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Food Chemistry 52 (1995) 249–253

0308-8146/95/$9.50

Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils

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(Received 16 February 1994; revised version received and accepted 26 April 1994).

The oxidative stability of refined, bleached and deodorized canola and soybean oils was evaluated over a 30-day dark storage period at 65°C. Peroxide value (PV), conjugated diene (CD) and triene (CT) contents, 2-thiobarbituric acid reactive substances (TBARS) and *p*-anisidine values were determined. In addition, NMR spectroscopy was used to monitor relative changes in the proton absorption pattern of the fatty acids of oils during storage. Canola oil showed higher PV, CD, CT and TBARS as compared with those for soybean oil. The ratio of aliphatic to olefinic protons in both oils, determined by NMR spectroscopy, increased steadily over the entire length of the storage period, indicating progressive oxidation of unsaturated fatty acids in both oils.

INTRODUCTION

The development of genetically improved, low erucic acid, canola varieties has boosted the use of canola oil in food applications. The content of saturated fatty acids in canola oil is lowest among vegetable oils and it contains *c.* 94% unsaturated fatty acids (Ackman, 1990). Canola oil has a high content (8–12%) of α-linolenic acid (C18:3 ω3) as compared to other vegetable oils such as soybean (8·0%), sunflower (0·2%), olive (0·8%) and corn (0·7%) (Sheppard *et al.*, 1978). However, the content of linoleic acid (18:2 ω6) is reasonably low in canola (22–25%) compared to soybean (57–60%). The other important property of canola oil is the ratio of linolenic (C18:3 ω3) to linoleic (C18:2 ω6) acid which is *c.* 1 to 2; this is considered to be nutritionally favourable (Ackman, 1990).

The high content of unsaturated fatty acid in canola, especially C18:3 ω3, influences the stability and quality of the oil (Hawrysh, 1990). Fresh canola oil is odourless, bland in taste and light in colour; however, it develops off-flavours during storage or upon heating (Tokarska *et al.*, 1986; Eskin *et al.*, 1989). Oxidative deterioration of the oil primarily involves autoxidation accompanied by various reactions having oxidative and nonoxidative characteristics (Gray, 1978). Primary products of autoxidation are lipid hydroperoxides which decompose readily to a range of secondary products, such as aldehydes, ketones and alcohols, that may cause strong undesirable flavours in oils (Labuza, 1971). However, susceptibility of individual fatty acids to oxidation depends on their degree of unsaturation. For example, the oxidation rate of linolenic acid is 25 times faster than that of oleic acid (C18:1 ω9) and twice that of linoleic acid (Labuza, 1971). Forss (1972) concluded that linoleic and linolenic acids are most important precursors for off-flavour development as they readily form degradable hydroperoxides.

In order to determine the oxidative state and quality of an oil/fat, a number of stability tests including accelerated storage tests, such as the Schaal oven test (Joyner & McIntyre, 1938), fluorescent light test (Sattar *et al.*, 1976) and practical storage test (Evans *et al.*, 1973), are frequently employed. Chemical, instrumental and sensory techniques are used to measure the oxidation of lipids (Hawrysh, 1990).

Since hydroperoxides are the primary products of lipid oxidation, peroxide value (PV) is often used as an indicator of the initial stages of oxidation (Gray, 1978). However, hydroperoxides decompose rapidly during storage and upon heating. Therefore, PV may not necessarily be indicative of the actual extent of lipid oxidation (Sherwin, 1968). Measurement of the content of conjugated dienes (CDs) (at 234 nm) and conjugated trienes (CTs) (at 268 nm) is a quick physical method which may also be employed to assess the oxidative stability of vegetable oils (St. Angelo *et al.*, 1975). Both linoleic and α-linolenic acids form hydroperoxides which absorb UV radiation at 234 nm. Conjugated

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dienes are also formed upon decomposition of hydroperoxides from α-linolenic acid, absorbing at 234 nm, whereas secondary oxidation products, particularly diketones, absorb at 268 nm (Patterson, 1989). The classical method to estimate secondary lipid oxidation products is the 2-thiobarbituric acid (TBA) test (Gray, 1978). This test measures the concentration of malonaldehyde and other TBA-reactive substances (TBARS). The *p*-anisidine value (AnV) also measures secondary products of lipid oxidation, namely, carbonyl compounds. The latter analysis is based on the reaction of *p*-anisidine with unsaturated aldehydes and subsequent measurement of the optical density of the resulting yellow adduct at 350 nm (IUPAC, 1987).

