Studies of Chemical Methods for Assessing Oxidative Quality and Storage Stability of Feeding Oils

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ABSTRACT

Peroxide value (PV), thiobarbituric acid number (TBA), anisidine value (AV), percent free fatty acids (FFA), and carbonyl value (CV) methods were compared in different experiments for their sensitivity and practicality in assessing the oxidative quality of four feeding oils: salmon oil, soybean oil, canola oil (oil from low erucic acid, low glucosinolate rapeseed), and canola soap stocks. In the first experiment, among the four methods (PV, AV, TBA and FFA) studied, PV appeared to be the most practical for its sensitivity, simplicity, and economy when the four oils were oxidized by bubbling air through at room temperature for 792 hr. In the second experiment, using herring oil, all four methods tested (PV, AV, TBA and FFA) were sufficiently sensitive once the oxidation of herring oil had passed the induction period and the oil was highly oxidized. In the third experiment, of the four methods (PV, AV, TBA and CV) compared, AV was the most sensitive for measuring the oxidative quality of canola oil aerated at 100 C for 240 hr. Results of further studies suggested that herring and canola oils stored under commercial conditions were stable for at least one year.

INTRODUCTION

Oxidative quality of feeding oils is known to have significant effects on the storage stability and nutritional value of feeds. The toxicity of oxidized oil to animals has been of considerable interest for the last few decades and the nutritional properties of such oil have been studied extensively (1-6).

Oxidation of oils results in development of off-flavors which can be evaluated by organoleptic tests. The routine evaluation of flavor by taste panel is tedious, subjective, expensive and difficult to compare among laboratories (7). Furthermore, it is not feasible to test oxidative quality of animal feeding oils by taste panel.

Different chemical methods have been suggested for assessing objectively the oxidative quality of oils either by measuring their primary or secondary deteriorative products (8) and some of these methods have been reviewed by Dugan (9) and Gray (7). However, little information is available comparing the sensitivity and practicality of these methods for assessing the oxidative quality of feeding oils. The objective of the present studies was to compare a few common analytical methods for measuring the oxidative quality of four different feeding oils. The storage stability of two oils was also studied.

The oils studied herein are of interest to feed manufacturers. Since manufactured fish feeds normally contain a high level (5-10%) of added oil or fat, a knowledge of the oxidative quality of the products employed in such diets is a matter of real concern. The oils used in these studies were crude oils containing no additives unless indicated in the text.

EXPERIMENTAL PROCEDURES

Autoxidation of Oils

The initial experiment was designed to compare four different chemical methods: peroxide value (PV), anisidine value (AV), thiobarbituric acid number (TBA), and percent free fatty acids (FFA), for measuring the oxidative quality of four different oils: salmon oil (SMO), soybean oil (SBO), canola oil (CLO) (the official name adopted for low erucic acid, low glucosinolate rapeseed oil by the Canola Council of Canada), and canola soap stocks (CSS).

About 10 kg of each oil was aerated (air flow rate ca. 10 L/min) for 0-800 hr in an all-plastic apparatus, with the laboratory air supply, at room temperature. Oils were sampled every 5-7 days and kept frozen at -17 C for future chemical determinations. Triplicate determinations with each method were performed on each sample. Peroxide value and FFA in the oils were measured by the AOAC methods (10). Thiobarbituric acid number was measured by the method of Yu and Sinnhuber (11), and AV was determinated by the method of List et al. (12).

Autoxidation of Herring Oil

A second experiment was conducted with 3 kg of herring oil (HFO) aerated in the same manner described previously but with the oxidation being carried much beyond the induction period. A sample of the oil was taken every 24 hr for 600 hr and kept frozen (-17 C) for later chemical determinations. Peroxide value, AV, TBA and FFA of the samples were measured as described in the initial experiment.

Thermal Oxidation of Canola Oil

Due to the very slow oxidation of the two vegetable oils (CLO and SBO) when they were aerated at room temperature, a third experiment was undertaken to study the effect of oxidation of CLO by aeration at an elevated temperature. About 3 kg of CLO was aerated in a stainless steel beaker which was placed within a heating mantle and heated to 100 C. The heated oil was sampled every 24 hr for 240 hr and the PV, AV and TBA of the samples were measured as previously described. The carbonyl value (CV) was determined by the method of Bhalerao et al. (13).

