Published November 21, 2014

Methods to create thermally oxidized lipids and comparison of analytical procedures to characterize peroxidation[1]

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ABSTRACT: The objective of this experiment was to evaluate peroxidation in 4 lipids, each with 3 levels of peroxidation. Lipid sources were corn oil (CN), canola oil (CA), poultry fat, and tallow. Peroxidation levels were original lipids (OL), slow-oxidized lipids (SO), and rapid-oxidized lipids (RO). To produce peroxidized lipids, OL were either heated at 95°C for 72 h to produce SO or heated at 185°C for 7 h to produce RO. Five indicative measurements (peroxide value [PV], p-anisidine value [AnV], thiobarbituric acid reactive substances [TBARS] concentration, hexanal concentration, 4-hydroxynonenal [HNE] concentration, and 2,4-decadienal [DDE]) and 2 predictive tests (active oxygen method [AOM] stability and oxidative stability index [OSI]) were performed to quantify the level of oxidation of the subsequent 12 lipids with varying levels of peroxidation. Analysis showed that a high PV accurately indicated the high level of lipid peroxidation, but a moderate or low PV may be misleading due to the unstable characteristics of hydroperoxides as indicated by the unchanged PV of rapidly oxidized CN and CA compared to their original state (OL). However, additional tests, which measure secondary peroxidation products such as AnV, TBARS, hexanal, HNE, and DDE, may provide a better indication of lipid peroxidation than PV for lipids subjected to a high level of peroxidation. Similar to PV analysis, these tests may also not provide irrefutable information regarding the extent of peroxidation because of the volatile characteristics of secondary peroxidation products and the changing stage of lipid peroxidation. For the predictive tests, AOM accurately reflected the increased lipid peroxidation caused by SO and RO as indicated by the increased AOM value in CN and CA but not in poultry fat and tallow, which indicated a potential disadvantage of the AOM test. Oxidative stability index successfully showed the increased lipid peroxidation caused by SO and RO in all lipids, but it too may have disadvantages similar to AnV, TBARS, hexanal, DDE, and HNE because OSI directly depends on quantification of the volatile secondary peroxidation products. To accurately analyze the peroxidation damage in lipids, measurements should be determined at appropriate time intervals by more than 1 test and include different levels of peroxidation products simultaneously.

Key words: hydroperoxides, lipid oxidation, secondary oxidation products

 J. Anim. Sci. 2014.92:2950–2959
doi:10.2527/jas2012-5708

INTRODUCTION

Energy is one of the most expensive components of swine diets. Lipids are commonly added to swine diets as concentrated energy sources to improve feed efficiency (Pettigrew and Moser, 1991) but may also reduce feed dust, supply fat soluble vitamins and essential fatty acids, and improve diet palatability. Lipids used in animal production not only vary in fatty acid composition because of their origin but may also contain various concentrations of primary and secondary lipid peroxidation products depending on fatty acid composition, storage length and conditions, and effects of processing (Canakci, 2007).

Lipid peroxidation, especially when subjected to heat during processing or cooking, may deplete endogenous antioxidants (Seppanen and Csallany, 2002) and generate an assortment of peroxidation

[1]This research was financially supported by the National Pork Board (Des Moines, IA) and the Fats and Proteins Research Foundation (Alexandria, VA). Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA or the University of Minnesota and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

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Received August 3, 2012.
Accepted April 14, 2014.

products (Lin et al., 1989; Adam et al., 2008), which increase intestinal oxidative stress (Suomela et al., 2005; Ringseis et al., 2007), damage small intestinal structure (Dibner et al., 1996a,b), and impair immune function (Takahashi and Akiba, 1999). In addition, DeRouchey et al. (2004) reported that feeding thermally oxidized choice white grease to pigs may also reduce growth performance. Therefore, accurately evaluating lipid peroxidation is essential for optimizing the value of lipids as an energy source in swine diets.

Although many analytical methods are commonly used to characterize lipid peroxidation, limitations of each method should not be overlooked due to the complexity of lipid peroxidation reactions, which produce a chemically diverse group of oxidation compounds. In the current experiment, 4 types of lipids, varying in their fatty acid composition, were thermally oxidized by 2 different heating methods to assess commonly used methodology to evaluate lipid peroxidation for use in animal feeds.