Unfortunately, all of the above methods have limitations (Gray, 1978). Sensory methods are considered to be the most sensitive for assessing the oxidative state of food lipids; however, their use is not practical for routine analysis (Gray, 1978). Generally, both primary and secondary products of lipid oxidation are evaluated by a variety of tests in order to assess the quality of lipids. Therefore, it is desirable to attain a single nondestructive method for evaluating the extent of lipid oxidation in food systems.

Nuclear magnetic resonance (NMR) spectroscopy has been used to evaluate oxidative deterioration of edible oils (Shahidi, 1992). NMR absorption peaks of olefinic and aliphatic protons were found to appear at δ 5·1–5·6 and δ 0·6–2·5, respectively. Since the number of protons under each peak is determined by integration, it has been suggested that the NMR technique may offer a useful means for measuring the oxidative deterioration of lipids (Saito & Udagawa, 1992; Shahidi, 1992). Relative changes in the NMR absorption pattern of lipid fatty acids and formation of both primary and secondary oxidation products may be monitored. The objective of this study was to assess the oxidative stability of canola and soybean oils, using classical and novel indicators of lipid oxidation.

MATERIALS AND METHODS

Materials

Freshly processed refined, bleached and deodorized (RBD) oils were obtained from CanAmera Foods (canola from Saskatoon, SK, and soybean from Hamilton, ON, Canada). All chemicals used in this study were ACS grade or of better quality.

Storage conditions

Oil (25 ml) samples were kept in open clear Pyrex containers (20 mm diameter and 50 mm height) in the dark in a Precision (Model 2) oven at 65°C for a period of 30 days. To estimate oil stability, samples were removed periodically (0, 2, 3, 5, 10, 15, 20, 25 and 30 day intervals and separate sample containers for each day from each oil) for analyses.

Chemical analyses

Official methods (AOCS, 1989) were used for determination of iodine (method Cd 1-25), peroxide (method Cd 8-53) and TBARS (method Cd 19-90) values. The AnV (method 2·504), CD (method 2·505) and CT (method 2·505) values were determined using IUPAC (1987) methods of analyses. The fatty acid composition of both oils was determined according to Keough & Kariel (1987). Total oxidation (TOTOX) values were calculated as 2PV + AnV.

NMR spectroscopy

NMR spectra of oil samples, in $CDCl_3$, were recorded using a NMR spectrometer (General Electric GN-300) at 300 MHz, using tetramethylsilane as internal standard. Exactly 35 mg of oil were dissolved in $CDCl_3$ for NMR analysis. The total number of protons under each peak was determined on the basis of integration of terminal methyl protons of triacylglycerol molecules.

Statistical analyses

All experiments and/or measurements were replicated three times. Analysis of variance and Tukey's studentized range test (Snedecor & Cochran, 1980) were performed on Statistical Analysis System (SAS, 1990) to evaluate the significance of differences between mean values. Relationships of parameters were established using a linear regression method.

RESULTS AND DISCUSSION

Both refined, bleached and deodorized (RBD) canola and soybean oils used in this study had good initial qualities, i.e. having iodine values of 111 and 126 g iodine/100 g oil and peroxide values of 0·91 and 0·54 meq/kg oil, respectively (Table 1). The fatty acid composition of both oils showed that canola oil had a reasonably high content of monoenes as compared with soybean oil, but the latter had a higher total content of

Table 1. Chemical properties of RBD canola and soybean oils

Parameter	Canola	Soybean
Iodine value (g iodine per 100 g oil)	111 ± 2·01	126 ± 3·20
Peroxide value (meq/kg oil)	0·91 ± 0·02	0·54 ± 0·01
Fatty acid composition (area %)		
C16:0	4·20 ± 0·02	10·2 ± 0·04
C16:1	0·26 ± 0·01	—
C18:0	1·91 ± 0·02	4·22 ± 0·20
C18:1	57·6 ± 0·51	24·4 ± 0·11
C18:2	23·4 ± 1·00	52·0 ± 0·22
C18:3	9·10 ± 0·11	7·70 ± 0·20
C20:0	0·63 ± 0·01	0·88 ± 0·01
C20:1	2·00 ± 0·10	0·30 ± 0·04
C22:0	0·34 ± 0·01	0·30 ± 0·05
C22:1	0·38 ± 0·01	—

