Evaluation of Oxidative Stability of Canola Oils by Headspace Analysis

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ABSTRACT: Lipid oxidation is a major factor affecting flavor quality and shelf life of vegetable oils. Oxidative stability is therefore an important criterion by which oils are judged for usefulness in various food applications. In this study a method based on headspace analysis was developed to evaluate relative oxidative stability of canola oils. The method does not require the use of chemicals, involves minimal sample preparation, and can be performed on a relatively small sample size in comparison with traditional wet chemical methods. Canola oils freshly extracted in the laboratory from different seed samples were subjected to accelerated oxidation and analyzed for PV by standard methods and headspace volatiles by solid phase microextraction/GC–MS. Forward stepwise regression analysis of the data revealed a relationship between PV and headspace concentration of the volatile lipid oxidation products hexanal and *trans,trans*-2,4-heptadienal. The PV calculated using this formula correlated (R^2 = 0.73) with those measured by conventional methods.

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KEY WORDS: Accelerated oxidation, canola oil, headspace analysis, hexanal, lipid oxidation, oxidative stability, peroxide value, solid-phase microextraction, *trans,trans*-2,4-heptadienal, volatile compounds.

Lipid oxidation is probably the single most important factor affecting the shelf life of edible oils. The hydroperoxides formed by lipid oxidation degrade into various smaller molecules such as aldehydes, ketones, alcohols, and acids. Some of these volatile lipid oxidation products affect flavor at extremely low concentrations, often below 1 ppm, whereby both the oil and the food prepared with it become unpalatable (1). Thus, oxidative stability is a key factor in developing new oils for food applications.

There are well-established analytical methods to assess the extent of lipid oxidation in stored or used oils. These include methods that measure primary oxidation products (e.g., PV) and secondary oxidation products (e.g., anisidine value and polar material). In contrast, methods to predict the oxidative stability of fresh oils are less well developed. A few empirical methods that have been attempted measure the time required to attain a predetermined value for one or more lipid oxidation parameters, such as PV, under accelerated oxidation conditions. The now-obsolete AOCS Fat Stability, Active Oxygen method (AOCS Official Method Cd 12-57) (2) was based on the above principle. Alternatively, PV at a particular time point during accelerated oxidation may be used to compare the oxidative stabilities of oils.

The current AOCS-approved Oil Stability Index (OSI) method (Oil Stability Index, Cd 12b-92) (2) is based on the determination of the induction period of oils subjected to accelerated oxidation. Instruments for measuring the OSI are available from commercial suppliers, for example, the Oxidative Stability Instrument (Omnion Inc., Rockland, MA) and Rancimat (Brinkmann Instruments, subsidiary of Sybron Corp., Westbury, NY). Broadbent and Pike (3), who recently evaluated the usefulness of the OSI as an oxidative stability test for canola oil, concluded that although the relationship that exists between OSI and sensory induction period is sufficiently strong to warrant the use of OSI in industry applications, it may not be ideal for more precise studies of canola oil shelf life. Furthermore, the OSI and other empirical methods outlined above require relatively large quantities of oil (>5 g) and are not suitable in situations when only minute quantities of oil (<1 g) are available for analysis. An example of such a situation is where a large number of experimental seed cultivar lines are grown in glasshouse conditions and only a few drops of oil are available for stability testing.

Some of the secondary products of lipid oxidation are volatile and can be detected and measured by headspace (HS) analysis. HS analysis can be performed with standard GC equipment commonly used by lipid chemists. A range of different, well-established techniques such as static HS sampling (SHS), dynamic HS sampling (DHS), and solid-phase microextraction (SPME) are available for the extraction of volatile compounds from oils prior to GC or GC–MS analysis (4,5). HS analyses have been used to determine volatile compounds in vegetable oils in studies aimed at differentiating oils according to geographic origin (6–8), variety (9) and sensory quality (10), as well as for assessing the oxidative status of oil (11,12).

HS methods are rapid and suitable for routine, consecutive analyses of many samples. The availability of various types of very reliable HS autosamplers has enabled the HS analysis of a large number of samples with minimal operator intervention.

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The SHS method has the important advantage of not requiring that the instrument be cleaned between sample injections, because only volatile compounds are injected to the gas chromatograph and the nonvolatile portion of the sample is retained in the vial (13). This also applies to SPME where the volatiles are extracted from the SHS onto an adsorbent fiber prior to GC analysis.

