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Oxidative Stability of Commodity Fats and Oils: Modeling Based on Fatty Acid Composition

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Abstract Although fatty acid (FA) composition is known to be of fundamental importance to oxidative stability in lipids, consistent quantifications of the magnitude of this association have proved elusive. The objective of this study was to quantify the relationship between FA composition and stability on a large scale within comparable lipid systems, with the numerical effects of individual outcome factors (e.g. output of a singular assay, oxidative products after a brief period of time, etc.) attenuated by incorporation into a comprehensive summation of stability. The stability of 50 plant-based oils and fats was modeled according to FA composition, utilizing a quantification of stability that encompassed the complete oxidation curves of four distinct classical assays (two 1° and two 2° oxidation assessments) throughout 2 months of accelerated storage (60 °C). In our models, the concentrations of monounsaturated FA (MUFA), diunsaturated FA (DiUFA), and triunsaturated FA (TriUFA) together demonstrated a very strong correlation with our consolidated measure of stability ($r^2 = 0.915$; greater than observed with our assessments by individual assays). The resultant model also indicated the relative effect upon magnitude of oxidation of MUFA:DiUFA:TriUFA to be approximately 1:3:12—substantially greater than the 1:2:3 ratio of their relative unsaturation.

Keywords Lipid oxidation · Autoxidation · Oxidative stability · Peroxide value · Conjugated dienes and trienes · 2-Thiobarbituric acid reactive substances · *p*-Anisidine value · Mathematical modeling

Introduction

The oxidative deterioration of lipids in food products can often result in the release of volatile and semi-volatile flavor compounds responsible for undesirable or 'rancid' notes in foods [1–3]. Many contributing factors to lipid oxidation progress have been reported in the scientific literature including: degree of fatty acid unsaturation, the presence of metal-ion catalysts, as well as concentrations of antioxidants or pro-oxidants, to name a few. Paramount among these factors has historically been the fatty acid composition of the sample [4]. In most commodity fats and oils, a greater proportion of unsaturated fatty acids will lead to a more rapid on-set and progress of autoxidation [5].

Although the association between fatty acid unsaturation and oxidation is well accepted, studies have often demonstrated substantial deviations from the simple expectation that more unsaturated samples will generally show lesser oxidative stability [6, 7]. In certain cases, such variations in oxidative behavior may be reasonably attributed to known influencing factors, such as significant variations in the concentration of antioxidants. A portion of these variances can also often be explained by the chemistry of the fatty acids—as controlled studies have stimulated oxidation and have found lipid oxidation rates associate linearly not with the double bonds, but with the total number of

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bis-allylic sites (the methylene CH directly adjacent to two double bonds) [8, 9]. Single allylic sites have been found to be much less reactive (i.e., roughly 1/40th that of a *bis*-allylic site), thereby rendering monounsaturated fatty acids (MUFA) far less susceptible to oxidation than their degree of unsaturation may otherwise suggest [10].

Still, studies carried out on seemingly simple lipid systems, such as bulk vegetable oil, can nevertheless note dramatic deviations in expected oxidative stability [11]. Figure 1 depicts an example of such an observation from a study upon common bulk vegetable oils [7]. The association between calculated iodine value and the rapid assessment of oxidative stability index in this study is statistically significant, but shows substantial aberrations.

Although some observed inconsistencies in oxidative performance can likely be attributed to true (as yet unexplained) variations in behavior among sample types, another potential reason for aberrant observed behaviors may lie in the incomprehensibility of the assessments and definitions of 'oxidative stability'. Oxidative stability in food science is a qualitative term without consistent quantitative definition, and studies may determine stability assessments according to a relatively small number of samples, draw conclusions after only abbreviated timeframes, or assess stability rapidly by measures that may influence oxidative chemistry [12, 13]. Such study designs may be appropriate for the specific research questions of the investigations, but the incomprehensibility may lead to a misrepresentation of the magnitude of divergent behavior to be typically expected within such systems. The net effect is that although the importance of unsaturated fatty acid composition in determining bulk lipid stability is unquestioned, the magnitude of this importance has continued to be quantitatively
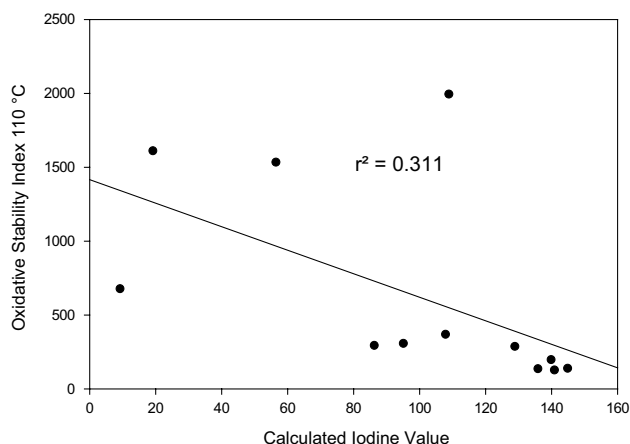
highly uncertain. Inversely, the quantitative importance of the sum of other contributing factors (both known and unknown) is similarly undefined.