MATERIALS AND METHODS

Lipid Preparation

In the current experiment, 4 lipids, each with 3 levels of peroxidation, were evaluated. Lipid sources were corn oil (**CN**; ADM, Decatur, IL), canola oil (**CA**; ConAgra Foods, Omaha, NE), poultry fat (**PF**; American Protein, Inc., Hanceville, AL), and tallow (**TL**; Darling International, Wahoo, NE). Peroxidation levels were original lipids (**OL**), slow-oxidized lipids (**SO**), and rapid-oxidized lipids (**RO**). To generate peroxidized lipids, vesicles containing the required amount of each OL were either heated at 95°C for 72 h to produce SO or heated at 185°C for 7 h to produce RO. Using an air pump, both heating processes were accompanied with a constant flow of air of 12,000 cm^3/min and a temperature of 22 to 24°C. All of the OL, SO, and RO sources were stored at –20°C and no antioxidant was added before laboratory analysis.

Analysis of Chemical Properties of Lipids

Crude fat (method 920.39 A; AOAC, 2010), free fatty acids (method 940.28; AOCS, 2009), moisture (method Ca 2c-25; AOCS, 2009), insolubles (method Ca 3a-46; AOCS, 2009), unsaponifiables (method Ca 6a-40; AOCS, 2009), and fatty acid profile (method 996.06; AOAC, 2010) of experimental lipids were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). The vitamin E concentration of experimental lipids was analyzed at a commercial lab (Minnesota Valley Testing Laboratories, New Ulm, MN) using a modified method of 971.30 (AOAC, 2006) by HPLC with a fluorescence detector. The term "vitamin E" is the generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of α-tocopherol (Ball, 2006).

Peroxide value (**PV**; method Cd 8-53; AOCS, 1993), thiobarbituric acid reactive substances (**TBARS**) concentration (Buege and Aust, 1978), and 4-hydroxynonenal (**HNE**; Zanardi et al., 2002; Fitzmaurice et al., 2006) were analyzed at the University of Minnesota. The active oxygen method (**AOM**) is a predictive method where purified air is bubbled through a lipid sample at 97.8°C, and the PV of the lipid is determined at regular intervals to determine the time required to reach a PV of 100 mEq/kg lipid (recorded as h), or the PV of the lipid is determined at a predetermined time endpoint, such as the 20-h time period used in this study (recorded as mEq/kg lipid). In principle, the oxidative stability index (**OSI**) method is similar to the AOM whereupon air passes through a lipid under a specific temperature, at which point volatile acids decomposed from lipid peroxidation are driven out by the air and subsequently dissolved in water, thereby increasing its conductivity. The conductivity of the water is constantly measured, and the OSI value is defined as the hours required for the rate of conductivity to reach a predetermined level. Unlike most other tests described herein, for the OSI test a high value means that less peroxidation has occurred (i.e., more hours to reach a predetermined level of peroxidation). Active oxygen method (PV at 20 h of method Cd 12-57; AOCS, 2009), oxidative stability index (method Cd 12b-92; AOCS, 1997), p-anisidine value (**AnV**; method Cd 18-90; AOCS, 2009), and hexanal (gas chromatography/mass spectrometry methodology and proprietary methodology) were analyzed by a commercial laboratory (Eurofins Scientific Inc., Des Moines, IA), while 2, 4-decadienal (**DDE**) was analyzed by gas chromatography using a flame ionization detector (detector temperature, 260°C, and injection temperature, 250°C), using a ramp temperature program from 40°C to 320°C with a run time of 12 min, a column (Zebron ZB Column; Phenomenex, Torrance, CA), and 1.0-μL injection at a another commercial laboratory (Kemin, Des Moines, IA).

Statistical Analysis

Relationships between measures of lipid peroxidation and various variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis using the CORR procedure of SAS (SAS Inst. Inc., Cary, NC). The mean of triplicate samples from each lipid peroxidation measures was used as the experimental unit. Treatment effects were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

Compositional Changes of Lipids

Lipid peroxidation is a complex process and is generally considered to consist of 3 phases: 1) an initiation phase, which involves the formation of free lipid radicals and hydroperoxides as primary reaction products; 2) a propagation phase where hydroperoxides formed are decomposed into secondary peroxidation products; and 3) a termination phase involving the formation of tertiary peroxidation products (Gutteridge, 1995; Yong and McEneny, 2001). As such, the increased and decreased amount of various lipid peroxidation products over time during each of these phases increases the difficulty of accurately measuring and assessing the extent of lipid peroxidation. Therefore, it is essential to understand the disadvantages of different methodologies used to evaluate lipid peroxidation.

The current experiment used 4 types of dietary lipids that varied in fatty acid composition depending on their origin (Table 1). Compared to PF and TL, CN and CA had greater concentrations of unsaturated fatty acids (89 and 84% vs. 64 and 37%, respectively), greater ratios of unsaturated fatty acids to SFA (14.2 and 6.2 vs. 2.1 and 0.7, respectively), and, accordingly, greater iodine values (**IV**; 103 and 121 vs. 75 and 34, respectively). As expected, the most abundant unsaturated fatty acid in CA was oleic acid (66%), while linoleic acid was the most abundant in CN (54%). Of the animal fat sources, PF had a greater concentration of unsaturated fatty acids (64%) compared with TL (37%), and subsequently had a greater unsaturated fatty acids to SFA ratio (2.1:1) and a greater IV (75) compared to TL (0.7:1 and 34, respectively). In general, the fatty acid composition of the 4 OL was in agreement with data reported in previous publications (NRC, 1998; AOCS, 2006).

