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# Challenges for Use of PeroxySafe™ MSA Kit for Analysis of Poultry Meat

Annie J. King and Lok T. M. Suen

**Abstract:** The rapid SafTest PeroxySafe™ MSA Kit (PeroxySafe method) was approved by the AOAC to determine peroxide values (PVs) in foods. Studies were conducted to remedy challenges (reaction time, lack of turbidity in samples, effect of prooxidant [NaCl]) for use of the method when analyzing PVs in turkey meat. Initially, PVs could not be consistently obtained after a reaction time of 10 min (per directions) for variously processed meat. However, trends indicated that heated and heated/stored samples generally had higher numerical values than Fresh ones. This trend agreed with that of other investigators, suggesting usefulness of the method if consistent data could be obtained. Data for PVs of all treatments within processing conditions were recorded at 10, 20, and 30 min. There was a highly significant ( $P \leq 0.0001$ ) effect for reaction time with 30 min > 20 min > 10 min. An increase in PVs was noted for heated samples when lipids and oxidation products were released by homogenization, rather than vortexing with glass beads, and data were recorded at the 30 min. It is likely that these precautions may promote more accurate determination of PVs from samples with NaCl, a prooxidant. Comparison of extraction procedures for the PeroxySafe method and that of Grau and others (2000) showed that the extraction procedure (homogenization) of the latter method produced numerically greater PVs for fresh/stored samples than that of the former. However, it was concluded that the PeroxySafe method could be used for comparative analyses of samples when adequate extraction (turbidity) occurred and measurements were recorded after a 20 to 30 min reaction time.

**Practical Application:** A plethora of processing and storage conditions are used to ensure a nutritious and safe food supply. Processing/storage conditions and additives may increase or decrease lipid oxidation in foods having a high unsaturated fatty acid content. Rapid and consistent determination of peroxide values (PVs) could be used by investigators to quickly analyze the effect of various conditions during early stages of unsaturated fatty acid oxidation. Of particular interest is the use of agri-food chain horticultural by-products, containing antioxidants, in feed of poultry and the capacity of the antioxidants to reduce oxidation of heated and stored post mortem poultry meat. Use of rapid methods to assess oxidation of fatty acids in raw and processed foods as well as the efficacy of dietary antioxidants in variously processed and stored poultry products would be valuable to scientists and food manufacturers.

## Introduction

Dark raw turkey muscle without skin contains 8.96% total fat and 0.61% total polyunsaturated fatty acid (PUFA). The most abundant of the PUFAs is linoleic acid, which can be changed by dietary lipid source (Zollitch and others 1996; Badinger and others 2003; USDA 2015). When subjected to various processing, heating and storage conditions, high quantities of PUFAs in dark turkey meat undergo rapid lipid oxidation due to the quantity of free iron (a prooxidant) and low concentration of the antioxidant,  $\alpha$ -tocopherol (Sklan and others 1983; Kanner and others 1988; Mercier and others 1998). Niki and others (2005) noted that among the 5 elementary reactions associated with the free radical-mediated peroxidation of PUFAs, 1 key reaction is the formation of hydroperoxides from the lipid peroxyl radical; these hydroperoxides are measured as peroxide values (PVs).

Analysis of PVs, once time-consuming and labor intensive, has been improved and is available as the Fox Determination Kit (Gülğün and others 2003). The SafTest PeroxySafe™ MSA Kit is

another commercially available kit for determining PVs in conjunction with the MP SafTest® Photo Detector Analyzer™. Use of the Kit and the Analyzer are hereafter referred to as the

PeroxySafe method, recognized by the AOAC for rapid analysis of lipid peroxides in food (Certification Report 2003). The correlation coefficient for the standard titration for PVs and the PeroxySafe method was 0.993 (Certification Report 2003). The test is based on the Fenton reaction where ferrous ion ( $\text{Fe}^{2+}$ ) is oxidized to ferric ion ( $\text{Fe}^{3+}$ ) by hydroperoxides in acidic conditions. The ferric ion reacts with xylenol-orange to form a blue-purple complex, having a  $\lambda_{\text{max}}$  at 570 nm.