This study employs a large-scale study design [i.e., 50 samples, four methods to detect oxidation products, 2 months of moderately accelerated storage (60 °C), and greater than 100 data points per sample] upon standard bulk lipid systems (commodity fats and oils of current commercial use that are refined and free of synthetic antioxidants). The methods chosen are among the most historic and common for the assessment of oxidation: peroxide value (PV), conjugated dienes and trienes (CDT), 2-thiobarbituric acid reactive substances test (TBARS), and *p*-anisidine value assay (*p*-AnV). PV and CDT served as measures of primary oxidation products (hydroperoxides and conjugated hydroperoxides, respectively), and TBARS and *p*-AnV served to measure "representative" markers of secondary oxidation (malondialdehyde-type products and predominantly unsaturated aldehydes, respectively). For a detailed discussion of the degradation of fatty acids into oxidation products, and the chemistry of these four assessment techniques, please consult a recent review on the subject [14]. The oxidation data of each of the aforementioned four assays is modeled according to fatty acid unsaturation, and additional predictive models are made *versus* unsaturation for a single consolidated quantitative summation of oxidative stability. This consolidated term is derived to comprehensively incorporate every acquired data point. Unsaturation of the samples in these models is represented both by calculated iodine value (CIV) and as a delineation of the concentrations of fatty acids containing one, two, and three double bonds.

The objective is to produce a rigorous assessment of what proportion of oxidative stability behavior can be meaningfully mathematically associated with the unsaturation of commercial-use bulk lipid plant-based samples such as those used in our study, and also to determine the quantitative importance of the combined presence of multiple double bonds within individual fatty acids upon oxidative stability. An additional objective is to discern the differences in correlating behavior when modeling output data that incorporates multiple assays simultaneously.

Materials and Methods

Lipid Sample Selection and Handling

Commercially available fat and oil samples (*n* = 50, see Table 1) were provided by the Nestlé Research Center/ NESTEC Ltd. in Lausanne, Switzerland. All samples were verified by HPLC analysis to be free of added synthetic antioxidants, according to AOAC official method 983.15

Fig. 1 Correlation between Oxidative Stability Index assessment and CIV (calculated iodine value indicative of sample unsaturation) for bulk vegetable lipid samples assessed by Tan *et al.* [7]

Table 1 Concentrations of mono- (MUFA), di- (DiUFA), and tri-unsaturated fatty acids (TriUFA) contained within the test lipid samples as well as their calculated iodine values (CIV)

Sample	CIV[a]	MUFA (% FA)[b]	DiUFA (% FA)[b]	TriUFA (% FA)[b]
Medium chain triacylglycerols 1	0.00	0.0	0.0	0.0
Medium chain triacylglycerols 2	0.00	0.0	0.0	0.0
Oil Mix A	0.10	0.1	0.0	0.0
Cocoa butter substitute	0.15	0.1	0.0	0.0
Hydrogenated oil blend	1.33	0.8	0.2	0.0
Palm kernel stearin oil	6.25	5.3	0.9	0.1
Hydrogenated palm blend	6.71	5.9	0.9	0.0
Coconut oil	8.45	6.5	1.6	0.0
Palm kernel oil	16.2	14.3	2.2	0.0
Palm kernel olein oil	22.5	19.4	3.3	0.0
Palm stearin oil	30.7	24.1	5.6	0.1
Cocoa butter substitute	31.5	28.6	1.1	0.0
Cocoa butter equivalent 1	31.6	30.2	3.1	0.1
Palm/coconut mix	31.6	24.2	6.0	0.1
Cocoa butter equivalent 2	31.9	30.5	3.2	0.0
Cocoa butter equivalent 3	32.1	30.9	3.1	0.1
Shea oil/palm	40.2	32.2	7.0	0.1
Palm oil 1	42.1	33.6	7.3	0.2
Palm oil 2	42.1	34.6	6.8	0.2
Palm oil 3	47.8	36.7	9.2	0.1
Palm/coconut high oleic	47.9	35.9	9.6	0.1
Palm olein oil 1	48.1	37.2	9.1	0.1
Cocoa butter replacer	49.0	43.7	1.6	0.0
Palm oil 4	50.0	37.8	9.8	0.2
Palm olein oil 2	52.0	41.6	9.0	0.2
Palm olein oil 3	53.7	40.6	10.6	0.2
Palm olein oil 4	54.5	40.4	11.2	0.2
Palm/soybean/canola	57.1	36.5	13.5	0.9
Palm olein oil 5	57.2	41.4	12.1	0.2
Palm olein oil 6	58.6	42.8	12.2	0.3
Oil mix B	62.5	33.5	16.5	2.0
Oil mix C	64.6	36.2	16.7	1.8
Olive oil 1	74.9	73.2	6.0	0.5
Olive oil 2	76.6	70.2	8.5	0.6
Sunflower oil high oleic 1	81.7	77.7	8.5	0.0
Sunflower oil high oleic 2	86.0	71.8	13.8	0.1
Oil mix D	94.9	66.4	16.5	3.5
Canola oil 1	95.4	66.6	16.6	3.6
Corn/canola mix	96.3	40.4	30.5	3.4
Corn/canola mix D	96.4	41.6	29.9	3.4
Canola/sunflower/corn	97.6	59.0	20.8	4.1
Canola oil 2	103	58.5	18.7	7.9
Canola oil 3	104	57.0	19.6	8.0
Corn oil 1	107	33.6	44.1	0.7
Corn/canola mix E	109	43.8	33.4	5.4
Corn oil 2	110	31.3	47.1	0.8
Corn oil 3	111	29.0	48.4	0.8
Corn oil 4	118	26.9	53.4	1.0
Soybean oil	120	22.7	48.5	6.4

