oil in the automated AOM test.

TABLE I

Precision of the Automated AOM Method

Sample	No. of tests	Range h	Mean h	s h	CV %
Canola oil[a]	6	9.4-11.0	9.85	0.78	8.0
Canola oil[a]	6	8.8-10.3	9.38	0.60	6.4
Canola oil[a]	4	9.5-10.8	10.10	0.62	6.1
Canola oil	5	19.1-25.5	21.3	1.78	8.3
Olive oil	6	6.1- 6.9	6.5	0.34	5.2
Corn oil	6	18.5-19.0	18.92	0.20	1.1
Sunflower oil	6	11.8-12.9	12.15	0.38	3.2
Sunflower oil	6	10.8-12.0	11.38	0.43	3.8
Criso® oil	6	21.1-22.0	21.6	0.32	1.5
Crisco® shortening	6	38.1-39.7	39.18	0.58	1.5
Lard	6	44.4-46.7	45.67	1.00	2.2
Lard without antioxidant	6	9.3- 9.8	9.73	0.22	2.2

[a]Refined and bleached.

listed in Table I. The coefficients of variability ranged from 1.1% to 8.3%, which is considerably better than the figure of 13.4% listed in official method Cd 12-57. The precision of a similar automated AOM method using galvanometric endpoint detection was reported by van Oosten et al. (11). In this collaborative test, 5 oil and fat samples were examined by 9 laboratories. The repeatability of the method was 0.99 hr and the within laboratory standard deviation was 1.45 hr. Our tests were performed at a temperature of 97.8 C, in accordance with AOCS method Cd 12-57. However, European researchers use a temperature of 100 C (6,7, 10). Hadorn and Zürcher (6) investigated the effect of temperature and found a coefficient of 2.2 for each 10 C degree increase in temperature; Pardun and Kroll (7) reported a temperature coefficient of 2.5. The temperature coefficient of 2.2-2.5 established for the autoxidation

reaction under the conditions of this test is somewhat higher than the value of 2 usually applied for organic reaction rates.

Hadorn and Zürcher (6) also investigated the effect of sample size and volume of air flow. They found that using a smaller volume of oil is generally preferable because the curves show a more distinct endpoint. For the type of equipment used in our study, 5 g of sample was chosen because this amount filled the sample tube to a height just below the level of the heating block. The air flow in the AOCS standard method Cd 12-57 (1) is specified as 2.33 mL/sec. In our study we have used an air flow of 1.67 mL/sec. This is somewhat less than in the AOCS method but the AOCS method requires a volume of 20 mL of oil in each tube. Hadorn and Zürcher (6) investigated the effect of air flow and found virtually no effect from the flow rate using 0.64, 2.5 and 5.0 mL/sec and a sample size of 2.5 g. This result is not unexpected since the oxygen concentration is not rate limiting in autoxidation reactions except at very low concentrations.

The endpoint of the AOCS method is specified as the time in hours required for the sample to reach a peroxide value of 100 meq/kg. The endpoint of the automated version of the AOM method is the time in hours required for a sudden increase in formic acid production to occur. This point is taken as the end of the induction period. Pardun and Kroll (7) stated that a good correlation exists between the 100 meq/kg peroxide value endpoint and the length of the induction period found with the automated AOM system. To determine the change in peroxide value in the neighborhood of the endpoint, several of the oils listed in Table I were placed in the equipment and peroxide values determined in each of 6 sample tubes at selected times. The results of triplicate determinations are presented in Tables II-IV. For Canola oil, the endpoint was reasonably close to the peroxide value of 100. The endpoint of corn oil was closer to a peroxide value of 200. The peroxide value for lard was ca. 35 at the endpoint and went up to over 500 at 54 hours. The formation and decomposition of peroxides in oils and fats appear to follow a different pattern than the formation of formic acid, which is the basis for the automated AOM system.

The aqueous solutions of the volatiles of three oils were used to determine T.B.A. value. T.B.A. values ranged from 6 to 60 μg of malonaldehyde/g of fat for Canola, sunflower and olive oil at the inflection point. No apparent relationship was found between T.B.A. values of the volatiles' solutions and the peroxide values of the oils.

A rapid change in peroxide values occurs when the acidity of the aqueous solution of the volatiles changes from 0.019 g to 0.025 meq/100 mL or at a conductivity of about 200 μS. During this change of acidity, peroxide values change from 20 to over 200 meq/kg in a short time.

The automated AOM test represents a considerable improvement over the standard version. The automated method is more precise and represents a great saving in labor.

ACKNOWLEDGMENT

The Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture and Food provided financial support for this work.

TABLE II

Change in Peroxide value of Canola Oil Close to the Endpoint of the Induction Period (8.34 h) in the Automated AOM System

Time h	PV meq/kg
8	78
8.5	95
9	83
9.5	112
10	117
10.5	135

TABLE III

Change in Peroxide Value of Corn Oil Close to the Endpoint of the Induction Period (18.92 h) in the Automated AOM System

Time h	PV meq/kg
17	180
17.5	180
18	179
18.5	186
19	220
19.5	194

TABLE IV

Change in Peroxide Value of Lard Oil Close to the Endpoint of the Induction Period (45.67 h) in the Automated AOM System

Time h	PV meq/kg
46	35.1
48	36.6
50	69.0
52	62.8
54	518.3
56	99.5

REFERENCES

1. AOCS Official and Tentative Methods of Analysis Cd 8-53.
2. Marcuse, R. and K. Remi. First Int. Congr. Food Sci. Technol. 3:53 (1965).
3. Imaeda, K., K. Ohsawa and Y. Yoshimura. Bunseki Kagaku 29:426 (1980).
4. Pardun, H. Süsswarentechnik 3:25 (1980).
5. Loury, M. Comptes Rendus 253:2717 (1961).
6. Hadorn, H. and K. Zürcher. Dtsch. Lebensm. Rundschau 70:57 (1974).
7. Pardun, H. and E. Kroll. Fette Seifen Anstrichm. 74:366 (1972).
8. Lips, H.J. Can. J. Res. 28: Sec. F, 21 (1950).
9. Schroeder, W.F. and J.W. Draper. JAOCS 33:628 (1956).
10. Tarladgis, B.G., A.M. Pearson and L.R. Dugan. Ibid. 39:34 (1962).
11. van Oosten, C.W., C. Poot and A.C. Hensen. Fette Seifen Anstrichm. 83:133 (1981).