Generally, the PeroxySafe method has been used to determine oxidation in oil rather than meat (Yildiz and others 2003; Halvorsen and Blomhoff 2011). If this method could be used by investigators to conduct rapid and accurate comparisons of oxidation after processing/storage conditions and for the efficacy of antioxidants in solid foods, it would be a valuable tool in many laboratories. As we explored use of the PeroxySafe method in our laboratory, there was concern about the consistency of PVs obtained for variously processed and stored poultry meat when data were recorded after the 10 min reaction interval (reaction time) as directed (MP Biomedical; LLC 2014). In addition, for some processed and stored samples, PVs were 0 and NaCl, a prooxidant, caused no appreciable increase in lipid oxidation for some processing/storage conditions as compared to controls. We endeavored to

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**Table 1—Experimental protocol for comparison of the extraction procedures from methods for PeroxySafe™ MSA Kit (2003) and Grau and others (2000).**

Extraction method <sup>a</sup>	Extraction reagent			Separation	Sample size (g)	Dilution factor
		Volume (mL)	Homogenization			
A (Control) <sup>b</sup>	Preparation reagent	3	Vortex	Membrane	1	4
B (Control) <sup>c</sup>	Methanol	15	PT 1200 E	Centrifuge	3	6
C	Methanol	3	Vortex	Membrane	1	4
D	Preparation reagent	3	PT 1200 E	Membrane	1	4
E	Preparation reagent	3	Vortex	Centrifuge	1	4
F	Preparation reagent	15	PT 1200 E	Centrifuge	3	6
G	Same as C, but sample was heated (see above).					

<sup>a</sup>PV values were determined by PeroxySafe at 30 min reaction time.

<sup>b</sup>Complete method from the PeroxySafe™ MSA Kit (2003).

<sup>c</sup>Complete method of Grau and others (2000).

**Table 2—Preliminary determination of peroxide values (meq/kg)<sup>a</sup> measured at 15 min reaction time for treatment and processing conditions of turkey thigh meat.**

Processing conditions <sup>b</sup>	Treatments <sup>c</sup>					
	Control	NaCl <sup>d</sup>	GSO 8 mm <sup>e</sup>	GSO 15 <sup>e</sup>	GSO 8 mm + NaCl	GSO 15 mm + NaCl <sup>d</sup>
Fresh	0.064	0.000	0.033	0.006	0.000	0.007
Stored	0.002	0.000	0.012	0.005	0.005	0.003
Heated	0.013	0.021	0.014	0.017	0.022	0.010
Heated/stored	0.033	0.015	0.016	0.037	0.032	0.024
Treatment effect across processing conditions	0.028 <sup>a</sup>	0.007 <sup>a</sup>	0.019 <sup>a</sup>	0.016 <sup>a</sup>	0.015 <sup>a</sup>	0.011 <sup>a</sup>
	± 0.027	± 0.012	± 0.010	± 0.015	± 0.015	± 0.009

<sup>a</sup>Measured by the SafTest PeroxySafe™ MSA Kit and the MP SafTest® Photo Detector Analyzer.

<sup>b</sup>Heating conditions were Fresh (immediately frozen at -80 °C), Stored (7 d at 5 °C prior to storage at -80 °C), Heated (boiled in boiling bags for 2 min prior to storage at -80 °C) and Heated/Stored (boiled in boiling bags for 2 min, stored at 5 °C for 7 d prior to storage at -80 °C).

<sup>c</sup>Average values within rows were not statistically analyzed. Means within the row (treatment across processing/storage conditions) with different superscripts are significantly different at  $P \leq 0.05$ .

<sup>d</sup>NaCl (a prooxidant).

<sup>e</sup>GSOs (antioxidants, grape seed oils processed using 8 and 15 mm nozzles) were each added at 1.0% (w/w).

assess trends developed by use of the method during routine laboratory analysis (effect of antioxidants, prooxidants, heating/storage conditions) of turkey meat and remedy the aforementioned challenges. Thus, the purpose of this study was to (1) establish the most appropriate time interval before recording measurements for PVs when using the PeroxySafe method for analysis of variously processed/stored poultry meat; (2) understand the reason for lower than expected trends for PVs associated with (a) Heated/Stored samples and (b) the prooxidant effect of NaCl; and (3) compare the efficacy of PV extraction procedures from the PeroxySafe method and that of Grau and others (2000).

## Materials and Methods

Turkey thigh meat was obtained from a local grocery store on the day of arrival from the processing plant. An antioxidant, MegaNatural® Gold Grape Seed Extract (GSE), was obtained from Polyphenolics, Inc. (Madera, Calif., U.S.A.). Other antioxidants, Sauvignon Blanc Grape Seed Oils (GSOs), processed from 2 nozzles (8 and 15 mm), were from SonomaCeuticals (Santa Rosa, Calif., U.S.A.). NaCl was from Morten Salt (Chicago, Ill., U.S.A.). The PeroxySafe™ MSA Kit and SafTest® Photo Detector Analyzer™ were from MP Biomedicals (Solon, Ohio, U.S.A.). Methanol was from Chemical Stores (Univ. of California, Davis, Calif., U.S.A.).