A major advantage of SHS analysis, including SPME, over other methods is the ability to perform volatile analysis on as little as 0.1 g of oil. Moreover, HS methods do not require the use of chemicals or elaborate sample preparation. Therefore, a method based on SHS or SPME analysis would be useful for evaluating the relative oxidative stability of oils without resorting to wet chemical methods, particularly when only small amounts of sample are available. Propanal has been widely used for the evaluation of the oxidative stability of oils containing *n*-3 oils (14–19). The application of propanal for this purpose has been based on the fact that it is a degradation product of *n*-3 PUFA, but there appear to have been no studies to correlate propanal content with conventional oxidation measurements such as PV. In this paper, we report on the establishment of a correlation between HS volatiles and PV of canola oils.

EXPERIMENTAL PROCEDURES

Oil samples. All oils used in this study, except the commercial canola oil, were freshly extracted in the laboratory from different cultivar lines of *Brassica juncea* and *B. napus* seeds (50 g) grown at a variety of sites within four Australian states (Western Australia, South Australia, Victoria, and New South Wales). Oil extraction was performed by Soxhlet solvent extraction (AOCS Official Method, Cd 8b) (2). The extracted oils were stored under nitrogen at −18°C until required. The commercial canola oil (refined, but without added antioxidants) was supplied by a local oil processor (Peerless Holdings, Melbourne, Australia).

Chemicals. trans,trans-2,4-Heptadienal (90% pure) and all organic solvents (analytical grade) were purchased from Sigma-Aldrich (Sydney, Australia). Each oil sample was analyzed in triplicate. The SPME fibers (polydimethylsiloxane/divinylbenzene, PDMS/DVB) were purchased from Supelco (Sydney, Australia).

Accelerated oxidation. In the first part of the study aimed at establishing a relationship between PV and volatile oxidation products, freshly extracted oils (2.5 g) were placed in 35-mm diameter Petri dishes and heated at 60°C inside a dark oven for up to 210 h. Dishes were removed from the oven at predetermined time intervals for analysis of volatiles and measurement of PV. A separate dish was used at each time point. In a separate experiment to verify the suitability of the method to evaluate the oxidative stability of canola oils, freshly extracted oils (1.0 g) were placed in glass HS vials (10 mL) and heated as above for up to 170 h. Once removed, the vials were sealed as before and immediately analyzed for HS volatiles.

HS analysis. The volatile composition of oxidized oils was determined by SPME and GC–MS analysis. The oil sample (1.0 g) was placed in a 10-mL glass HS vial and sealed with a polytetrafluoroethylene/silicone septum and aluminum cap. The vial containing the oil was preheated at 60°C for 2 min, the SPME fiber (PDMS/DVB) inserted into the sample HS, and heating continued for a further 30 min. The fiber was then withdrawn and inserted into the GC injector to desorb the adsorbed compounds onto the GC column. The fiber remained in the injector for 7 min. The entire sequence of events was performed by the autosampler.

GC was conducted on a volatile organic compound (VOC) fused-silica capillary column (60 m, 0.32 mm i.d., 1.8 μm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector was operated in the splitless mode for the first 2 min, and its temperature was maintained at 220°C. The oven temperature was increased from −20 to 180°C at the rate of 50°C min^{-1}, to 210°C at the rate of 2°C min^{-1}, to 230°C at the rate of 5°C min^{-1}, and finally to 250°C at the rate of 20°C min^{-1}, where it was held for 2 min. The mass spectrometer was operated in electron ionization mode (70 eV), and data were acquired in the full-scan mode for the range *m/z* 29–250. The detector was operated at the auto-tune voltage. The temperatures of the source and the detector were 150 and 230°C, respectively, while the mass spectrometer transfer line was held at 280°C. The volatile compounds were identified by reference to the instrument's electronic library of mass spectra (Wiley 275). Quantifications were based on the abundance of characteristic fragment ions using Chemstation software (Agilent, Forest Hill, Victoria, Australia).