Table 1 continued

Sample	CIV[a]	MUFA (% FA)[b]	DiUFA (% FA)[b]	TriUFA (% FA)[b]
Sunflower oil	120	29.4	54.6	0.2

[a] Calculated iodine value (CIV): determined according to an official method Cd 1c-85 [15] using fatty acid (FA) composition data

[b] Summary data for the fatty acid (FA) compositions of lipid samples obtained via GC-FID according to [16]

[15]. After sample acquisition, the commercial lipids were immediately repacked in 600-mL aluminum bottles under a nitrogen headspace, sealed with high-density polyethylene (HD-PE) caps, and stored in a low-temperature (-40 °C) freezer until their inclusion in the storage study.

Oven Storage Test

An accelerated storage test was carried out according to oven-storage protocol AOCS Cg 5-97 [16]. Test lipid samples were dispensed in 4-mL aliquots into identical 20-mL amber glass vials and covered. Vials were then placed in a thermostatically-controlled gravity convection oven (Fisherbrand™ Isotemp Incubators) at a constant temperature of 60 °C. Oven temperatures were dually monitored with Fisherbrand™ Traceable™ Snap-in Module Thermometers with Probe and Fisherbrand™ Red-Spirit™ No-Roll Laboratory Thermometers. Samples were pulled from storage at 0, 1, 3, 7, 10, 14, 18, 23, 29, 36, 43, 50, and 57 days for the assessment of lipid oxidation progress. Peroxide value (PV) and conjugated dienes/trienes (CDT) tests were conducted to monitor the accumulation of primary oxidation products, whereas 2-thiobarbituric acid reactive substances (TBARS) and p-anisidine value (p-AnV) tests were utilized to determine the presence of secondary oxidation products. Multiple aliquots of the test lipid samples were stored in order to ensure that each remained entirely undisturbed prior to its assessment via the analytical regiment specified. Storage replicates (i.e., duplicate with mean reported) were achieved by the storage of aliquots under identical conditions within separate vials.

Fatty Acid (FA) Composition

Fatty acid compositions of the test samples were elucidated by gas chromatography with flame ionization detection (GC-FID) in accordance with a procedure for the production/quantification of fatty acid methyl esters (FAME) outlined by Badings and De Jong [17]. Summary data of total mono- (MUFA), di- (DiUFA), and tri-unsaturated fatty acids (TriUFA) contained in the test samples were calculated directly from the FAME data.

Calculated Iodine Value (CIV)

The total unsaturation contained within the lipid samples tested was determined according to the calculated iodine value (CIV) AOCS official method Cd 1c-85 [16]. CIV results are reported in grams of iodine per hundred grams of oil in reference to the traditional iodine-value assay of which these results are intended to closely approximate.

Oxidative Stability Assays

Peroxide Value (PV)

The concentration of hydroperoxides formed within the lipid samples tested was quantified according to AOCS official method Cd 8b-90 [16]. Results were calculated according the PV Eq. (1) below and were reported with the units meq (milliequivalents) active O_2/kg sample.

PV Equation: $$\text{Peroxide value} = [(S - B) \times N \times 1000]/m \tag{1}$$

where S, B, N, and m are the sample titration volume (mL), blank titration volume (mL, normality of the sodium thiosulphate solution used, and the mass of the test portion, respectively.

Conjugated Dienes and Trienes (CDT)

The concentrations of CDT within the stored lipids were determined according to IUPAC official method 2.505 using an Agilent 8453 UV–visible diode array spectrophotometer [18]. Results were calculated according to the CDT Eq. (2) below and reported as sample extinction coefficients (E1%). Extinction coefficients were calculated for both conjugated dienes and trienes separately and aggregated in order to obtain a global CDT value.

CDT equation: $$E1\% = A_\lambda/(c_L \times l) \tag{2}$$

where E, A_λ, c_L, and l represent the extinction value, the absorbance of the sample (measured at 232 nm for conjugated dienes, 268 nm for conjugated trienes), the concentration of the lipid solution tested (g/100 mL), and the path length within the 1.00-cm quartz cuvette, respectively.