## Accuracy of sample preparation

Procedures for production of all samples are presented to show precautions taken to ensure that observed trends were challenges of the PeroxySafe method and not due to lack of exact sample preparation and handling within each study.

## Preparation of turkey meat for study I—increase in reaction time

After skin, bone, and fat were removed, turkey thighs were cut into uniform pieces (600 g, 2-cm cubes). Samples (200 g) were homogenized (OMNI Macro Homogenizer, Model 17505, OMNI Intl., Kennesaw, Ga., U.S.A.) for 1 min at speed level 5, then transferred to air-tight storage bags inside aluminum pouches and stored at -80 °C until thawed (24 h at 12.22 °C), treated, processed (2 samples of 10 g each), and analyzed as Fresh. GSO, an antioxidant, processed from 2 nozzles (8 and 15 mm), was added at 1.0% (w/w). To enhance lipid oxidation, NaCl (1%, w/w) in deionized distilled water (1%, v/w) was added where indicated. Treatments were Control, NaCl, GSO 8 mm, GSO 15 mm, GSO 8 mm + NaCl and GSO 15 mm + NaCl. Meat and additives were thoroughly mixed with a spatula for 1 min before placing into storage bags followed by massaging for an additional 15 s to ensure uniform distribution of additives. Processing/storage conditions were fresh, stored (7 d at 5 °C), heated (boiled in boiling bags for 2 min), and heated/stored (boiled in boiling bags for 2 min, stored at 5 °C for 7 d). Immediately after processing, all treatments were stored at -80 °C until analyzed. Due to lack of consistency in previous measurements at 10 min, data for Study I were recorded at 15 min reaction time. The experimental design was 6 treatments × 4 processing/storage conditions × 2 samples.

## Initial preparation of turkey meat for studies II to V

Skin, bone, and fat from turkey thighs were removed. Meat was cut into uniform pieces (2-cm cubes) and homogenized (1.2 kg) in a food processor (Model 70610 Food processor, Type

FP14, Hamilton Beach, Southern Pines, N.C., U.S.A.) at medium power for 1 min, allowed to stand for 1 min, and processed for an additional min. Samples (200 g) were homogenized (OMNI Macro Homogenizer) for 45 s at speed level 5, then transferred to air-tight bags inside aluminum pouches and stored at  $-80^{\circ}\text{C}$  until thawed.

### Study II—comparison of reaction times

Prooxidant, NaCl (1%, w/w) and/or GSE (an antioxidant, w/w at 0.25%, 0.5%, and 1.0%), and distilled water (1%, v/w) were added to meat while mixing with a spatula for 1 min before placing mixtures into storage bags. Mixtures in bags were massaged for an additional 15 s to insure uniform distribution of additives. Eight treatments were Control (no additives), 0.25% GSE, 0.5% GSE, 1.0% GSE, NaCl, NaCl + 0.25% GSE, NaCl + 0.5% GSE, and NaCl + 1.0% GSE. Processing/storage conditions were as delineated above except that samples were stored for 5 d. Data were recorded at 10, 20, and 30 min reaction times. The experimental design was 8 treatments  $\times$  4 processing/storage conditions  $\times$  2 samples  $\times$  3 reaction times  $\times$  2 runs.

### Study III—effect of 30 min reaction time for heated/stored samples

Fresh samples were labeled as 0 to 5 d. Samples designated day 0 were stored at  $-80^{\circ}\text{C}$  immediately until further analysis. Others were stored at  $4^{\circ}\text{C}$  for 1 to 5 d prior to storage at  $-80^{\circ}\text{C}$  and designated as Fresh 1 to Fresh 5 (Table 3). Heating and storage of samples followed procedures delineated in Study I. Data were recorded at 30 min reaction time. The experimental design was 2 processing conditions  $\times$  3 samples  $\times$  5 storage conditions  $\times$  2 runs.

### Study IV—effect of homogenization and reaction times for heated/stored samples

Ground turkey meat was subjected to the 4 processing/storage conditions (no treatments) delineated in Study I with the exception that samples were stored for 4 d. Before analysis, samples were mixed for 30 s with a hand-held PT1200 E Homogenizer (Polytron<sup>®</sup> PT1200 E, Kinemati, Inc., Bohemia, N.Y., U.S.A.). Data were recorded at 10, 20, and 30 min reaction time. Hydrogen peroxide was used as an equipment and reagent control. The experimental design was 4 processing/storage conditions  $\times$  3 samples  $\times$  3 reactions times  $\times$  2 runs.