Verification of 2,4-heptadienal isomers. trans,trans-2,4-Heptadienal was isomerized by heating it in the presence of *p*-toluene sulfinic acid using a modification of the procedure reported by Snyder and Scholfield (20). In brief, 250 μL of a 0.1% (wt/vol) solution of *trans,trans*-2,4-heptadienal in ethylene glycol was mixed with 250 μL of a 0.1% (wt/vol) solution of *p*-toluene sulfinic acid in the same solvent in a 1.0 mL pressure vial and heated at 158°C for 6 h.

PV. PV was determined according to the SafTest® PeroxySafe® procedure (http://www.saftest.com). The colorimetric measurements were made at 570 nm using reagents supplied by SafTest Inc. (Tempe, AZ) after validating the method with the AOCS standard method Cd 8b (2) ($R^2 = 0.93$).

FA composition. The oil (0.06 g) was dissolved in THF (1 mL) and mixed with sodium methoxide (0.5 M, 2 mL). The mixture was heated at 40°C for 1 h. On cooling, the reaction was terminated by addition of glacial acetic acid (100 μL) and water (5 mL). The FAME were extracted into heptane (2 × 2 mL) and dried over a mixture of sodium sulfate and sodium bicarbonate (9:1 w/w) (21).

Statistical analysis. To establish a model relating ln PV to the volatile oxidation products, an initial selection of explanatory variables was made using "forward stepwise regression analysis." This approach started with the explanatory variable that made the most significant contribution to the model, then the variable that made the most significant additional contribution was added, and so on. Variables whose correlation coefficient with ln PV was between –0.2 and 0.2 were excluded from consideration. The criterion for entry was an *F*-ratio of 2, which

corresponds to a *P*-value of around 0.16. Five variables entered the model: acetic acid, pentanal, 1-penten-3-ol, hexanal, and *trans,trans*-2,4-heptadienal. Following this initial selection, variables were progressively omitted from the model if their contribution was not significant at the 0.05 level when corrected for the other variables in the model. The variable 1-penten-3-ol was omitted first, followed by acetic acid, and then pentanal. This resulted in a final model, which included only hexanal ($P < 0.001$) and *trans,trans*-2,4-heptadienal ($P < 0.01$). The fitted equation was $\ln PV = -2.50 + 0.308 \times \ln[\text{hexanal}] + 0.229 \times \ln[\textit{trans,trans}\text{-2,4-heptadienal}]$ ($R^2 = 0.26$).

The interactions of the two variables with seed variety (and cultivar line) and the growing location were then examined. A significant interaction would mean that the model could differ, depending on the sample, preventing general application of the formula for all sample types. Results showed that neither the site effect nor variety/cultivar line effect (and their interaction with either variable) was significant at the 0.05 level.

RESULTS AND DISCUSSION

Table 1 shows the average FA composition of *B. napus* and *B. juncea* oils used in this study. The average levels of the PUFA linoleic (18:2) and linolenic (18:3) in the *B. napus* oils were 19.1 and 9.6%, respectively. The corresponding values for *B. juncea* oils were 28.8% and 13.1%, respectively. Both these PUFA undergo oxidative deterioration and produce volatile degradation products that lead to oxidative rancidity in canola oils. In this respect, linolenic acid is more undesirable than linoleic acid for two reasons: (i) Linolenic acid undergoes autoxidation faster than linoleic acid, and (ii) the volatiles derived from linolenic acid have lower flavor threshold values than those derived from linoleic acid, thereby causing undesirable odors and flavors at a much lower concentration than linoleic acid (13).

In this study, 77 volatile compounds were identified in oxidized canola oil. Several other volatile components were also detected in relatively low concentrations but were not fully characterized. The number of volatile compounds detected by HS methods depends on the particular technique and operating conditions (e.g., the sampling temperature and time) used for HS extraction. SHS methods are suitable for detecting highly volatile compounds but are not particularly suited for detecting components with relatively low volatility. In the present study, HS sampling using SPME was used, enabling the detection of many more volatile compounds than is possible by SHS analysis. Even with SPME, the type and range of compounds detected differ depending on the particular fiber used. In this study, we used PDMS/DVB fiber. It detects a wider range of volatiles than, for example, a PDMS/Carboxen fiber, which favors lower M.W. compounds. The abundance of volatile peaks in GC–MS is also a function of the type of SPME fiber used for analysis. The actual concentrations of volatiles present in oil can only be obtained by calibrating for each and every compound present using authentic standards. This was neither practical nor necessary for the present investigation. Instead, relative peak abundances were used, which were more than adequate for comparing the amounts of volatiles present in oils oxidized to different extents.