Each OL was heated in 2 ways. The SO process applies a relatively low temperature (95°C) for a relatively long period of time (72 h), reflecting the peroxidation that might occur during the rendering of animal fats (Meeker and Hamilton, 2006). The RO process was used to simulate the heating process that normally occurs in the restaurant industry where lipids may be heated in fryers for up to 18 h at a temperature of approximately 180°C (Frankel, 1984). Unsaturated fatty acids, especially PUFA, are highly susceptible to oxidation (Frankel et al., 1984; Linfield et al., 1985) and their oxidation can ultimately lead to the generation of free radicals, peroxides and hydroperoxides, and undesirable substances with intrusive odor and toxic properties (Lin et al., 1989; Adam et al., 2008). In addition to the fatty acid composition of lipids from different origins, the thermal oxidation conditions applied (e.g., temperature and duration of heating, addition of oxygen and catalysts, and water activity) play an important role in determining the variable concentration of lipid peroxidation products (Chang et al., 1978). Generally, autoxidation of lipids at a low temperature without catalysis mainly results in the formation of primary lipid peroxidation products such peroxides and hydroperoxides, while large quantities of secondary oxidation products such as aldehydes, carbonyls, and ketones will be produced when lipids are heated at relative high temperatures because hydroperoxides are not heat stable and will further break down into numerous secondary oxidation products when exposed to prolonged heating (Esterbauer et al., 1991).

After the heating processes, small changes were noted in crude fat, FFA, and the sum of moisture, impurities, and unsaponifiables among OL, SO, and RO in all sources of lipids. In general, the concentration of PUFA (namely linoleic and linolenic acid) was reduced for all lipids, which was likely due to the PUFA being highly susceptible to lipid peroxidation (Sherwin, 1978; Seppanen and Csallany, 2002) forming hydroperoxides and further decomposing into other oxidation products compared to SFA. Except for these 2 minor changes, the fatty acid composition within each lipid source was unaltered.

Traces of total tocopherols were only measurable in the original CA (0.40 IU/g) and CN (0.29 IU/g), whereas the total tocopherols in original PF or TL was lower than the detection limit (<0.10 IU/g) of the assay. Slow oxidation and RO decreased the total tocopherol level of original CA and CN to a level lower than the detection limit (<0.10 IU/g). The depletion of antioxidants in lipids has been observed in other studies (Lea and Ward, 1959; Seppanen and Csallany, 2002), where the loss of vitamin E is partly due to its antioxidant effect because antioxidants can be quickly consumed by lipid free radicals derived from fatty acid peroxidation at high temperatures (Ullrey, 1981) and partly because of the heat sensitive characteristics of antioxidants (Park et al., 2004). These results indicate that although the presence of the total tocopherols helped minimize lipid peroxidation, their protective potential was greatly diminished by both SO and RO heating processes used in the current experiment.

Indicator Measures of Lipid Peroxidation

Peroxide value measures lipid peroxides and hydroperoxide, which are primarily formed in the initial phase of lipid peroxidation. Because peroxides and hydroperoxides are well documented for their potential toxicity (Holman and Greenberg, 1958; Kaneko et al., 1988), PV may provide useful information for predicting animal growth performance. Peroxide value analysis in the current experiment showed that SO of lipids led to a relatively greater PV compared to OL or RO lipids. The lack of a high PV in lipids that had been RO indicates that the

Table 1. Characteristics of the experimental lipids[1]