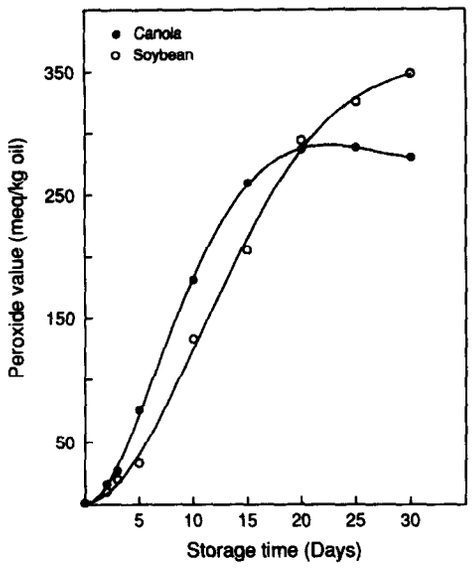

Fig. 1. PVs of canola and soybean oils during accelerated dark storage at 65°C.

polyunsaturated fatty acids. The slightly higher initial PV of the canola oil tested as compared with that of soybean oil may be due to the high content of α-linolenic acid and formation of more hydroperoxides during the extraction process.

Figure 1 shows peroxide formation over the 30-day storage period at 65°C. Canola oil produced slightly higher amounts of peroxides than soybean oil for up to 20 days of storage, after which the trend was reversed. While the induction period of canola oil was 2 days, a 3-day induction period was noted for soybean oil. Although it is understood that oils with PVs exceeding 50 meq/kg oil may produce unacceptable odours and cause quality deterioration of the oils, the experiments were continued beyond 10 days of storage in order to establish correlations with other indices of oxidation. Canola oil had a typical lipid oxidation curve showing induction, propagation and termination stages during the 30-day storage period. The propagation stage for soybean oil still continued after the 30-day period. The high PV of canola oil during the initial stage may be attributed to its higher content of α-linolenic acid compared with that of soybean oil. Frankel (1980) suggested that low levels of linolenate hydroperoxides might catalyse the oxidation of linoleate, the predominant fatty acid in soybean oil, hence producing more hydroperoxides during long term storage.

Formation of CD during storage of both canola and soybean oils is shown in Table 2. A higher level of CD was reached for canola oil as compared with soybean oil over a 15-day storage period at 65°C. However, in the later stages of storage (after 15 days), soybean oil showed CD values higher than those of canola oil. As stated previously, the higher CD values for canola oil during the initial stage may also be due to its high linolenic acid content as compared with soybean oil. Linolenic acid contains two inner allylic methylene groups

Table 2. Conjugated diene and triene values of RBD canola and soybean oils during accelerated dark storage at 65°C

Storage time (days)	Conjugated diene value (optical density at 234 nm)[a]		Conjugated triene value (optical density at 268 nm)[a]	
	Canola	Soybean	Canola	Soybean
0	3·69 ± 0·06	4·90 ± 0·10	0·78 ± 0·04	0·80 ± 0·01
2	6·01 ± 0·10	5·00 ± 0·24	0·89 ± 0·03	1·16 ± 0·02
3	7·90 ± 0·12	6·05 ± 0·33	0·98 ± 0·02	1·19 ± 0·01
5	12·6 ± 0·15	9·41 ± 0·34	1·04 ± 0·01	1·23 ± 0·03
10	21·8 ± 0·82	19·6 ± 0·65	1·82 ± 0·03	1·61 ± 0·08
15	27·0 ± 0·90	27·8 ± 1·78	3·42 ± 0·01	2·31 ± 0·07
20	30·2 ± 0·31	38·1 ± 1·44	4·86 ± 0·02	3·03 ± 0·02
25	30·8 ± 1·21	48·3 ± 1·35	5·72 ± 0·02	5·45 ± 0·03
30	30·3 ± 1·16	52·2 ± 2·50	6·14 ± 0·01	8·33 ± 0·02

[a]1% oil in iso-octane.

(—CH=CH—CH_2—CH=CH—CH_2—CH=CH—), thus double bonds in C18:3 are twice as likely to shift and form a CD than C18:2 (Frankel, 1985). The levelling off of CD formation after 15 days of storage may be due to termination of shifting of C18:3 double bonds in canola oil, which has a lower content of C18:2 as compared with soybean oil. The CD values of soybean oil after 15 days tended to follow the degree of polyunsaturation with a higher overall CD formation (Liu & White, 1992).