Storage Stability of Herring Fish Oil

A study of the storage stability was conducted in collaboration with a commercial fish feed manufacturer (Martin Feed Mills Ltd., Elmira, Ont.).

This company had purchased 14,000 kg of good quality herring oil on May 19th, 1978 and stored it under nitrogen in an outdoor stainless steel tank. This batch continued to be used until December 20th, 1978. An antioxidant mixture (FC4C, Canada Packers Ltd., Toronto, Ont.) containing 20% hydroxyanisole, 20% hydroxytoluene and 10% monoglyceride citrate in soybean oil was added at the 0.05% level to the oil with nitrogen bubbling through to mix the antioxidant with the oil and to eliminate oxygen in the oil. The nitrogen pressure in the tank was maintained, as oil was drawn off through a tap at the bottom of the tank.

Oil was sampled during the seven-month period which was required for the manufacturer to use up that amount

of oil. Peroxide values of the samples were measured by the AOAC method (10).

Storage Stability of Canola Oil

Canola oils with no antioxidant added were stored in 45-gal commercial drums at room temperature for one, two, or three years. The PV, AV and TBA of these oils were determined as previously described.

Determination of Fatty Acid Composition

The weight percentages of fatty acids in SMO, SBO, CLO, CSS and HFO were determined by gas liquid chromatography after the oils were methylated and the esters extracted according to the method of Morrison and Smith (14). Identification of individual fatty acids was conducted by comparing their retention times with known fatty acid standards (Nu-Chek-Prep, Elysian, MN).

Statistical Analysis

Regression analyses between aeration time and different measurements were carried out for SMO, HFO, SBO, CLO and CSS according to Steel and Torrie (15).

RESULTS AND DISCUSSION

Autoxidation of Oils

The PV, AV, TBA and FFA of SMO, SBO, CLO and CSS aerated from 0 to 800 hr at room temperature are shown in Figure 1. The coefficient of determination (r^2) between aeration time and different chemical measurements showed that only PV was highly correlated ($r^2 > 0.90$) with aeration time in the four oils. The coefficient of determination was highly significant ($r^2 = 0.93$) between TBA and aeration time only in SMO. The regressions were also highly significant ($p < 0.01$) between PV and hours of aeration for the four oils studied and between TBA and hours of aeration for SMO. These results suggested that PV was sufficiently sensitive for measuring oxidative quality of the four oils and TBA was sensitive enough only for SMO with an oxidation period of up to 800 hr.

Regression analyses showed that the slopes of regression lines between aeration time and peroxide value was highest in SMO (0.06), followed by CSS (0.04), CLO (0.02), and SBO (0.002). These values indicate that both SMO and CSS were very susceptible to oxidation by aeration whereas CLO and SBO were not. The high susceptibility of SMO and CSS to oxidation may result from the high level of highly unsaturated fatty acids (HUFA) (fatty acids with three or more double bonds) in the SMO (27%) (Table I) and the high level of FFA (40%) in the CSS (Fig. 1) since it is known that both unsaturation and molecular structure (free fatty acid vs triglyceride) have significant effects on the susceptibility of the oil to oxidation (16).

Salmon oil also showed a significant coefficient of determination ($r^2 = 0.93$) and a significant slope ($p < 0.01$) of regression (0.2) between aeration time and TBA. These phenomena may be related to the high level of HUFA in this oil (Table I) as compared with the other oils. The TBA is known to be suitable for measuring oxidation in oils with fatty acids having three or more double bonds (7).

Among the four analytical methods studied, PV appeared to be the most practical because it proved sensitive enough for measuring the oxidative quality of all four oils during the induction period. This method has the further advantages of being very simple and inexpensive. These results agree with a previous study (17).