	Corn oil			Canola oil			Poultry fat			Tallow		
Item	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO
Crude fat, %	99.34	99.36	99.26	99.16	99.50	99.26	95.52	96.42	98.23	98.04	98.68	99.02
Free fatty acids, %	0.28	0.48	0.65	0.36	0.57	0.58	3.62	3.65	3.17	1.99	3.10	2.28
Total MIU[2]	1.00	1.02	1.22	1.01	0.89	0.96	2.24	1.01	1.23	0.78	0.60	0.64
Moisture, %	0.06	0.00	0.06	0.08	0.00	0.00	0.19	0.02	0.07	0.15	0.10	0.07
Insolubles, %	0.02	0.04	0.08	0.02	0.02	0.02	1.08	0.08	0.22	0.22	0.16	0.23
Unsaponifiables, %	0.92	0.98	1.06	0.91	0.87	0.94	0.97	0.93	0.94	0.41	0.34	0.34
Fatty acids, %												
Myristic (14:0)	0.06	0.06	0.07	0.08	0.09	0.08	0.63	0.63	0.65	3.03	3.21	3.29
Palmitic (16:0)	10.76	11.90	12.11	3.95	4.39	4.43	24.69	24.49	24.68	24.50	24.68	25.94
Palmitoleic (16:1)	0.10	0.10	0.12	0.22	0.23	0.23	7.11	7.39	7.19	2.55	2.71	2.55
Stearic (18:0)	1.71	1.91	1.93	1.78	1.93	1.95	5.93	5.62	5.80	21.59	20.00	21.97
Oleic (18:1)	27.70	29.84	29.80	64.57	65.47	66.82	38.07	39.16	39.20	32.03	33.48	30.62
Linoleic (18:2)	57.18	52.73	52.32	17.90	16.51	15.93	18.50	17.59	17.10	2.80	1.83	1.84
Linolenic (18:3)	0.79	0.62	0.63	7.09	5.73	5.01	0.77	0.67	0.69	0.22	0.12	0.11
UFA:SFA[3]	6.85	6.01	5.87	15.45	13.72	13.62	2.06	2.11	2.06	0.77	0.80	0.69
Iodine value[4]	125	119	118	105	100	98	73	73	72	35	35	32
Vitamin E, IU/g	0.40	<0.10	<0.10	0.29	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Oxidation products												
PV,[5] mEq/kg	1	151	2	1	239	12	1	57	2	1	29	3
p-Anisidine[6]	<1	61.4	142.9	1	37.0	154.8	3	88	22	4	120	19
TBARS,[7] μmol/kg	16	225	119	45	968	622	79	151	58	58	61	41
Hexanal, mg/kg	<1	390	83	1	180	59	3	88	22	4	120	19
2, 4-decadienal, mg/kg	72	3,728	1,345	7	1,091	511	30	442	169	47	261	125
HNE,[8] μmol/kg	0	194	594	0	105	221	0	2	0	0	13	6
AOM,[9] mEq/kg	103	575	528	112	419	533	4	298	5	<2	6	446
OSI,[10] h	8.4	<1.0	<1.0	9.2	<1.0	<1.0	24.6	<1.0	<1.0	12.1	<1.0	<1.0

[1]OL = original lipids (lipids were stored as received without antioxidants or heating); SO = slow-oxidized lipids (lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min); RO = rapid-oxidized lipids (lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min).

[2]Total of moisture, insolubles, and unsaponifiables (MIU).

[3]Unsaturated fatty acid (UFA) to SFA ratio.

[4]Iodine value was calculated by the following equation: iodine value = (C16:1) × 0.95 + (C18:1) × 0.86 + (C18:2) × 1.732 + (C18:3) × 2.616 (method Cd 1-25; AOCS, 1998).

[5]PV = peroxide value.

[6]There is no unit for p-anisidine value.

[7]TBARS = thiobarbituric acid reactive substances.

[8]HNE = 4-hydroxynonenal.

[9]AOM = active oxygen method (measured as the PV at 20 h of oxidation).

[10]OSI = oxygen stability index (time to exponential increase in conductivity when assayed at 110°C).

production of peroxides and hydroperoxides may have been further degraded to secondary or tertiary oxidation products. This is supported by the results from AnV and TBARS tests, which are measures of carbonyl-containing secondary lipid oxidation products formed from the decomposition of hydroperoxides. These results were expected because CN and CA are lipids, which are more prone to oxidation, compared with PF and TL, lipids that are less prone to oxidation. As a result, a single PV analysis may cause a misleading assessment of lipid oxidation because the hydroperoxides formed were unstable at a high temperature. As expected, the increased PV in CN or CA caused by SO was much greater than that TL, with PF being intermediate. These responses are consistent with their concentrations of unsaturated fatty acids because unsaturated fatty acids are more susceptible to lipid oxidation than SFA.

Measures such as AnV, TBARS, HNE, and hexanal have also been used to determine the production of secondary oxidation products derived from the decomposition of initially produced hydroperoxides. The AnV measures the amount of high molecular weight saturated and unsaturated aldehydes. In the current experiment, the AnV of all OL were low (CN, <1.0; CA, 1.4; PL, <1.0; and TL, 4.3), but the SO process and RO resulted in a greater AnV compared to the OL. The greater level of lipid oxidation caused by RO, as indicated by the increased AnV, were in contrast to their unchanged PV, which indicates that

the measurement of secondary oxidation products, such as AnV, may provide a better assessment of lipid oxidation than PV for the lipids that have experienced a relatively high level of peroxidation. However, measurement of only AnV may not provide reliable information of lipid peroxidation because AnV of lipids exposed to extreme oxidative conditions have been shown to exhibit a bell shaped curve (DeRouchey et al., 2004), and thus their respective values are time dependent.