### Study V—comparison of the extraction procedures from methods for peroxySafe and Grau and others (2000)

Fresh samples were divided into equal portions and transferred to air-tight storage bags, labeled 0 to 5 d. Samples (3) labeled day 0 (Fresh) were stored at  $-80^{\circ}\text{C}$  immediately until further analysis; those labeled 1 to 5 d were stored at  $4^{\circ}\text{C}$  prior to storage at  $-80^{\circ}\text{C}$ . Extraction procedures were divided into 3 parts as shown in Table 1 and Figure 1. Comparisons were made by singularly substituting parts from Grau and others (2000) into the PeroxySafe method. Where indicated, homogenization was performed with the PT1200 E Homogenizer (Polytron<sup>®</sup> PT1200 E, Kinemati, Inc.) at medium speed. The PeroxySafe method is delineated above and designated as Method A in Table 1. For the method of Grau and others (2000) (Method B, Table 1), meat (3 g) was weighed into a 50 mL centrifuge tube, followed by the addition of 15 mL HPLC grade methanol ( $-20^{\circ}\text{C}$ ). After homogenizing for 30 s at medium speed followed by centrifuging for 3 min at 1400 rpm, the supernatant was transferred to a glass tube for PV analysis.

Data were recorded at 30 min reaction time. Triplicates of each extraction method were performed.

### Analysis of lipid oxidation—PeroxySafe method

Variouly treated, prepared, and stored ground turkey meat (1 g) was weighed into conical tubes, followed by the addition of 3 mL preparation reagent and 10 glass beads. Content was vortexed for 1 min at highest speed with 10 glass beads. Tubes were placed in the heating block for 15 min. Content was separated by membrane filtration with a vacuum pump and the extract was collected in a glass tube for PV analysis. Data were converted to PV as:

Measurement  $\times$  dilution factor (usually 4) = PV as meq/kg (MP Biomedicals; LLC 2014).

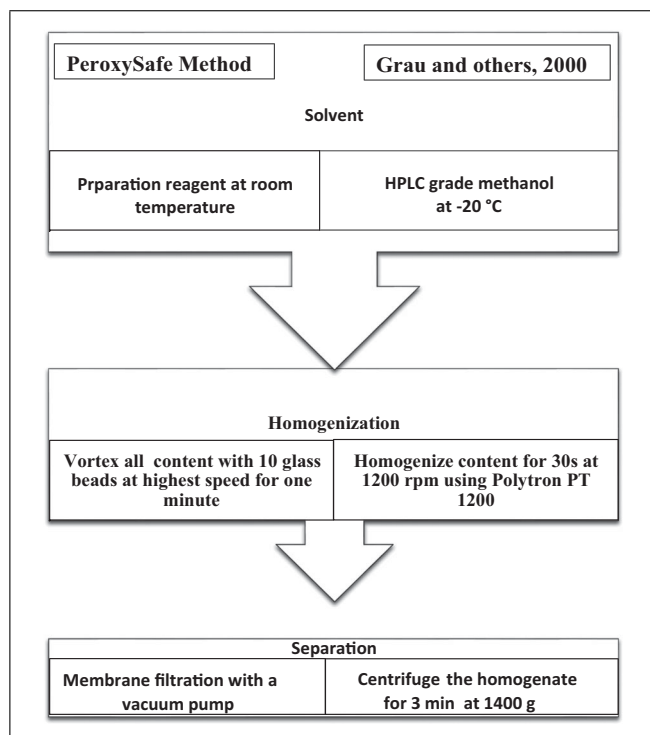


Figure 1—Extraction procedures for PeroxySafe<sup>™</sup> MSA Kit (2003) and Grau and others (2000).

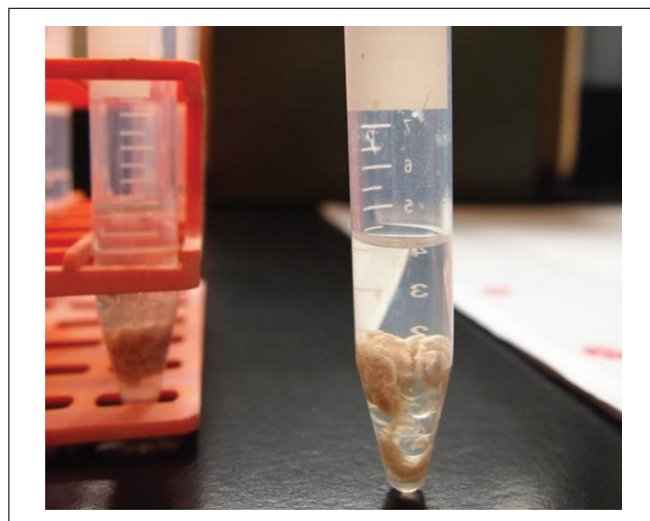


Figure 2—Clarity of supernatant after centrifugation of heated meat sample.