Peroxide value provides a clear indication of the initial oxidation potential of different lipids since hydroperoxides are the primary products of lipid oxidation (Labuza, 1971). Conjugated diene value may also be used to determine the initial rate of oxidation (Gray, 1978). Accordingly, both of these methods indicated that canola oil was more prone to oxidation than soy bean oil. Excellent correlations were calculated between PV and CD for canola oil (r = 0·997) and for soybean oil (r = 0·989) (Fig. 2). Jackson (1981) reported that formation of peroxides normally coincides

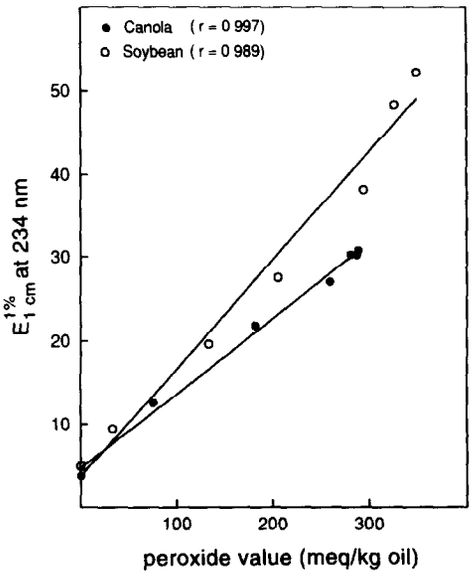

Fig. 2. Relationship between PV and CD value of canola and soybean oils.

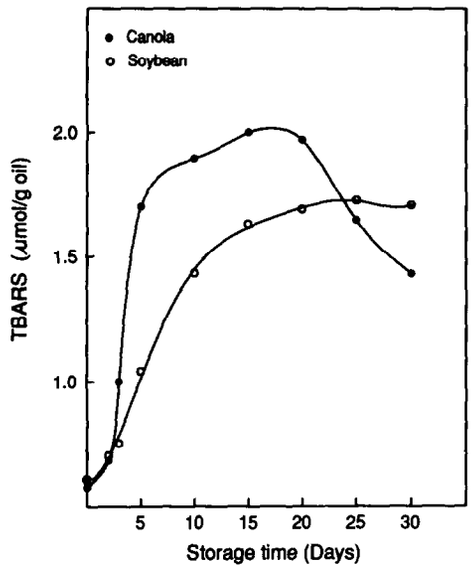

Fig. 3. TBARS values of canola and soybean oils during accelerated dark storage at 65°C.

with CD formation in oils upon oxidation. The CD assay is faster than PV determination and does not depend on chemical reactions such as colour development for its determination. Therefore, CD content may be used as a measure of primary oxidation products for both canola and soybean oils.

Formation of CTs during oxidation of canola and soybean oils is illustrated in Table 2. Soybean oil had higher CT values than canola oil during the first 5 days of storage, after which canola oil produced more CTs (up to 25 days). Conjugated trienes may be produced by dehydration of CD hydroperoxides (Fishwick & Swoboda, 1977). Canola oil produced more CD (Table 2) up to 15 days; this may be the reason for higher CT values in canola during the extended storage period.

Production of the TBARS in both oils is presented in Fig. 3. Canola oil had significantly ($P < 0{\cdot}05$) higher TBARS values as compared with soybean oil, which may also be due to its higher linolenate content. It was noted that TBARS of canola oil reached a maximum on day 18 and then declined, perhaps due to volatilization of carbonyl compounds or their further reactions.

Changes in TOTOX value of canola and soybean oils during the 30-day storage period are shown in Fig. 4. Canola oil for up to 25 days had TOTOX values higher than those of soybean oil. Changes of TOTOX value provide information regarding progression of formation of primary and secondary oxidation products. According to these results, formation of both primary and secondary lipid oxidation products was higher in canola oil than in soybean oil. The TOTOX value is often considered to have the advantage of combining evidence about the past history of the oil (as reflected in the AnV) with its present state (as evidenced in the PV). Therefore determination of TOTOX value has been used extensively to estimate oxidative deterioration of lipids (Rossell, 1983). However, it is worth noting that despite its practical advantages, the TOTOX value does not have any sound scientific basis since it combines variables with different dimensions.