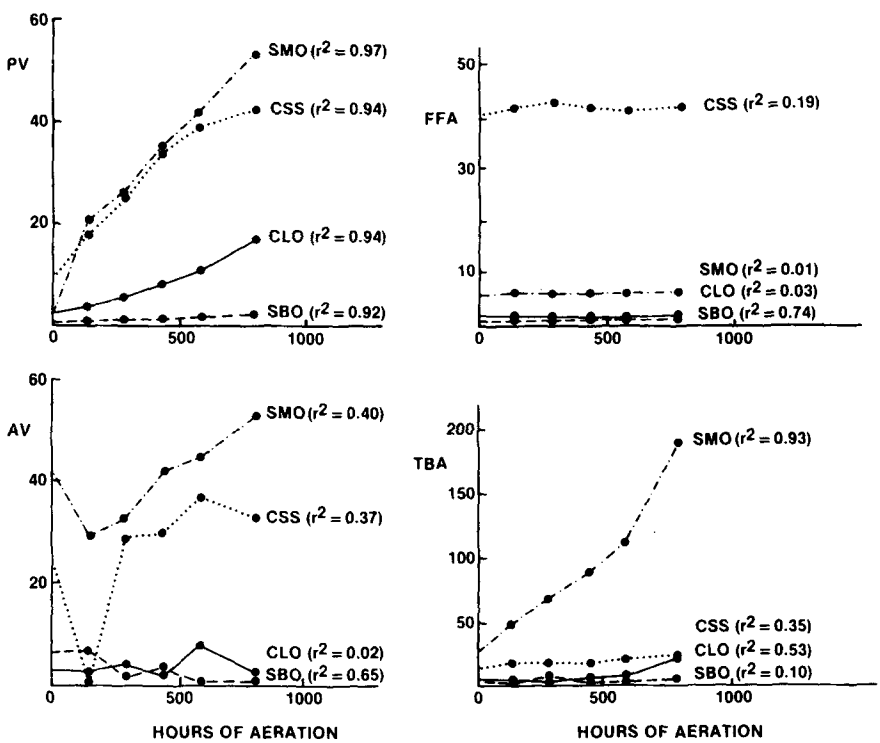

FIG. 1. Relationship between various methods for assessing the oxidative quality of salmon oil (SMO), canola oil (CLO), soybean oil (SBO), and canola soap stocks (CSS). PV, peroxide value in meq/kg oil; AV, anisidine value, defined as 100 times the optical density measured in a 1-cm cell of a solution resulting from reaction of 1 g of oil in 100 mL of a mixture of solvent and reagent; TBA, thiobarbituric acid number in mg of malonaldehyde/kg oil; and FFA, as percent of free fatty acids expressed as oleic acid. r^2 is the coefficient of determination between aeration time and different measurements.

Autoxidation of Herring Oil

The PV, AV, TBA and FFA of hearring oil aerated from 0 to 600 hr at room temperature are shown in Figure 2. All four parameters showed the same pattern of having a long induction period of ca. 216 hr before they began a sharp rise which was followed by either a plateau or a decline. These results showed that all four chemical methods were sensitive enough once the oxidation passed the induction period. However, only PV and TBA were sufficiently sensitive to measure the oxidative changes in herring oil during the induction period (0 to 216 hr) which represents the usual range of oxidation under practical conditions. The coefficients of determination between aeration time (up to 216 hr) and oxidation parameters were: PV, 0.99; TBA, 0.98; AV, 0.59; and FFA, 0.26. These results again agree with those in the initial study.

The FFA showed very irregular changes (Fig. 2). This may have resulted partly from the nature of the method which requires "vigorous shaking until a permanent faint pink color appears and persists for more than 1 min" (10) as the endpoint of titration. The endpoint determined in this manner was not sharp and was greatly affected by the shaking and timing. The irregular results may also have been due to the nature of the fat rather than the method, and changing the solvent may help solve this problem. Furthermore, FFA resulted from hydrolytic rather than oxidative degradation of the oils and do not respond well to aeration. The FFA was much more uniform in the initial experiment (Fig. 1) than in this experiment. This is probably due to the much milder oxidation in the initial study. However, the exact cause of the difference between the two experiments is not known.

The induction periods in salmon oil (Fig. 1) and herring oil (Fig. 2) were around 800 and 250 hr, respectively. The more than 3-fold differences between these oils may have resulted from several factors which cannot be controlled, e.g., the presence of prooxidants (trace heavy metals), or natural antioxidants, or the degree of unsaturation of the oils.