Accelerated oxidation of the commercial canola oils was conducted at 60°C for up to 672 h, and the volatile compounds were measured at 0, 48, 120, 480, and 672 h. During this period, the volatile compounds increased to a maximum before falling off. The time taken to reach the maximum was different for each compound. Most volatiles attained concentration maxima between 120 and 672 h of oxidation (data not shown). One exception was 2-pentyl furan, which continued to increase in concentration during the whole period of accelerated oxidation. The PV of the oils also increased until approximately 480 h when they began to fall. Some of the more dominant compounds present in commercial canola oil subjected to accelerated oxidation included hexanal, heptanal, nonanal, 2-octenal, 2-decenal, *cis,trans*-2,4-heptadienal, *trans,trans*-2,4-heptadienal, hexanoic acid, 1-octen-3-ol, and 2-pentyl furan. Neither *cis,trans*-2,4-decadienal nor *trans,trans*-2,4-decadienal was evident in the earlier stages of oxidation, but steadily increased in concentration toward the later stages (480–672 h).

There were no significant qualitative or quantitative differences in the volatile compounds formed when the oils extracted from *B. napus* and *B. juncea* seeds were subjected to accelerated oxidation. As with the refined, commercial canola oil, the amount of each volatile compound formed was a function of time. Among the most abundant peaks in the average volatile profile of the laboratory-extracted canola oils oxidized for 168 h were pentanal, hexanal, nonanal, 2-pentenal, *cis,trans*-2,4-heptadienal, *trans,trans*-2,4-heptadienal, 6-methyl,5-hepten-2-one, *cis,trans/trans,cis*-3,5-octadien-2-one, *trans,trans*-3,5-octadien-2-one, propanoic acid, hexanoic acid, and 2-ethyl furan.

TABLE 1
Average FA Composition of Unrefined Oils Solvent-Extracted in the Laboratory from *Brassica napus* (*n* = 86) and *B. juncea* (*n* = 38) Seeds, and Refined, Commercial Canola Oil (*n* = 1)

	FA composition (wt%)											
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
B. napus	0.2	4.4	0.3	2.3	61.6	19.1	9.6	0.7	1.4	0.2	0.2	0.1
Std. dev.	*0.2*	*0.2*	*0.1*	*0.4*	*2.5*	*1.5*	*1.8*	*0.1*	*0.2*	*0.1*	*0.1*	*0.2*
B. juncea	0.2	3.8	0.3	2.5	48.0	28.8	13.1	0.7	1.6	0.2	0.5	0.4
Std. dev.	*0.2*	*0.6*	*0.1*	*0.3*	*4.3*	*2.8*	*1.8*	*0.1*	*0.9*	*0.1*	*0.2*	*1.5*
Commercial canola	0.1	4.3	0.2	2.0	60.5	21.3	9.3	1.6	0.2	0.3	0.0	0.2

Interestingly, the unrefined laboratory-extracted oils contained a number of volatile compounds that were either absent or present in much lower concentrations in the refined canola oil oxidized under the same accelerated conditions. They included appreciable amounts of the *cis,trans*-3,5-octadien-2-one and *trans,trans*-3,5-octadien-2-one (Fig. 1). These compounds occurred at a much lower level in the commercial canola oil and disappeared as the oxidation progressed. 3,5-Octadien-2-one is derived from the oxidation of *n*-3 PUFA such as linolenic acid (22) and 4,7,10,13,16,19-DHA (23). In a separate study, we observed that *cis,trans/trans,cis*-3,5-octadien-2-one and *trans,trans*-3,5-octadien-2-one are formed in abundance during accelerated oxidation of cold-pressed, unrefined flaxseed oil (linolenic acid content 56%). In addition to 3,5-octadien-2-one, significant amounts of limonene and 6-methyl,5-hepten-2-one were also present in the unrefined oil. Jelen *et al.* (10) found 6-methyl, 5-hepten-2-one in both cold-pressed and refined rapeseed oil, but limonene only in cold-pressed oil. The latter compounds are natural components of vegetable oils rather than oxidation products and may be lost during industrial oil processing.