2-Thiobarbituric Acid Reactive Substances Test (TBARS)

A TBARS test was carried out according to AOCS official method Cd 19-90 using the Agilent 8453 UV–visible diode array spectrophotometer [16]. Results were calculated according to the TBARS value Eq. (3) below and are reported in magnitude only:

TBARS equation: $TBARS_{value} = [50 \times (A - B)]/m$

$$(3)$$

where A, B, and m are equivalent to the absorbance of the test solution, absorbance of the reagent blank, and mass of the test portion [g], respectively.

p-Anisidine Value Assay (p-AnV)

The *p*-AnV assay was carried out on lipid samples according to AOCS official method Cd 18-90, utilizing the Agilent 8453 UV–visible diode array spectrometer [16]. The resulting data were calculated according to the following *p*-AnV Eq. (4) with values reported in magnitude only:

p-AnV equation: $p\text{-}AnV = [25 \times (1.2A_s - A_b)]/m$

$$(4)$$

where A_s, A_b, and m are equivalent to the absorbance of the fat solution post reaction with the *p*-anisidine reagent, absorbance of the initial fat solution, and the test sample mass in grams, respectively.

Data Analysis

Area under the Curve (AUC)

Plotted curves for the performance of each sample within oxidative stability assays were computed according to the mean values of the repetitions for each data point. The summary data area-under-the-curve (AUC) values were then computed using integral calculus computations within SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA).

Standardized Sum Area-under-the-Curve (ssAUC)

For a more comprehensive quantification of the oxidative stability of test lipid samples, a 'standardized sum area-under-the-curve' (ssAUC) value was calculated. This ssAUC value is a weighted composite score obtained via the combination of the four oxidative product assay curves for each individual sample. The relationship between ssAUC and oxidative stability is an inverse one (i.e., higher ssAUC values can be interpreted as lower oxidative stability). The purpose of the term is to provide a singular numerical output that encompasses the value of each data point, equally represents both primary and secondary oxidation,

does not overemphasize the specificities of a particular assay, and is influenced both by the magnitude of observed products and also the time period of their observed presence (a natural mathematical feature of the determination of AUC). The term was calculated according to the equation below (Eq. 5) with the coefficients derived according to the relative magnitude of the AUC values for each assay within the complete data set:

$$ssAUC = [PV_{AUC} + 6.31(CDT_{AUC}) + 2.60(TBARS_{AUC}) + 2.87(p\text{-}AnV_{AUC})]/4 \qquad (5)$$

The coefficients in the above equation are for the purpose of providing approximately equal consideration of each of the four assays in the final composite value. For example, the sum of PV AUC values within our data set was 6.31 times greater than the sum of CDT AUC values, and thus, the $6.31(CDT_{AUC})$ term appears in the equation above.

Multiple Linear Regression Modeling

A multiple linear regression analysis (REG protocol) was performed on the oxidative stability data obtained in this study using the statistical analysis software (SAS) package (SAS Institute Inc., Cary, NC). The criterion for statistical significance for inclusion within models was $\alpha = 0.05$.

Results and Discussion

FA Composition and CIV for the Test Lipid Samples

CIV results for the tested lipids appear in Table 1; summary data for concentrations of MUFA, DiUFA, and TriUFA are included. Among our sample set, MUFA were most abundant in oils containing relatively intermediate levels of unsaturation (i.e., CIV of approximately 50–90), and were of the highest concentrations in olive oil samples as well as high-oleic sunflower oil samples. DiUFA were most abundant in the relatively highly unsaturated oils (i.e., CIV of approximately ≥90), and were generally highest in corn oil, sunflower oil, and soybean oil samples. Canola oils were unique in that they exhibited relatively low concentrations of DiUFA, despite having a high CIV (~100). TriUFA were the least abundant class of unsaturated lipids contained within the samples tested. In general, TriUFA levels appeared more abundant in the samples with high CIV, though they did not necessarily increase with increasing CIV. Canola oils, and lipid blends including canola oil, were the most abundant sources of TriUFA analyzed in this study. Canola oils are known for their

Table 2 Area-under-the-curve (AUC) values of the test samples as exhibited within the peroxide value (PV), conjugated dienes and trienes (CDT), 2-thiobarbituric acid reactive substances (TBARS) and *p*-anisidine value (*p*-AnV) assays over the storage period