The TBARS analysis is another method used to quantify secondary lipid oxidation products. Compared to AnV, the TBARS assay was developed to detect malondialdehyde, although other carbonyl compounds can also contribute to TBARS values (Gutteridge, 1981). However, the TBARS assay may provide useful information regarding the quality of dietary lipids because malondialdehyde is highly reactive and potentially mutagenic (Marnett, 1999), which can be a factor in causing intestinal oxidative stress (Suomela et al., 2005; Ringseis et al., 2007) and ultimately lead to poor growth performance in animals fed thermally oxidized lipids (DeRouchey et al., 2004). As expected, all of the OL had a low TBARS value, and the increase in TBARS caused by SO in CN, CA, and PF was greater than TL due to these lipids having a greater concentration of unsaturated fatty acids compared to TL. Although original PF had the potential to produce a greater TBARS value because of its high amount of unsaturated fatty acids (7% of palmitic acid, 38% of oleic acid, and 18% of linoleic acid), the TBARS of RO PF was only slightly greater than that of the OL PF, which may be due to the loss of the secondary oxidation products because of their volatile characteristics (Seppanen and Csallany, 2002). These results indicate that a single low TBARS value may also be misleading because of the loss of volatile secondary oxidation products.

Hexanal is one of the major secondary lipid oxidation products produced from the termination phase during the oxidation of linoleic and other ω-6 fatty acids. Hexanal has become a well-known indicator of lipid peroxidation (Shahidi et al., 1987; Ha et a., 2011) because the production of hexanal is directly related to oxidative off-flavors (Shahidi and Pegg, 1994; van Ruth et al., 2000; Kalua et a., 2007). The hexanal content of the initial CN (<1.0 mg/kg), CA (1 mg/kg), PF (3 mg/kg), and TL (4 mg/kg) were all increased by the SO and RO heating processes. Heated CN was expected to result in a greater hexanal value compared to CA, PF, and TL because CN contains the greatest concentration of linoleic acids among all the lipids evaluated and because ω-6 fatty acids are prone to peroxidation. However, because hexanal is volatile at high temperatures, a single low hexanal value may not necessarily indicate a low level of peroxidation in a lipid sample. As with other secondary lipid oxidation products, hexanal can be lost during subsequent processing and storage.

The compound DDE is a byproduct derived from oxidized linoleic acid formed during storage or heating (USDHHS, 1993; Wu et al., 2001). Low levels of DDE result in a deep-fried flavor, but excessive amounts of this specific dienaldehyde induce many undesirable effects, including oxidative stress and proinflammatory reactions in human lung cells (Chang et al., 2005), cellular toxicity in liver and kidney tissues (Hageman et al., 1991), cellular proliferation in gastrointestinal epithelial cells (National Toxicology Program, 1993), and carcinogenic effects in the gastrointestinal tract (Hageman et al., 1991). In the current study, low levels of DDE were detected among all OL and were increased by SO and RO. Because of the potential negative influence of DDE and its common presence in heated lipids, monitoring the concentration of these compounds provides additional information regarding the level of lipid peroxidation.

The compound HNE is an α,β-unsaturated lipophilic aldehyde formed from the lipid peroxidation of polyunsaturated ω-6 fatty acids, such as arachidonic or linoleic acid. The HNE compound is known for its cytotoxic and mutagenic effects (Witz, 1989; Esterbauer et al., 1991). The reactive groups (an aldehyde, a double bond at carbon-2, and a hydroxy group at carbon-4) of HNE have generated attention as a potential marker of lipid peroxidation (Zarkovic, 2003). In the current study, no HNE was detected in any of the OL, while SO and RO caused HNE generation mainly in CN (194 and 594 μmol/kg, respectively) and CA (105 and 221 μmol/kg, respectively). Although HNE was produced in TL (13 and 6 μmol/kg for SO and RO, respectively), it was only produced by SO in PF (2 μmol/kg). Because HNE is a secondary peroxidation product derived from peroxidation of *n*-6 fatty acids and CN contained the greatest concentration of linoleic acid among all 4 lipid sources, the concentration of HNE was expected to be greater after SO and RO in CN than in the other heated lipid sources. Although the original PF contained about 18% of linoleic acid (18:2, *n*-6), which was much greater than that in the original TL (3%), the HNE concentration in SO and RO of PF (2 and 0 μmol/kg, respectively) was slightly less than that in SO and RO of TL (13 and 6 μmol/kg, respectively). One explanation for the lower HNE in heated PF might be due to the HNE being already volatized before the analysis (Seppanen and Csallany, 2002), which indicates that HNE analysis has a similar disadvantage as with PV and TBARS to evaluate oxidative deterioration in dietary lipid sources. Another disadvantage of using the HNE analysis as an indicator of lipid oxidation might relate to its high cost and complexity of analysis.