TABLE I

Fatty Acid Composition of Salmon Oil (SMO), Herring Oil (HFO), Soybean Oil (SBO), Canola Oil (CLO) and Canola Soap Stocks (CSS)[a]

Fatty acid[b]	SMO	HFO	SBO	CLO	CSS
	wt % of total fatty acids[c]				
14:0	5.2	5.0	–	–	–
16:0	15.9	18.1	11.1	3.5	7.5
16:1	5.8	8.1	3.2	–	–
17:0	–	2.3	–	–	–
18:0	3.3	3.2	–	–	–
18:1ω9	22.7	29.7	24.6	52.1	56.6
18:2ω6	1.6	1.1	51.6	25.5	24.0
18:3ω3	1.2	–	8.2	14.8	8.2
18:4ω3	2.2	1.1	–	–	–
20:1ω9	7.7	8.0	–	1.7	2.2
20:3ω3	11.5	–	–	–	–
20:5ω3	–	6.7	–	–	–
22:1ω9	–	–	–	1.4	–
22:1ω11	7.3	9.1	–	–	–
22:5ω3	1.9	–	–	–	–
22:6ω3	10.6	4.7	–	–	–

[a]Values given are means of 3 determinations.

[b]X:YωZ abbreviation for fatty acid with X carbon atoms; Y double bonds between adjacent carbon atoms, and Z after ω is the location of the first double bond from the methylene end.

[c]Fatty acids less than 1% by wt were omitted from this table.

Thermal Oxidation of Canola Oil

The PV, AV, TBA and CV of CLO aerated at 100 C from 0 to 240 hr are shown in Figure 3. The PV increased very sharply once the aeration started and this value reached the

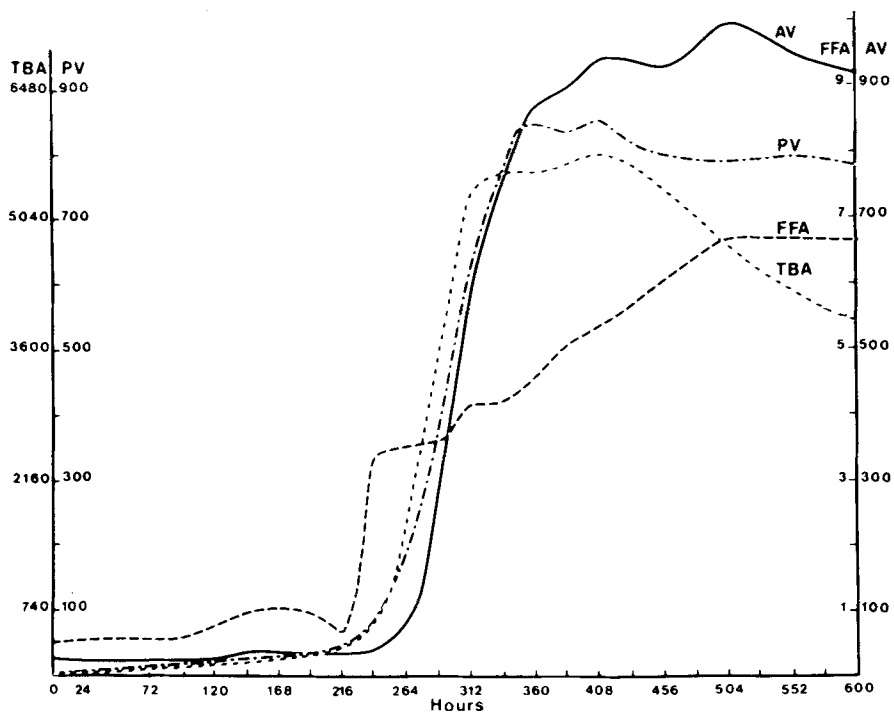

FIG. 2. Relationship between various methods for assessing the oxidative quality of herring oil. PV (—·—), peroxide value in meq/kg oil; TBA (····), thiobarbituric number in mg malonaldehyde/kg oil; FFA (– – –), percent of free fatty acids expressed as oleic acid; and AV (——), anisidine value defined as 100 times the optical density measured in a 1-cm cell of a solution resulting from reaction of 1 g of oil in 100 mL of a mixture of solvent and reagent.