Sample	PV	CDT	TBARS	*p*-AnV
Medium chain triacylglycerols 1	58	120	135	39
Medium chain triacylglycerols 2	13	96	77	23
Oil mix A	222	69	71	13
Cocoa butter substitute	212	69	93	13
Hydrogenated oil blend	163	96	143	29
Palm kernel stearin oil	58	77	24	42
Hydrogenated palm blend	175	91	73	36
Coconut oil	103	87	249	117
Palm kernel oil	460	46	350	228
Palm kernel olein oil	1328	154	92	70
Palm stearin oil	449	208	281	323
Cocoa butter substitute	203	50	175	105
Cocoa butter equivalent 1	386	140	575	71
Palm/coconut mix	1727	269	630	212
Cocoa butter equivalent 2	806	201	458	475
Cocoa butter equivalent 3	1253	253	521	436
Shea oil/palm	2117	108	418	534
Palm oil 1	1412	297	251	187
Palm oil 2	1521	43	314	134
Palm oil 3	2261	503	759	432
Palm/coconut high oleic	3725	660	945	880
Palm olein oil 1	2599	749	416	368
Cocoa butter replacer	216	208	252	38
Palm oil 4	3054	602	448	1160
Palm olein oil 2	4375	581	819	1856
Palm olein oil 3	2687	706	508	1073
Palm olein oil 4	7101	702	1266	808
Palm/soybean/canola	7606	162	1175	673
Palm olein oil 5	5248	968	755	1714
Palm olein oil 6	5913	560	1062	1554
Oil mix B	4938	412	1031	934
Oil mix C	7371	1000	2829	2660
Olive oil 1	6104	493	1757	635
Olive oil 2	3967	510	2763	2784
Sunflower oil high oleic 1	7578	607	1015	697
Sunflower oil high oleic 2	8587	854	1523	2658
Oil mix D	9772	1904	3521	3145
Canola oil 1	9524	1278	2926	6195
Corn/canola mix	6066	1330	4292	2483
Corn/canola mix D	10,129	1508	4564	3536
Canola/sunflower/corn	10026	1927	4207	2574
Canola oil 2	4005	907	5411	4256
Canola oil 3	5757	872	4724	5827
Corn oil 1	11,890	1900	2275	1314
Corn/canola mix E	9322	2095	4853	3520
Corn oil 2	11,939	1577	2811	2414

Table 2 continued

Sample	PV	CDT	TBARS	*p*-AnV
Corn oil 3	9618	1313	5265	5701
Corn oil 4	10,793	1985	2721	4459
Soybean oil	8587	1159	4583	5718
Sunflower oil	5372	1505	5240	2040

Determined over a period of two months accelerated oven storage ($T = 60$ °C). Test lipid samples were analyzed by the four named oxidative stability assays at 0, 1, 3, 7, 10, 14, 18, 23, 29, 36, 43, 50, and 57 days of storage. PV and CDT were also analyzed at 63 days of storage

relatively high quantities of α-linolenic acid (18:3n-3), which likely explains the magnitude of these data points [19, 20].

Regression Modeling of AUC Data Within Individual Oxidative Stability Analyses

AUC values for the lipid samples tested within the four oxidative stability assays appear in Table 2. Samples were arranged in ascending unsaturation (i.e., from top to bottom) just as they appeared in Table 1. It can be noted that as the samples proceed to higher unsaturation (and higher CIV) there appears a clear, yet not fully consistent, trend towards higher AUC values across all four assays. Notable deviations from this trend are in some cases observed simultaneously by all four assays (e.g. see the 'Cocoa Butter Replacer' sample). In other cases deviations from this trend can be seen only within a subset of the analyses data. For example, see the 'Palm/Soybean/Canola' sample, which exhibits a notably lower AUC (or less oxidation) in the *p*-AnV and CDT assays in comparison to its performance in the PV and TBARS assays.

Correlations (r^2) observed from the linear regression modeling of CIV versus AUC within the four oxidation assays appear in Table 3. The strength of correlations observed in the models for PV, CDT, and TBARS are fairly comparable ($r^2 = 0.771$, $r^2 = 0.752$, $r^2 = 0.758$, respectively), while the correlation between AUC and CIV within the *p*-AnV assay is notably weaker ($r^2 = 0.661$). A potential explanation for this discrepancy in strength of correlation between the TBARS model and the *p*-AnV model can be attributed to the performance of certain samples (e.g. corn oil 1, corn oil 2, and soybean oil) within the *p*-AnV assay. The three named oils above all exhibited dramatically lower *p*-AnV AUC values than was expected according to their high CIV, in addition to having relatively low concentrations of TriUFA (refer back to Table 1). This may be an indication of a relationship between the presence of higher levels of TriUFA, resulting in higher levels of

Table 3 Linear regression correlations (r^2) between the calculated iodine values (CIV) of the lipid samples and their area-under-the-curve (AUC) values over the storage period as determined within the below-named oxidative stability assays

Assay tested	Correlation (r^2)
PV: peroxide value	0.771
CDT: conjugated dienes and trienes	0.752
TBARS: 2-thiobarbituric acid reactive substances	0.758
p-AnV: p-anisidine value	0.661

Calculated iodine value (CIV): determined according to an official method Cd 1c-85 [15] using fatty acid (FA) composition data

release of unsaturated aldehydes with which the p-anisidine reagent are most reactive [21].

Results of regression modeling and the development of correlations between AUC data within the oxidative stability assays according to their inherent FA composition appear in Table 4. As seen in Table 4, there exists a divergent pattern of behavior between the models generated for the primary oxidation analysis assays (PV and CDT) and the secondary oxidation analyses (TBARS and p-AnV). In the case of the primary oxidation assays, the strength of the correlations of the models considering MUFA, DiUFA, and TriUFA composition are roughly comparable to the models based solely upon CIV. Conversely, the secondary oxidation assay models are each strengthened (increase in r^2 of approximately 0.10) by the more in depth approach of considering summary FA composition data.