Our results are in agreement with those reported by Brandsch et al. (2004) where SO (heating a mixture of

sunflower oil and linseed oil at 50°C over 16 d) led to a greater production of total primary lipid peroxidation products, such as peroxides and hydroperoxides reflected by relatively high PV, and total secondary lipid peroxidation products, such as aldehydes, carbonyls, and ketones reflected by a relatively high TBARS. Rapid oxidation in the current study contributed to a greater PV value than that of OL only in CA but not in other 3 lipid sources, and the PV of RO of each source of lipid were much lower than that of SO of the corresponding lipid. This lower PV in RO compared with SO can be explained by the fact that hydroperoxides are thermally unstable under high temperature heating (Frankel, 1998) and the hydroperoxides generated had possibly been decomposed into secondary peroxidation products during the RO process. In addition, the increased TBARS concentration in RO compared to those in OL of CN and CA also indicate that the decomposition of hydroperoxides into secondary peroxidation products occurred. Heating vegetable oils were expected to increase the production of primary and secondary lipid peroxidation products compared to heating animal fats because of the heat sensitive characteristics of PUFA and because PUFA are more concentrated in vegetable oils than in animal fats. The results from these chemical analyses of experimental lipids indicate that lipids from different origins vary in fatty acid composition, and the 2 different heating processes altered the peroxidation products of these dietary lipids through the formation of both primary and secondary peroxidation products.

Predictive Measures of Lipid Peroxidation

In addition to the indicator measurements of lipid peroxidation previously discussed, predictive tests also are used to measure the stability or susceptibility of lipids to oxidation. In these tests, a lipid is subjected to a specific accelerated condition and an endpoint is defined to determine the level of peroxidation damage. In the current experiment, after 20-h of accelerated conditions, the AOM of OL, SO, and RO of CN or CA were greatly increased (as reflective by a greater PV) and the AOM of SO and RO were much greater than that of OL in CN or CA. These results are in agreement with the results obtained using TBARS, AnV, and hexanal analysis. Within PF, only SO but not RO had a greater AOM compared to the original PF, while within TL only RO but not SO had an increased AOM compared to the original TL. The unchanged AOM of RO PF and SO TL may have been due to the decomposition of primary hydroperoxides formed during the heating process, which has been suggested as a reason for the relatively low reproducibility of the AOM test (Jebe et al., 1993).

Another limitation of AOM is that it is labor intensive and time consuming (Jebe et al., 1993). Therefore, a faster and more automated method has been developed. Advantages of the OSI over the AOM include the fact that multiple samples can be analyzed easily and simultaneously because a computer software program controls the instrument configuration and data collection and that the results from OSI tests are highly reproducible because the volatile acids are relatively stable tertiary oxidation products compared to hydroperoxides (Jebe et al., 1993; Mendez, 1996). Like AOM, the OSI test provides useful information regarding the changes in the concentration of volatile lipid peroxidation products over time by constantly monitoring the conductivity. Therefore, OSI can provide a better evaluation of the level of lipid peroxidation than a single indicator assay. As expected, the OSI of original CN (8.4 h) and CA (9.2 h) were comparable and were lower than the OSI of original PF (24.6 h) and TL (12.1 h) due to their unsaturated fatty acid composition differences. These results are in agreement with the AnV and hexanal analysis, indicating that SO and RO caused lipid peroxidation as indicated by the low OSI values for CN, CA, PF, and TL (less than 1 h for all lipids). However, because the OSI test depends on monitoring conductivity by quantification of the volatile fatty acids, the OSI test may have a similar disadvantage as AnV, TBARS, HNE, and hexanal because of the VFA derived from lipid peroxidation being lost before the OSI test.

Correlation among Measures of Peroxidation

Lipid peroxidation is a complex process and is affected by several factors including level of saturation, temperature, oxygen, heavy metals, undissociated salts, water, and other nonlipidic compounds (Kamal-Eldin and Korory, 2005). Lipid hydroperoxides initially formed during the lipid peroxidation process not only have a potential impact on lipid quality, and therefore on animal health and performance, but the formation of secondary and tertiary oxidation products (aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds) often has additional effects on lipid quality and animal productivity. Peroxidation reactions occur concurrently with a wide range of oxidative compounds produced and modified during the peroxidation process (Liu, 1997). To date, however, limited data regarding the relationship between these tests have been published and, under practical conditions, there may be advantages in time and cost savings in predicting lipid peroxidation by understanding these relationships.