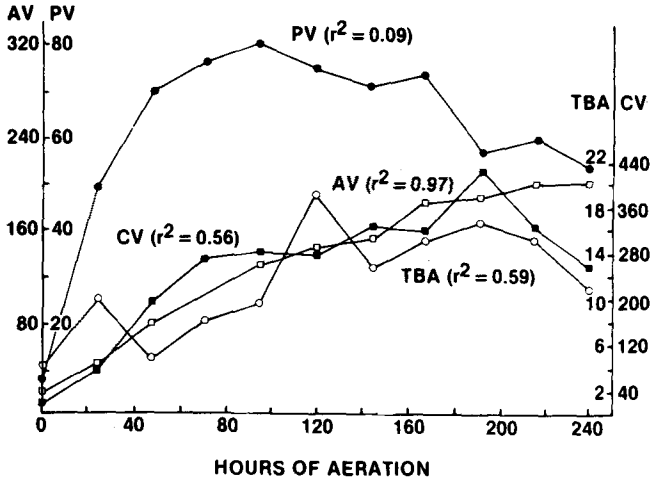

FIG. 3. Relationship between various methods for assessing the thermal oxidation of canola oil. PV (—·—), peroxide value in meq/kg oil; TBA (—○—), thiobarbituric acid number in mg malonaldehyde/kg oil; CV (—▪—), carbonyl value in meq carbonyl/kg oil; and AV (—□—), anisidine value defined as 100 times the optical density measured in a 1-cm cell of a solution resulting from reaction of 1 g of oil in 100 mL of a mixture of solvent and reagent. r^2 is the coefficient of determination between aeration time and the different measurements.

maximum at around 100 hr and declined slowly and irregularly thereafter. The TBA and CV were quite variable but the general trends were similar to the peroxide values. Only AV increased in a linear fashion for the whole 240-hr period of aeration.

Among the four analytical methods studied, only AV had a significant coefficient of determination (r^2=0.97) and slope of regression (0.83) with aeration time. These values suggested that AV was the best method for measuring oxidation of CLO aerated at 100 C up to 240 hr. However, the maximal values for PV, AV and TBA in the heat-aerated CLO (Fig. 3) were much lower than the corresponding maximal values in the room temperature aerated herring oil (Fig. 2). This apparent discrepancy may have resulted from differences in the two oils and temperatures at which the oxidations were carried out.

Storage Stability of Herring Oil

It has been shown in a previous report (17) and previous experiments in the present studies that peroxide value was the best method for assessing oxidative quality of fish oil under practical conditions. Thus, only PV was determined in this study. The initial peroxide value of the HFO was 2.3±0.3 meq/kg of oil (mean ± SEM, n=3) when purchased. The peroxide values were 2.8±0.1, 2.9±0.1 and 4.1±0.1 meq/kg of oil (means ± SEM, n=3) after 2, 5 and 7 months storage, respectively. These results indicated that HFO stored under the present conditions was very stable even during the hot summer months. The stability of HFO may be attributable to: (a) the high levels of α-tocopherol, a natural antioxidant, in this oil (17,18); (b) the antioxidant mixture added to the oil; (c) eliminating contact of air with the oil by replacement with nitrogen; or (d) a combination of these factors.

Storage Stability of Canola Oil

Studies with canola oil stored under conditions similar to those in commercial practice for 1, 2 or 3 years showed that these oils were very stable (Table II). It is of interest that with the exception of one oil stored for 2 years at room temperature, the remaining oils, including the three oils which had been held for three years, were still of reasonable quality, even though none had been stabilized by addition of antioxidants. The stability of these oils appeared to be due to the relatively high levels of naturally occurring tocopherols in canola oil as reported (19). The reason for the poor oxidative quality of the oil which had been stored for 2 years is not known, but this oil may have been oxidized before being received in our laboratory.

TABLE II

Peroxide Value (PV), Anisidine Value (AV) and Thiobarbituric Acid Number (TBA) of Canola Oils Stored at Room Temperature for Different Time Periods

Storage time (yr)	PV[a] (meq of peroxide/kg oil)	AV[a,c]	TBA[b] (mg malonaldehyde/kg oil)
1	2.5 ± 0.03	2.5 ± 0.21	0.6
2	19.5 ± 0.02	13.5 ± 0.40	25.9
3	3.2 ± 0.03	7.4 ± 0.35	2.9
3	1.6 ± 0.06	6.8 ± 0.25	2.2
3	3.3 ± 0.12	9.1 ± 0.69	2.2

[a]Values are given as means ± SEM of 3 determinations for each sample.

[b]Values from a single determination.

[c]AV of an oil is defined by convention as 100 times the optical density measured in a 1-cm cell of a solution resulting from the reaction of 1 g of oil in 100 mL of a mixture of solvent and reagent.

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