**Table 3—Peroxide values (meq/kg)<sup>a</sup> measured after 10, 20, and 30 min (reaction time).**

Processing/storage condition <sup>c</sup>	Reaction time <sup>d</sup> (min)	Control	Treatments <sup>b</sup>						
			GSE (%) <sup>e</sup>			NaCl <sup>f</sup> + GSE (%)			
			0.025	0.5	1.0	0.0	0.25	0.5	1.0
Fresh	10 <sup>a</sup>	0.000	0.036	0.000	0.028	0.000	0.000	0.040	0.008
	20 <sup>b</sup>	0.012	0.084	0.024	0.052	0.012	0.028	0.096	0.060
	30 <sup>c</sup>	0.072	0.160	0.056	0.100	0.076	0.084	0.128	0.132
Fresh/stored	10 <sup>a</sup>	0.000	0.024	0.000	0.036	0.000	0.000	0.000	0.008
	20 <sup>b</sup>	0.016	0.096	0.000	0.088	0.012	0.000	0.076	0.072
	30 <sup>c</sup>	0.072	0.160	0.040	0.152	0.076	0.076	0.132	0.136
Heated	10 <sup>a</sup>	0.364	0.000	0.196	0.060	0.244	0.000	0.000	0.048
	20 <sup>b</sup>	0.516	0.056	0.224	0.068	0.368	0.012	0.092	0.144
	30 <sup>c</sup>	0.624	0.144	0.296	0.136	0.492	0.080	0.164	0.208
Heated/stored	10 <sup>a</sup>	0.000	0.024	0.000	0.144	0.140	0.000	0.000	0.032

<sup>a</sup>Measured by the SafTest PeroxySafe™ MSA Kit and the MP SafTest® Photo Detector Analyzer.

<sup>b</sup>Heating conditions were fresh (immediately frozen at  $-80^{\circ}\text{C}$ ), stored (5 d at  $5^{\circ}\text{C}$  prior to storage at  $-80^{\circ}\text{C}$ ), heated (boiled in boiling bags for 2 min then stored at  $-80^{\circ}\text{C}$ ), and heated/stored (boiled in boiling bags for 2 min, stored at  $5^{\circ}\text{C}$  for 5 d prior to storage at  $-80^{\circ}\text{C}$ ).

<sup>c</sup>Means of reaction time for each processing/storage condition are highly significant at  $P \leq 0.0001$ , SE = 0.2827.

<sup>d</sup>GSE, grape seed extract.

<sup>e</sup>NaCl (1.0%, w/w).

**Table 4—Peroxide values (Meq/kg)<sup>a</sup> obtained at 30 min reaction time.**

Processing conditions <sup>b</sup>	Days of storage <sup>c</sup>					
	0 <sup>c</sup>	1	2	3	4	5
Fresh	0.000 <sup>a</sup>	0.264 <sup>b</sup>	0.580 <sup>c</sup>	0.600 <sup>c</sup>	0.682 <sup>d</sup>	0.690 <sup>d</sup>
Heated	0.024 <sup>a</sup>	0.000 <sup>a</sup>	0.044 <sup>a</sup>	0.000 <sup>a</sup>	0.000 <sup>a</sup>	0.000 <sup>a</sup>

<sup>a</sup>Measured by the SafTest PeroxySafe™ MSA Kit and the MP SafTest® Photo Detector Analyzer.

<sup>b</sup>Processing conditions for all treatments were fresh (stored at  $-80^{\circ}\text{C}$  until analyzed) and Heated (boiled in boiling bags for 2 min).

<sup>c</sup>Storage at  $4^{\circ}\text{C}$  for 1 to 5 d prior to storage at  $-80^{\circ}\text{C}$ . Means of processing condition with different superscripts are significantly different at  $P \leq 0.05$ .

## Statistical analysis

Data from Study I were preliminary and averages of duplicate samples are shown. Means for Studies II and III were analyzed by ANOVA (SAS Inst. 2004). Statistical differences among means for Studies II and III were performed using the Student's *t*-test at  $P \leq 0.05$ . Means for PVs in Studies IV and V were used to develop comparative curves.

## Results and Discussion

### Study I—comparison of reaction times

Previous trials for use of the PeroxySafe method in our laboratory indicated that PVs were not measurable for untreated, stored samples at the 10 min reaction time as directed (MP Biomedicals; LLC 2014). For some processing/storage conditions in our previous work, PVs (data not shown) were close to 0; however, results of other investigators noted that this was unlikely for stored samples and for those with added NaCl (1–3%, w/w), known to accelerate oxidation (Niki and others 2005; Brannan 2009; Gheisari 2011; Kashyap and others 2012).