Correlations among lipid peroxidation measurements are summarized in Table 2. Even though some correlations were found to be significant among various composition and peroxidation measures, caution must be taken when interpreting these data because signifi-

Table 2. Correlation matrix among lipid composition and various oxidation measures[1]

	CF	FFA	MIU	Mo	In	Usap	Myr	Pal	Pmo	Ste	Ole	Lin	Linol	US	IV	VE	PV	AnV	TBARS	Hex	DDE	HNE	AOM	OSI
CF	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FFA	–0.81 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MIU	–0.66 (0.02)	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Moi	–0.57 (0.05)	0.50 (0.10)	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ins	–0.77 (0.01)	0.60 (0.04)	0.80 (0.01)	0.77 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Usap	NS	NS	0.58 (0.05)	NS	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Myr	NS	NS	NS	NS	NS	–0.97 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pal	–0.64 (0.03)	0.89 (0.01)	NS	0.57 (0.05)	0.51 (0.09)	–0.52 (0.08)	0.69 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pmo	–0.86 (0.01)	0.93 (0.01)	NS	NS	0.60 (0.04)	NS	NS	0.77 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ste	NS	NS	NS	NS	NS	–0.96 (0.01)	0.99 (0.01)	0.71 (0.01)	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ole	NS	NS	NS	NS	NS	NS	NS	–0.66 (0.02)	NS	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–
Lin	NS	–0.56 (0.06)	NS	NS	NS	0.68 (0.02)	–0.68 (0.02)	NS	NS	–0.68 (0.01)	NS	1.0	–	–	–	–	–	–	–	–	–	–	–	–
Linol	NS	–0.54 (0.07)	NS	NS	NS	NS	NS	–0.80 (0.01)	NS	–0.49 (0.10)	0.95 (0.01)	NS	1.0	–	–	–	–	–	–	–	–	–	–	–
US	0.52 (0.09)	–0.76 (0.01)	NS	–0.51 (0.09)	NS	NS	–0.65 (0.02)	–0.96 (0.01)	–0.63 (0.03)	–0.67 (0.02)	0.83 (0.01)	NS	0.94 (0.01)	1.0	–	–	–	–	–	–	–	–	–	–
IV	NS	–0.72 (0.01)	NS	–0.51 (0.09)	NS	0.85 (0.04)	–0.92 (0.01)	–0.79 (0.01)	NS	–0.93 (0.01)	NS	0.85 (0.01)	NS	0.66 (0.02)	1.0		–	–	–	–	–	–	–	–
VE	NS	–0.47 (0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0								
PV	NS	NS	NS	–0.57 (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	–	–	–	–	–	–	–
AnV	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	–	–	–	–	–	–
T-BARS	NS	NS	NS	–0.58 (0.05)	NS	NS	NS	–0.59 (0.04)	NS	NS	0.70 (0.01)	NS	0.60 (0.04)	0.62 (0.03)	NS	NS	0.75 (0.01)	NS	1.0	–	–	–	–	–
Hex	NS	NS	NS	–0.57 (0.06)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.50 0.10	NS	0.76 (0.01)	NS	NS	1.0	–	–	–	–
DDE	NS	NS	NS	–0.53 (0.08)	NS	NS	NS	NS	NS	NS	NS	0.56 (0.06)	NS	NS	NS	NS	0.61 (0.04)	NS	NS	0.94 (0.01)	1.0	–	–	–
HNE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.54 (0.07)	NS	NS	NS	NS	NS	0.67 (0.02)	NS	NS	0.49 (0.10)	1.0	–	–
AOM	NS	–0.51 (0.09)	NS	–0.75 (0.01)	NS	NS	NS	NS	–0.50 (0.10)	NS	NS	NS	NS	NS	NS	NS	NS	0.53 (0.08)	0.51 (0.09)	0.57 (0.06)	0.65 (0.02)	0.66 (0.02)	1.0	–
OSI	–0.60 (0.04)	NS	0.70 (0.01)	0.81 (0.01)	0.78 (0.01)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	–0.57 (0.05)	NS	NS	NS	NS	–0.58 (0.05)	1.0

[1]CF = crude fat; MIU = moisture, insolubles, and unsaponifiables; Moi = moisture; Ins = insolubles; Unsap = unsaponifiables; Myr = myristic acid; Pal = palmitic acid; Pmol = palmitoleic acid; Ste = stearic acid; Ole = oleic acid; Lin = linoleic acid; Linol = linolenic acid; US = unsaturated:saturated ratio; IV = iodine value; VE = vitamin E; PV = peroxide value; AnV = p-anisidine value; TBARS = thiobarbituric acid reactive substances; Hex = hexanal; DDE = 2,4-decadinal; HNE = 4-hydroxynonenal; AOM = active oxygen method; OSI = oxidative stability index. The top value represents correlation and bottom value in parentheses represents the *P*-value. If no value is given, it was not found to be different at $P \leq 0.10$ and listed as NS = nonsignificant.