It can be noted that the models generated using data of the primary oxidation analyses also exhibit a dramatically lower emphasis on the concentrations of TriUFA contained in the test lipids than is observed in the models generated using the data of the secondary oxidation analyses. This tendency is not well documented in the scientific literature, but may be attributable to the 'inverse-U' shaped curve that results from the long-term assessment of primary oxidation as hydroperoxides decompose [22]. In turn, AUC values of plots that depict both product proliferation and degradation (PV/CDT) could be speculated to be less affected by rate of accumulation than those that depict only accumulation (TBARS/p-AnV). To further investigate this hypothesis, the time (days) of maximum value in the oxidation progress

assays for all samples was gathered and appears in Table 5 in order of CIV (i.e., as with Table 1). These data for time of maximum value within assay were modeled according to TriUFA concentration and it was found that TriUFA concentration was a significant negative predictor of time of maximum value in both of the primary oxidation analyses (i.e., $p = 0.021$ for PV and $p = 0.007$ for CDT), but was not a significant predictor ($\alpha = 0.05$) for time of maximum value in the secondary oxidation analyses (TBARS/p-AnV). This result supports the hypothesis that samples with higher concentrations of TriUFA were reaching their maximum concentrations of primary oxidation products earlier in storage, and therefore, were also exhibiting degradation of those primary products sooner in the storage period.

Regression Modeling of a 'Standardized Sum Area-Under-the-Curve' (ssAUC)

ssAUC data for the test lipid samples were computed according to Eq. (5) and are reported in Table 6 in ascending order of CIV. As with the AUC data gathered within oxidation assay (refer back to Table 2), there appears to be a clear, yet not fully consistent, trend towards higher ssAUC values as a sample's CIV increases. Some notable exceptions to this trend include the 'Cocoa Butter Replacer' sample and the 'Palm Oil 2' sample; both of which exhibited a much greater stability than would be expected according to their CIV (refer back to Table 1). In contrast, sample 'Oil Mix C' exhibits a much lower oxidative stability than would be expected according to its CIV.

Given this apparent close relationship between CIV and the generated ssAUC aggregated value for sample oxidative stability, these two values were modeled together and their correlation ($r^2 = 0.873$) appear in Fig. 2. The Eq. (6) for this model between CIV and ssAUC for the test lipids is as follows:

$$\text{ssAUC} = -1382 + 99.3(\text{CIV}) \tag{6}$$

This correlation is quite strong and is substantially stronger than the correlations generated for the models of the AUC values within the individual oxidation assays (refer back to Table 3). The correlation may also be considered to surpass expectations of strength, considering the

Table 4 Linear regression models of AUC values for the below-named oxidative stability assays *versus* the concentration of MUFA, DiUFA, and TriUFA contained in the test lipid samples

Assay tested	Correlation ($r^2_{\text{adj.}}$)	Linear regression model
PV: peroxide value	0.778	$\text{PV}_{\text{AUC}} = -880 + 80.3[\text{MUFA}] + 174[\text{DiUFA}] + 11[\text{TriUFA}]$
CDT: conjugated dienes & trienes	0.771	$\text{CDT}_{\text{AUC}} = -52.1 + 7.91[\text{MUFA}] + 29.2[\text{DiUFA}] + 37.7[\text{TriUFA}]$
TBARS: 2-thiobarbituric acid reactive substances	0.870	$\text{TBARS}_{\text{AUC}} = -198 + 13.6[\text{MUFA}] + 62.4[\text{DiUFA}] + 411[\text{TriUFA}]$
p-AnV: p-anisidine value	0.762	$p\text{-AnV}_{\text{AUC}} = -260 + 16.5[\text{MUFA}] + 52.1[\text{DiUFA}] + 426[\text{TriUFA}]$

Table 5 Time (days of storage at $T = 60$ °C) of maximum value exhibited by test lipid sample within the peroxide value (PV), conjugated dienes and trienes (CDT), 2-thiobarbituric acid reactive substances (TBARS) and *p*-anisidine value (*p*-AnV) assays

Sample	PV	CDT	TBARS	*p*-AnV
Medium chain triacylglycerols 1	63	36	29	18
Medium chain triacylglycerols 2	63	7	29	14
Oil mix A	50	36	36	18
Cocoa butter substitute	57	36	18	7
Hydrogenated oil blend	63	36	29	43
Palm kernel stearin oil	3	36	29	57
Hydrogenated palm blend	29	50	57	57
Coconut oil	36	43	57	50
Palm kernel oil	63	63	57	57
Palm kernel olein oil	63	57	57	57
Palm stearin oil	29	63	57	57
Cocoa butter substitute	63	18	57	57
Cocoa butter equivalent 1	50	57	57	57
Palm/coconut mix	29	63	43	57
Cocoa butter equivalent 2	57	63	43	57
Cocoa butter equivalent 3	63	14	43	57
Shea oil/palm	29	50	57	57
Palm oil 1	43	50	57	57
Palm oil 2	57	10	57	57
Palm oil 3	43	63	29	57
Palm/coconut high oleic	43	63	57	57
Palm olein oil 1	57	57	57	57
Cocoa butter replacer	57	18	57	57
Palm oil 4	50	57	50	57
Palm olein oil 2	50	57	36	36
Palm olein oil 3	63	43	57	57
Palm olein oil 4	36	57	57	50
Palm/soybean/canola	57	50	57	57
Palm olein oil 5	43	43	57	57
Palm olein oil 6	63	63	36	57
Oil mix B	50	57	57	57
Oil mix C	36	43	43	50
Olive oil 1	36	43	43	43
Olive oil 2	50	57	57	57
Sunflower oil high oleic 1	36	43	57	50
Sunflower oil high oleic 2	36	43	43	57
Oil mix D	43	36	57	57
Canola oil 1	23	23	29	43
Corn/canola mix	36	36	43	57
Corn/canola mix D	29	43	43	50
Canola/sunflower/corn	43	43	57	57
Canola oil 2	50	23	29	36
Canola oil 3	23	18	57	57
Corn oil 1	43	50	50	57
Corn/canola mix E	43	43	50	57
Corn oil 2	43	43	57	57