In our preliminary Study I, samples from several treatments (prooxidant [NaCl], antioxidant [GSOs], and processing/storage conditions) were analyzed to widely assess the use of an extended reaction time of 15 instead of 10 min (Table 2). Trends for effects of treatments were not clear. NaCl, a prooxidant, did not increase oxidation as compared to the control. PVs for each treatment across processing/storage conditions were not significantly different ( $P < 0.05$ ).

However, because heated and heated/stored samples generally had the highest numerical values, we surmised that the PeroxySafe method showed weak trends comparable to results of other investigators (Grau and others, 2000; Kashyap and others 2003;

Lau and King, 2003). Gheisari (2011) showed that PVs in chicken significantly increased over 0, 2, and 4 d of storage in refrigerated conditions. Also, Kashyap and others (2012) found that PVs numerically increased in chicken patties during 2 mo of frozen storage ( $-18 \pm 2.0^{\circ}\text{C}$ ). Although, hydroperoxides begin to decompose at  $<100^{\circ}\text{C}$ , investigators reported PVs for heated meat (Muik 2005; Gheisari 2011; Kashyap and others 2012). Due to lack of consistency of values in Study I and Study II was designed to evaluate PVs in variously processed/stored samples at longer reaction times.

### Study II—effect of 30 min reaction time for heated/stored samples

Table 3 shows results for effects of variously treated and processed samples when measured at 10, 20, and 30 min. Again, PVs were not always attainable at 10 min. There was a highly significant effect ( $P \leq 0.0001$ ; SE = 0.2827) when PVs were recorded at increasing reaction times where 30 min > 20 min > 10 min. Thus, 20 to 30 min intervals could be used to determine PVs in poultry (turkey) meat. One precaution with this assumption is that during initial oxidation, PVs increase with time; therefore, the quantity of PVs at 30 min may be greater than the initial production of PVs at a lower reaction time. However, in comparative studies, consistent PVs at 30 min would provide more reliable data for differences among treatments than inconsistent data with large standard deviations at 10 or 15 min. Because our studies were conducted to refine the AOAC approved PeroxySafe method, PVs were not compared to those obtained from traditional methods for determination. As noted above, the PeroxySafe method has been approved by the AOAC with a correlation coefficient for the standard titration for PVs and the PeroxySafe method of 0.993 (Certification Report 2003).

**Study III—effect of 30 min reaction time for heated/stored samples**

Due to inconsistency in trends for addition of NaCl and processing/storage conditions observed in Studies I and II, Study III was conducted to compare fresh and heated samples without confounding effects of treatment. Table 4 shows a steady significant increase in PVs of fresh/stored (4 °C) samples for 0 to 4 d when measured after 30 min. However, PVs for heated/stored samples remained generally at 0 with a slight increase at days 0 and 2,

much less than the effect reported by other investigators (Muik 2005; Gheisari 2011; Kashyap and others 2012).

One possible explanation for the inconsistent measurements of PVs for heated samples in our study was the variable effectiveness of the PeroxySafe method for extraction of peroxides from all heated meat samples. Because heated meat was more compact than fresh meat, lipids, and hydrogen peroxide may not have been evenly extracted from our heated samples by vortexing with glass beads or breaking up with a spatula for 1 min. As shown in Figure 2,