The volatile compounds in vegetable oils mainly result from lipid oxidation. The process starts with the reaction of oxygen with the double bonds in the unsaturated FA leading to the formation of lipid hydroperoxides, which then degrade into various lower M.W. secondary oxidation products such as alcohols, aldehydes, ketones, and acids. Although hydroperoxides themselves are not flavor-active, their degradation products have strong flavors and can influence the flavor characteristics of the oil, as well as that of the foods prepared in it. This influence can be positive or negative depending on the amount of the secondary oxidation products formed, as well as their flavor thresholds. Although a low concentration of some volatiles products are desirable or even essential to produce certain desirable flavor characteristics, e.g., fried food flavor, higher amounts of the same compounds may lead to unpleasant odors and flavors.

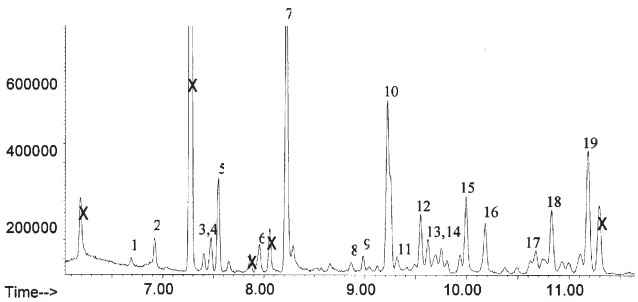

FIG. 1. Partial GC–MS chromatogram of laboratory-extracted canola oil subjected to accelerated oxidation. Peak designation as follows: (1) acetic acid, (2) propanoic acid, (3) 1-penten-3-ol, (4) 2,3-pentanedione, (5) 2-ethyl furan, (6) 2-pentenal/1-pentanol, (7) hexanal, (8) 2-heptanone/nonane, (9) heptanal, (10) limonene, (11) hexanoic acid, (12) 2-heptenal, (13) 1-octen-3-ol, (14) 2-pentyl furan, (15) *cis,trans/trans,cis*-2,4-heptadienal, (16) *trans,trans*-2,4-heptadienal, (17) 2-octenal, (18) *cis,trans*-3,5-octadien-2-one, (19) *trans,trans*-3,5-octadien-2-one/nonanal, and (X) artifacts from solid-phase microextraction fiber.

Aliphatic aldehydes constitute a major portion of the volatiles generated by lipid oxidation, as demonstrated in the present study for canola subjected to accelerated oxidation. Generation of aliphatic aldehydes from oil oxidation is of interest because they are major contributors to unpleasant odors and flavors in food products. Many of these aldehydes have strong, rancid-like flavors, and some, particularly dienals, have very low flavor thresholds (1). The volatile aldehydes derived from linolenic acid (soybean and canola oils) have significant sensory impact and lower threshold values than those derived from oils containing linoleic acid (cottonseed, corn, and sunflower oils). The most sensorily significant linolenate-derived aldehydes such as 2,4-heptadienal and 2,4-nonadienal are characteristic in having *n*-3 unsaturation (13).

Characterization of geometric isomers of 2,4-heptadienal. 2,4-Heptadienal is derived from oxidation of linolenic acid, and its presence in oxidized soybean, rape, and canola oils has been reported. The GC chromatograms for the volatile profile of these oils contain two well-resolved heptadienal peaks, which most authors have simply referred to as 'heptadienal' or heptadienal A and B. To the best of our knowledge, the geometric isomerism of these important secondary lipid oxidation products has not been investigated. We used a relatively nonpolar GC column (VOC) for volatile analysis, and the heptadienal isomer with the higher retention time on this column co-eluted with authentic *trans,trans*-2,4-heptadienal. Chemical isomerization of *trans,trans*-2,4-heptadienal with *para*-toluene sulfinic acid produced a mixture of geometric isomers corresponding to *cis,trans/trans,cis* and *trans,trans* isomers. Further heating at elevated temperatures produced higher amounts of the *cis,trans/trans,cis*-isomers at the expense of the *trans,trans* isomer. Only trace amounts of the *cis,cis*-isomer were produced. On this basis, the heptadienal isomer eluting first probably was *cis,trans/trans,cis*-2,4-heptadienal and the second was confirmed as *trans,trans*-2,4-heptadienal.