Table 5 continued

Sample	PV	CDT	TBARS	*p*-AnV
Corn oil 3	36	43	57	43
Corn oil 4	43	50	57	50
Soybean oil	18	18	57	57
Sunflower oil	23	50	36	29

Determined over a period of two months accelerated oven storage ($T = 60$ °C). Test lipid samples were analyzed by the four named oxidative stability assays at 0, 1, 3, 7, 10, 14, 18, 23, 29, 36, 43, 50, and 57 days of storage. PV and CDT were also analyzed at 63 days of storage

aforementioned observed deviations from this association in literature [6–11]. This greater strength of association for the aggregate ssAUC value may be due to its comprehensive nature, which mitigates the quantitative effect of outlier behaviors that can result from the specificities of the individual oxidation assays.

The aggregate ssAUC values were also modeled according to the concentrations of MUFA, DiUFA, and TriUFA within samples, and their correlation ($r^2_{\mathrm{adj.}} = 0.915$) appears in Fig. 3. All three independent variables are statistically significant ($\alpha = 0.05$). The Eq. (7) for this model between summary FA composition data and ssAUC is as follows (7):

$$\mathrm{ssAUC} = -618 + 53.2[\mathrm{MUFA}] + 167[\mathrm{DiUFA}] + 635[\mathrm{TriUFA}]$$

$$(7)$$

The correlation of this model is a notable improvement on the model based solely upon CIV, and is again of better strength than models of the AUC values for the individual assays. This model provides a good quantitative assessment of the importance of fatty acid unsaturation in the oxidative stability of bulk lipids. This is in support of the speculation that this importance has perhaps been previously quantitatively underestimated due to insufficient modulation of singular aberrations observed within oxidative stability assessments.

Using this model, we can also examine the ratio upon stability of the relative effects of MUFA, DiUFA, and TriUFA. As quantified by the coefficients in the model, the ratio of their correlating effect is approximately 1:3:12 (i.e., more specifically, 53.2:167:635). This is substantially different than the 1:2:3 ratio of their relative unsaturation. This suggests a synergistic oxidative effect attributable to the combined presence of multiple double bonds on individual fatty acids. This result has been demonstrated previously [5, 8], but has not been consistently or frequently quantified with rigor [9, 23, 24].

As discussed in our introduction, studies that have focused specifically on rates of oxidation (i.e., without

Table 6 The computed 'standardized sum area-under-the-curve' (ssAUC) of the lipid samples tested over the storage period[b]
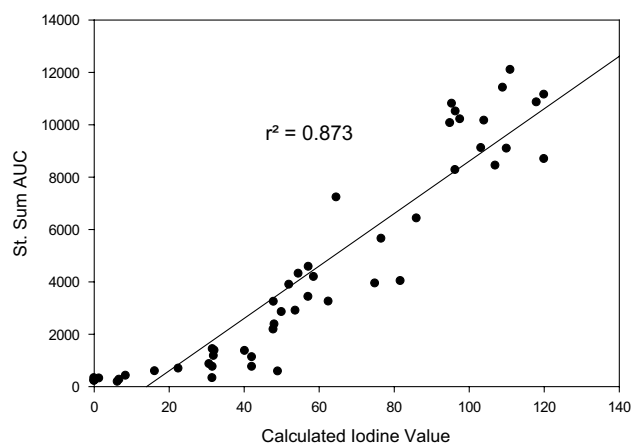
Sample	ssAUC
Medium chain triacylglycerols 1	320
Medium chain triacylglycerols 2	221
Oil mix A	220
Cocoa butter substitute	232
Hydrogenated oil blend	306
Palm kernel stearin oil	182
Hydrogenated palm blend	261
Coconut oil	409
Palm kernel oil	579
Palm kernel olein oil	685
Palm stearin oil	855
Cocoa butter substitute	319
Cocoa butter equivalent 1	742
Palm/coconut mix	1418
Cocoa butter equivalent 2	1157
Cocoa butter equivalent 3	1364
Shea oil/palm	1354
Palm oil 1	1119
Palm oil 2	748
Palm oil 3	2162
Palm/coconut high oleic	3218
Palm olein oil 1	2366
Cocoa butter replacer	573
Palm oil 4	2837
Palm olein oil 2	3874
Palm olein oil 3	2886
Palm olein oil 4	4285
Palm/soybean/canola	3404
Palm olein oil 5	4560
Palm olein oil 6	4167
Oil mix B	3225
Oil mix C	7168
Olive oil 1	3901
Olive oil 2	5590
Sunflower oil high oleic 1	4012
Sunflower oil high oleic 2	6391
Oil mix D	9992
Canola oil 1	10,744
Corn/canola mix	8186
Corn/canola mix D	10,415
Canola/sunflower/corn	10,128
Canola oil 2	9003
Canola oil 3	10,066
Corn oil 1	8391
Corn/canola mix E	11,315
Corn oil 2	9032
Corn oil 3	11,988
Corn oil 4	10,798