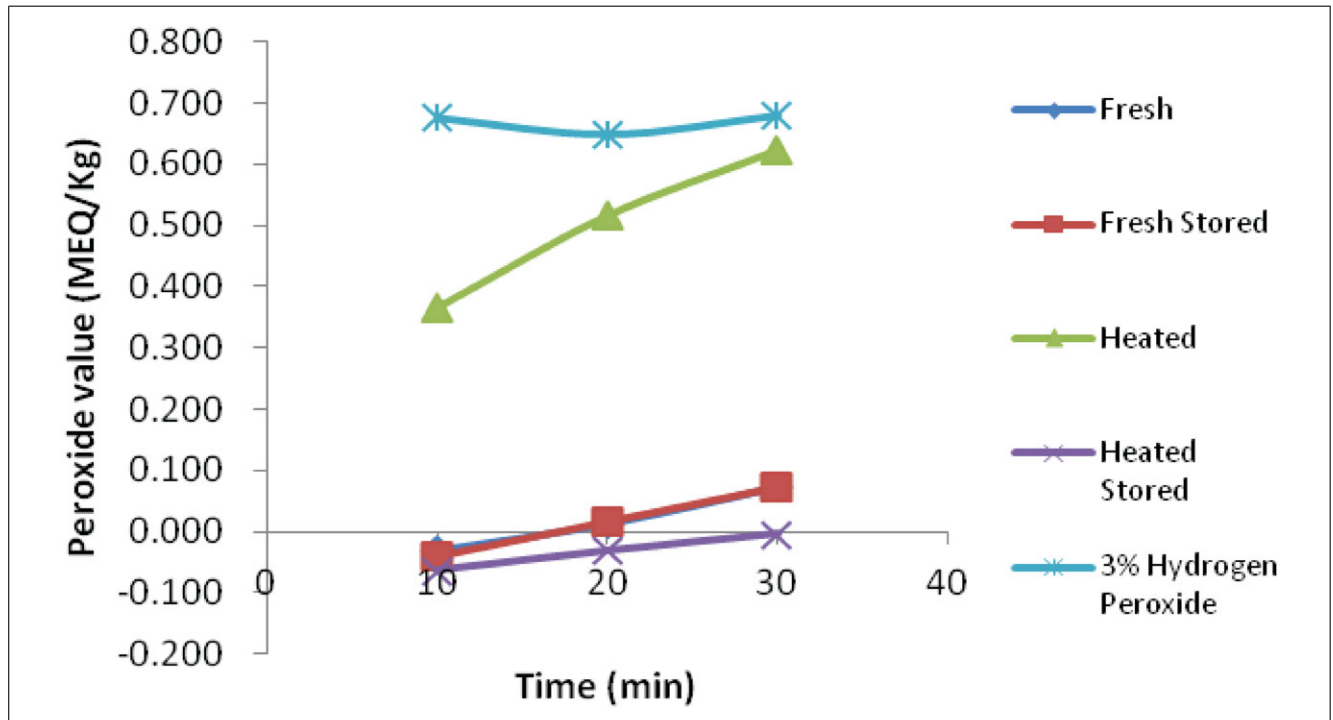


Figure 3—PVs (meq/kg)<sup>a</sup> for homogenized processed/stored samples after a 30 min reaction time.

(a) Measured by the SafTest PeroxySafe™ MSA Kit and the MP SafTest® Photo Detector Analyzer.

Heating conditions were fresh (immediately frozen at -80 °C), stored (4 d at 5 °C prior to storage at -80 °C), heated (boiled in boiling bags for 2 min prior to storage at -80 °C), and heated/stored (boiled in boiling bags for 2 min, stored at 5 °C for 4 d prior to storage at -80 °C).