The ^{1}H-NMR of canola and soybean oils exhibited changes in aliphatic, olefinic and diallylmethylene protons of their fatty acid constituents during storage. The ratios of aliphatic to olefinic protons (R_{ao}) and aliphatic to diallylmethylene protons (R_{ad}) were determined. It was found that R_{ao} and R_{ad} increased continuously during storage of both oils (Fig. 5). Canola oil showed a higher R_{ao} and R_{ad} than soybean oil, which may reflect lower numbers of total olefinic and diallylmethylene protons, respectively, in canola oil than in soybean oil. Furthermore, plotting of R_{ao} and R_{ad} against corresponding TOTOX values for both canola and soybean oils indicated a significant ($P < 0{\cdot}05$) linear correlation between both R_{ao} and R_{ad} and TOTOX values for both

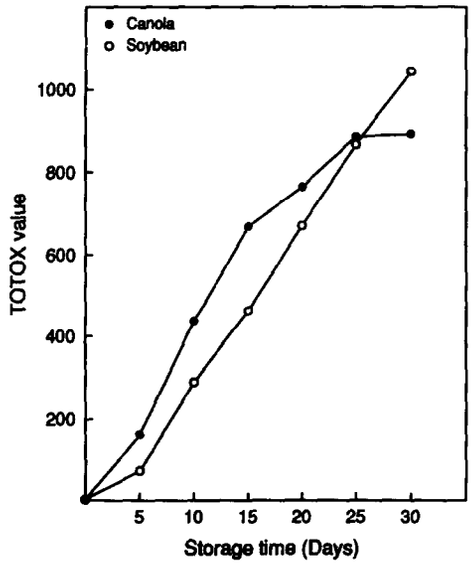

Fig. 4. TOTOX values of canola and soybean oils during accelerated dark storage at 65°C.

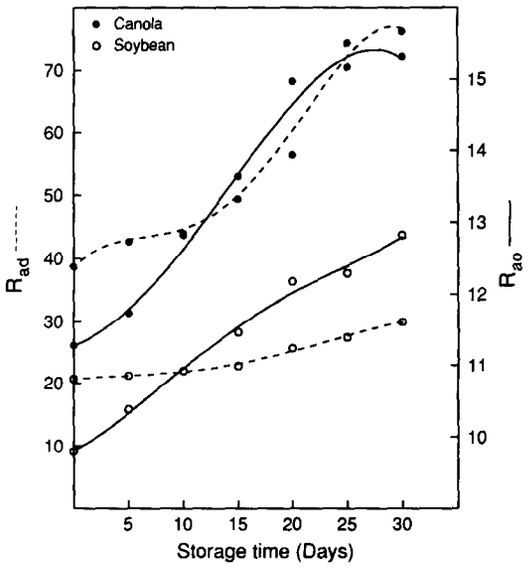

Fig. 5. Changes in aliphatic proton to diallylmethylene proton ratio (R_{ad}) and aliphatic proton to olefinic proton ratio (R_{ao}) of canola and soybean oils during accelerated dark storage at 65°C.

oils. Correlation coefficients were 0·974 and 0·926 for R_{ao} and R_{ad} for canola oil and 0·985 and 0·977 for R_{ao} and R_{ad} for soybean oil, respectively. Therefore, NMR methodology can offer an alternative method to estimate the overall primary and secondary changes in canola and soybean oils during oxidation. Use of NMR methodology for estimation of oxidative stability of brown fish meal has recently been reported (Saito & Udagawa, 1992). Good correlations were found between PVs and the NMR results. In this study, correlation coefficients between peroxide and R_{ad} and R_{ad} values were 0·848, 0·623, 0·880 and 0·820 for canola and soybean oils, respectively. However, better nonlinear correlations may exist between the PVs and the NMR data. TOTOX values for canola and soybean oils correlated better than PVs with R_{ao} and R_{ad} values. This is not surprising since both TOTOX and NMR data estimate the overall changes in fatty acid profile of oils and include both primary and secondary changes during lipid oxidation. The NMR methodology provides a rapid, nondestructive and simple procedure for evaluation of the oxidative changes during storage of edible oils.

ACKNOWLEDGEMENTS

We are grateful to the Canola Council of Canada for financial support. Grateful acknowledgement is also made to Mrs N. Brunet for her assistance in carrying out NMR analyses.

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