Table 6 continued

Sample	ssAUC
Soybean oil	11,057
Sunflower oil	8587

ssAUC values are weighted composite scores obtained via the combination of the four oxidative product assay curves for each test lipid sample according to the following equation: $ss_{AUC} = [PV_{AUC} + 6.31(CDT_{AUC}) + 2.60(TBARS_{AUC}) + 2.87(p\text{-}AnV_{AUC})]/4$

Determined over a period of two months accelerated oven storage ($T = 60\ °C$). Test lipid samples were analyzed by the four named oxidative stability assays at 0, 1, 3, 7, 10, 14, 18, 23, 29, 36, 43, 50, and 57 days of storage. PV and CDT were also analyzed at 63 days of storage

Fig. 2 Correlation between standardized sum AUC (ssAUC value for overall oxidative stability) and CIV (calculated iodine value indicative of sample unsaturation) for the lipid samples tested

examination of magnitude of proliferated oxidation products), have observed presence of *bis*-allylic sites to be better linear inverse predictors of stability than measures of double bonds [10]. This effect is due to a breakdown of energy distribution within individual fatty acids. The ratio of *bis*-allylic sites in DiUFA and TriUFA (1:2) is closer to our observed ratio of oxidative impact than the ratio of double bonds (2:3), but still underestimates the differences in oxidation progress observed in this study (1:4). These dissimilarities in ratio effect may not be construed as in contradiction, however, as the considerations of rate-assessment and assessment of long-term product accumulation are not expected to demonstrate direct equivalency. Given the documented capabilities of primary oxidation reaction products to promote the initiation reaction of autoxidation, the greater oxidative rate of TriUFA may be producing a less favorable environment within the bulk oil system in regards to oxidative stability [25–29]. The effect of such an action would be one of increased
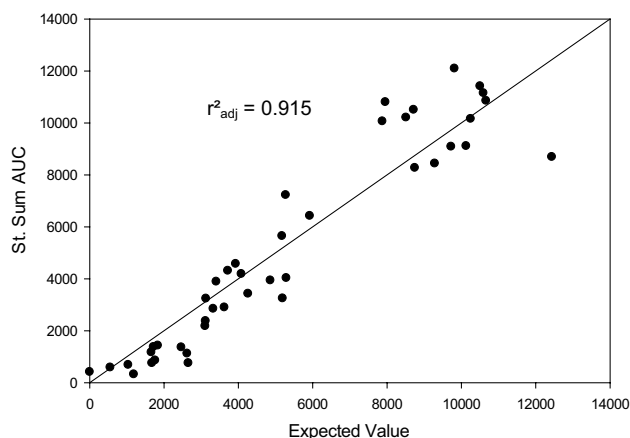
Fig. 3 Correlation between standardized sum AUC (ssAUC value for overall oxidative stability) and the expected value of a predictive model according to the inherent concentrations of MUFA, DiUFA, and TriUFA contained in the lipid samples tested

exhibition of oxidation products within the system throughout storage, as observed in this study.

Conclusion

It was determined in this study that the concentrations of mono- (MUFA), di- (DiUFA), and tri-unsaturated fatty acids (TriUFA) within the test lipids were all found to be statistically significant predictors of oxidative progress within the analytical assays conducted. Further, when used in combination, these fatty acid data exhibited a strong correlation ($r_{\text{adj.}}^2 = 0.915$) with a novel consolidated measure of oxidation progress; namely, the standardized sum area-under-the-curve (ssAUC) for the entire storage period. This model outperformed a model created using calculated iodine values (CIV) as the sole independent variable ($r^2 = 0.873$), and also outperformed analogous predictive models for the curves obtained in the individual oxidative assays conducted (i.e., $r^2 = 0.778$, $r^2 = 0.771$, $r^2 = 0.870$, and $r^2 = 0.762$ for predictive models based on PV, CDT, TBARS, and p-AnV, respectively). These results provide a comprehensive quantification of the association between fatty acid unsaturation and stability within bulk vegetable lipids, and suggest that this association has possibly been previously underestimated. The regression models built based on concentrations of MUFA, DiUFA, and TriUFA contained within the test samples indicated the relative effect upon oxidative stability of MUFA:DiUFA:TriUFA to be approximately 1:3:12—substantially greater than the 1:2:3 ratio of their relative unsaturation. This result suggests that the combined

presence of multiple double bonds on individual fatty acids is associated with impaired oxidative stability, even more so than a comparison of *bis*-allylic sites would predict.

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