Relationship between PV and HS volatiles. To determine volatile compounds that could be used as measures of lipid oxidation, correlations were sought between PV and the concentration of various volatile compounds formed during accelerated oxidation of canola oils. For this purpose, 114 samples of oils from *B. napus* and *B. juncea* seed (various cultivars and growing locations) and solvent-extracted in the laboratory were subjected to accelerated autoxidation and analyzed for PV and volatile oxidation products. Forward stepwise regression analysis of the data revealed a statistically significant correlation between PV and the concentration of hexanal and *trans,trans*-2,4-heptadienal represented by the formula

$$\ln \mathrm{PV} = -2.50 + 0.308 \times \ln[\text{hexanal}] + 0.229 \times \ln[\textit{trans,trans}\text{-2,4-heptadienal}] \quad [1]$$

where PV is the peroxide value determined by conventional methods, and [hexanal] and [*trans,trans*-2,4-heptadienal] are the HS concentrations of hexanal and *trans,trans*-2,4-heptadienal, respectively.

The PUFA linoleic and linolenic acids are the main sub-

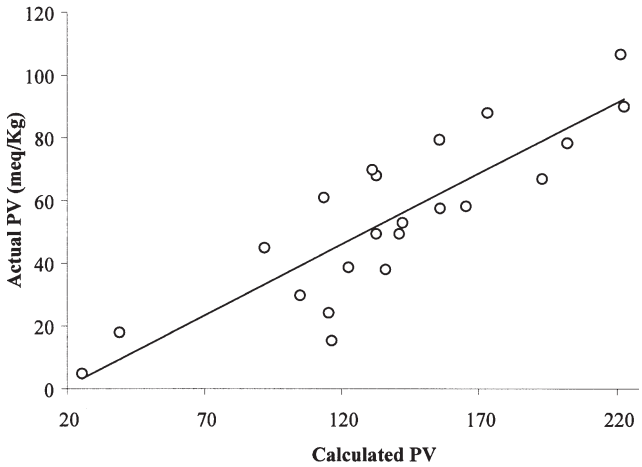

FIG. 2. Correlation between calculated PV and actual PV (mequiv/kg) for 22 samples of solvent-extracted, unrefined canola oils oxidized for different periods at 60°C.

strates for autoxidation in canola oils. The contents of linoleic and linolenic acids in canola oils used in this study were 20 and 10%, respectively, for *B. napus* and 32 and 13%, respectively, for *B. juncea*. As hexanal and *trans,trans*-2,4-heptadienal are degradation products of the hydroperoxides formed from the autoxidation of linoleic acid and linolenic acid, respectively, it is not surprising that PV of oxidized canola oil is associated with these two aldehydes.

Many aldehydes affect the flavor of vegetable oils and consequently the flavor of food prepared with the oil. The unsaturated aldehydes in particular are very potent flavor compounds, and have very low flavor threshold values. *trans,trans*-2,4-Heptadienal, the flavor of which has been variously described as rancid, fishy, and fatty, has a flavor threshold value of 0.04 ppb in oil (1). This compound, together with other aldehydes, has been associated with rancidity in fish oils and fish products (24–27).

The formula generated in the present study allows the calculation of PV for oils subjected to accelerated oxidation from the concentration of hexanal and *trans,trans*-2,4-heptadienal in the oil HS. However, the calculated PV depend on the actual experimental conditions used for the accelerated oxidation (sample size, surface area-to-volume ratio, etc.) as well as HS analysis (HS sampling conditions, SPME fiber type, peak integration parameters, etc.). Despite this, a linear relationship exists between PV calculated by the formula and those determined by standard chemical methods. For comparison of oxidative stabilities of different canola oils by the HS method, it is more convenient to conduct the accelerated oxidation in HS vials so that the oxidized sample can be used directly for HS analysis. When 1.0-g samples of fresh canola oils were oxidized in 10-mL HS vials and analyzed under the same conditions as before, a linear relationship ($R^2 = 0.73$) was observed between the calculated and experimental PV (Fig. 2). Thus, HS analysis can potentially be used as an alternative method to determine relative PV for comparing the oxidative stability of different canola oils.

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