cant correlations do not infer a cause and effect relationship because of the potential confounding of lipid source and the peroxidation method used. For example, moisture, insolubles, and MIU were positively correlated to OSI (r = 0.81, 0.78, and 0.70, respectively; P w< 0.01). In animal fats, however, the greater OSI was most likely because animal fats have a lower level of unsaturation and not because they had greater level of moisture and insoluble as shown in Table 1. In addition, the negative correlation noted between moisture and unsaturated fatty acids:SFA (r = –0.51, $P < 0.10$) was likely due to the fact that the CN and CA used for

this project were more refined compared to the PF and TL, which were obtained largely unprocessed from their respective rendering locations. Another example is that even though palmitic acid was negatively correlated to TBARS (r = –0.59, P < 0.01), lipids that had greater palmitic acid in the current study (PF and LT) also had a lower unsaturated fatty acids:SFA compared to CN and CA. As expected, there was a positive correlation of unsaturated fatty acids:SFA, oleic acid, and linolenic acid with TBARS (r = 0.62, 0.70, and 0.60, respectively; P < 0.05), but no significant association was noted between linoleic acid and TBARS. Moreover, there was a negative correlation between vitamin E and FFA (r = 0.47, P < 0.05), which was likely due to the presence of vitamin E preventing the production of FFA from lipid oxidation. However, no correlation was found between any peroxidation measurements and vitamin E. Linoleic acid (e.g., CN) was positively correlated to DDE and HNE (r = 0.56 and 0.54, respectively; P < 0.10) but not to other measures of peroxidation.

Table 2 also lists correlations among peroxidation measures, where PV was positively associated with TBARS, hexanal, and DDE (r = 0.75, 0.76, and 0.61, respectively; P < 0.05), AnV was correlated positively to HNE (r = 0.67, P < 0.05) and AOM (r = 0.53, P < 0.10) but associated negatively to OSI (r = –0.57, P < 0.05), TBARS was positively correlated to AOM (r = 0.51, P < 0.10), hexanal was positively associated to DDN (r = 0.94, P < 0.05) and AOM (r = 0.57, P < 0.10), DDN was positively correlated to HNE (r = 0.49, P < 0.10) and AOM (r = 0.65, P < 0.05), HNE was positively associated to AOM (r = 0.66, P < 0.05), and AOM was negatively correlated to OSI (r = –0.58, P < 0.05). It was not surprising that correlations among several of the oxidation measures were not always significant given that peroxidation reactions occur concurrently during the peroxidation process, with primary, secondary, and tertiary oxidation products being produced and degraded at different rates depending on the stage of oxidation (Gutteridge, 1995; Liu, 1997; Yong and McEneny, 2001). The negative correlation between AOM and OSI are in agreement with the findings by Woestenburg and Zaalberg (1986) and Laubli and Bruttel (1986).

Measurements of lipid peroxidation provide important information in assessment of lipid quality, which is important because of the potential impact on animal health and performance due to the effects of secondary and tertiary peroxidation products. Unfortunately, the assessment of the level of lipid peroxidation is challenging because of the drawbacks of each method used. These results indicate that there is no single method that seems to adequately describe or predict lipid peroxidation because of the complexity of lipid composition and the phases involved in lipid peroxidation. To accurately analyze the amount of lipid damage caused by peroxidation, it may be advantageous to determine the level of lipid peroxidation at several time intervals using more than 1 test. However, a high value of PV, AnV, TBARS, hexanal, DDE, HNE, or AOM as well as a low value of OSI indicate a high level of lipid peroxidation. If a lipid undergoes a mild level of lipid peroxidation and most of the hydroperoxides formed have not been decomposed, it is economical and feasible to use PV as a primary measure of peroxidation because there is less concern about decomposition of hydroperoxides and PV can accurately reflect the level of lipid peroxidation. However, if a lipid is subjected to a high level of lipid peroxidation and most of the hydroperoxides formed have already been decomposed to yield secondary or tertiary lipid peroxidation products, a single measure of secondary oxidation products, such as AnV or TBARS may be more acceptable and economical because the production of secondary lipid peroxidation products can be measured more accurately than a single PV analysis. Furthermore, for better evaluation of lipid peroxidation of lipids that have been subjected to extreme peroxidation, future research should focus on development of measures used to assess lipid peroxidation based on quantification of the triacylglycerol dimers or triacylglycerol polymers. The amount of dimers and polymers formed during lipid peroxidation keeps increasing as the heating time is increased (Sanchez-Muniz et al., 1993; Takeoka et al., 1997).

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