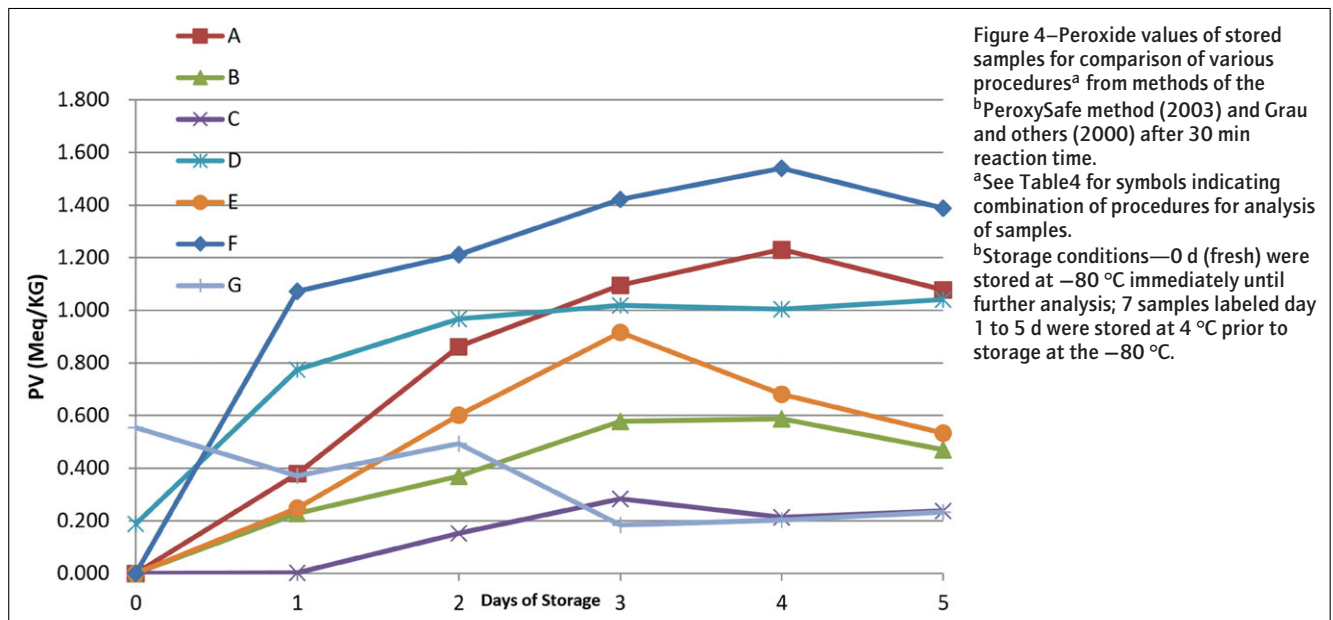


Figure 4—Peroxide values of stored samples for comparison of various procedures<sup>a</sup> from methods of the <sup>b</sup>PeroxySafe method (2003) and Grau and others (2000) after 30 min reaction time.

<sup>a</sup>See Table 4 for symbols indicating combination of procedures for analysis of samples.

<sup>b</sup>Storage conditions—0 d (fresh) were stored at -80 °C immediately until further analysis; 7 samples labeled day 1 to 5 d were stored at 4 °C prior to storage at the -80 °C.

some extracts for heated samples remained clear while those for fresh samples (observation not shown) were always turbid. Clearness of supernatants may have indicated incomplete extraction. Thus, it was decided that all heated and Heated/Stored samples needed to be separated by a small hand-held homogenizer to ensure consistent turbidity of extracts with measurement of data at 20 or 30 min reaction time.

For both Studies I and II (Tables 2 and 3), with the exception of NaCl heated/stored, the prooxidant did not seem to produce more PVs when compared to the control. Except for heated/stored samples, those with the prooxidant had similar or lower treatment effects than the Control or those with various quantities of GSE (no NaCl; Table 3). Results for NaCl were most likely confounded by our inability to assay early production of PVs caused by this prooxidants and/or due to inconsistencies caused by inadequate release of oxidation products from meat after NaCl changed the physical structure of the sample. This observation suggested that like any Heated samples, those containing NaCl should be homogenized before analysis. Clearly, this suggestion should be further investigated.

#### Study IV—effect of homogenization and reaction times for heated/stored samples

Study IV was conducted to determine if homogenization (with turbidity) helped to release PVs for variously processed/stored samples. As noted in Figure 3, fresh, fresh/stored, and heated/stored samples had less PVs than heated samples. Although there were likely less PVs in fresh samples and fresh/stored, less PVs in heated/stored samples were not expected. Possibly, there was a reduction in peroxides for heated/stored turkey meat (approximately 0.75 meq/kg) after 4 d as shown in Graph G of Figure 4 whereas other oxidation products, not detected in our study, were increasing. A steady increase in oxidation for heated samples after homogenization (with turbidity) seemed to verify our assumption that glass beads with vortexing were not adequate for release of peroxides from heated meat.

#### Study V—comparison of the extraction procedures from methods for peroxySafe and Grau and others (2000)

Various combinations of procedures from these 2 methods are shown in Table 1 and Figure 1. Results are shown in Figure 4. Visual observation for Graph G of Figure 4 indicated that homogenization from the method of Grau and others (2000) possibly increased extraction efficiency. However, although PVs are lower, the PeroxySafe method (Graph A) generally follows the same trend as that for Graph F and could be employed in comparative analysis for efficacy of antioxidant in unheated and heated meat after a reaction time of 20 or 30 min. Also, note that for Graph G (Heated) in Figure 4.1, (1) PVs (approximately 350 to 600 meq/kg for days 1 and 2) at 30 min reaction time are in the range of those for heated/stored samples in Figure 3 and (2) PVs decrease in Heated samples after day 3. Without adequate release by homogenization, inconsistent PVs could occur beginning at day 1 and certainly after day 3 when PVs begin to decline. Results of this study showed that the PeroxySafe method, with homogenization, could be employed in comparative PV analyses for variously treated and processed turkey meat at a reaction time of 20 to 30 min.

## Conclusions

The AOAC approved PeroxySafe method seemed to adequately determine PVs in poultry meat when there is homogenization of meat to release oxidation products (associated with turbidity) in the extract and data is recorded after 20 to 30 min reaction times. Challenges encountered when analyzing samples containing NaCl were likely due to inadequate detection of early production of PVs caused by the pro-oxidant. As well, samples containing NaCl may need to be homogenized for best results. Future investigations will determine if lower quantities of PVs can be detected in variously processed/stored hogenized samples without and NaCl at 10 to 20 min reaction times.

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## Authors' Contributions

A. King planned the initial research and prepared the final manuscript. L. Suen extended and conducted the research, then wrote the initial manuscript.

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