1991

Analysis of meat lipid oxidation with examination of polyphosphate interaction

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Analysis of meat lipid oxidation with examination of polyphosphate interaction

by

Michael Paul Wanous

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

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Departments: Food Science and Human Nutrition
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GENERAL INTRODUCTION

Implications and Mechanisms of Lipid Oxidation

Lipid oxidation is a major factor affecting flavor, color, texture, and consumer acceptibility of muscle foods, and therefore, exercises a major role in overall shelf-life. Many of these ramifications are not unique to meat products, and affect all lipid-containing foods as well.

In addition to influence on sensory factors of food, of major concern are the safety implications of lipid oxidation. Coronary heart disease continues to be the number one killer of adults in the United States (Addis, 1990). High correlations have been calculated between meat oxidative rancidity and cholesterol oxidation (Park and Addis, 1987; DeVore, 1988); cholesterol oxidation is recognized as a crucial step in atherosclerosis development (Kolata, 1988). In fact, Addis (1990) presented evidence suggesting that lipid oxidation products (dietary and/or formed in vivo) may be involved in the initiation, progession and termination phases of atherosclerosis.

Lipid oxidation is usually termed an "autocatalytic reaction" because it is a reaction whose rate increases with time, due to the formation of products which themselves catalyze the reaction (Lundberg, 1962). On the basis of kinetic and chemical observations, a free-radical chain

mechanism has been commonly accepted to explain the mode of autoxidation (Lundberg, 1962; Gray, 1978; Karel, 1980; Lillard, 1985, Hammond, 1986):

Initiation:

$RH \longrightarrow R\cdot + H\cdot$

$ROOH \longrightarrow$ free radicals ($R\cdot$, $RO\cdot$, $RO_2\cdot$, $\cdot OH$, etc.)

$ROOR \longrightarrow 2RO\cdot$

$(ROOH)_2 \longrightarrow$ free radicals

Propagation:

$R\cdot + O_2 \longrightarrow RO_2\cdot$

$RO_2\cdot + RH \longrightarrow R\cdot + ROOH$

Termination: [to stable (non-radical) end products]

$R\cdot + R\cdot \longrightarrow RR$

$R\cdot + RO_2\cdot \longrightarrow ROOR$

$RO_2\cdot + RO_2\cdot \longrightarrow ROOR + O_2$

Heat, light, transition metals and oxygen are regarded as promoters of the initiation step. Polyunsaturated fatty acids (PUFAs) with ≥2 unsaturated sites are the most susceptible to oxidative attack. PUFAs exist in greater proportions in phospholipids than in triglycerides. Researchers analyzing lipid class changes during oxidation have observed losses in phospholipid content with concomitant increases in lipid oxidation products (Keller and Kinsella, 1973; Rauch and Kas, 1983; Salih et al., 1989). Though total muscle is composed of

≤2% phospholipids, they are considered to be most responsible for meat oxidative rancidity (Wilson et al., 1976; Igene and Pearson, 1979; Igene et al., 1980).

Hydroperoxides (ROOH) are created in the propagation step as fatty acids are oxidatively deteriorating, and have been designated as the primary products of lipid oxidation. Hydroperoxides then undergo decomposition to form volatile and non-volatile secondary products. It is the volatile secondary products that are responsible for degradative flavor changes in food quality. (Figure 1)

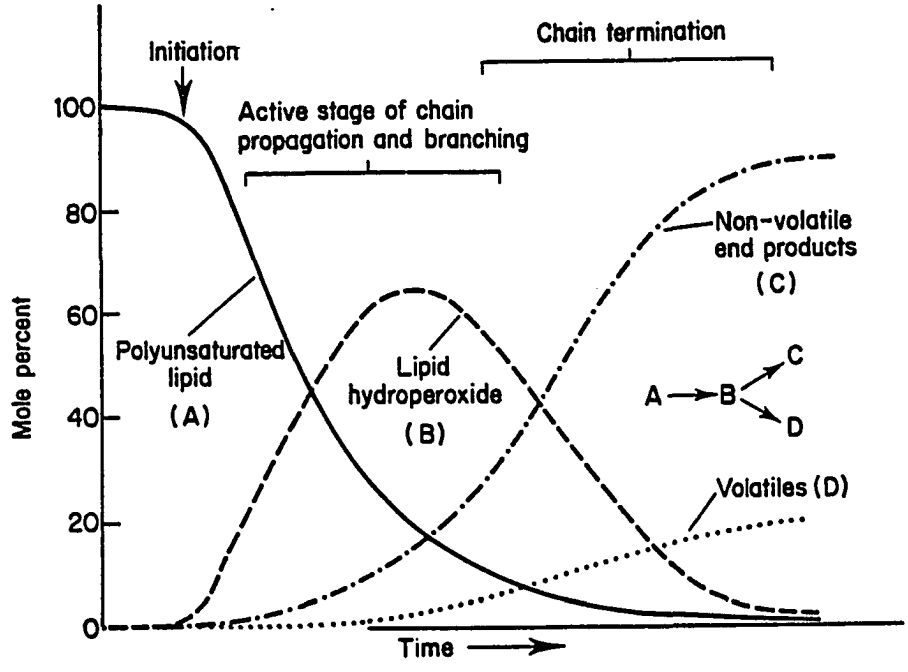

Figure 1. Lipid oxidation cycle of polyunsaturated lipids demonstrating reaction stages and product formation with time (Gardner, 1983)

Pure hydroperoxides are essentially stable at 40°C and lower temperatures, but catalysts such as transition metals attack the HOO- group. This leads to homolytic cleavage of the oxygen-oxygen bond or oxygen-hydrogen bond to yield alkoxy and hydroxy radicals or peroxy and hydrogen radicals (Frankel, 1980, 1984; Miyashita and Takagi, 1986; Grosch, 1987). Saturated and unsaturated aldehydes, alkanes, alkenes and alcohols are formed in the process, some of which contribute to the off-flavors. Unsaturated entities may undergo further oxidation. (Figure 2)

A more detailed review of the literature concerning endogenous and exogenous aspects of meat lipid oxidation has previously been completed (Wanous, 1986) and the reader is referred to that reference for additional information.

Meat Lipid Oxidation Assessment

2-Thiobarbituric acid test

The 2-thiobarbituric Acid (TBA) test is the most widely used assay for measuring the extent of lipid oxidative deterioration in muscle foods (Melton, 1983; Melton, 1985; St. Angelo et al., 1988; Hwang et al., 1990). Its use in assessing lipid oxidation has been reported for other fields, such as oil analysis (Das and Pereira, 1990) and medical/biochemical research (Janero and Burghardt, 1989) as well. Of the three main variations of the 2-thiobarbituric

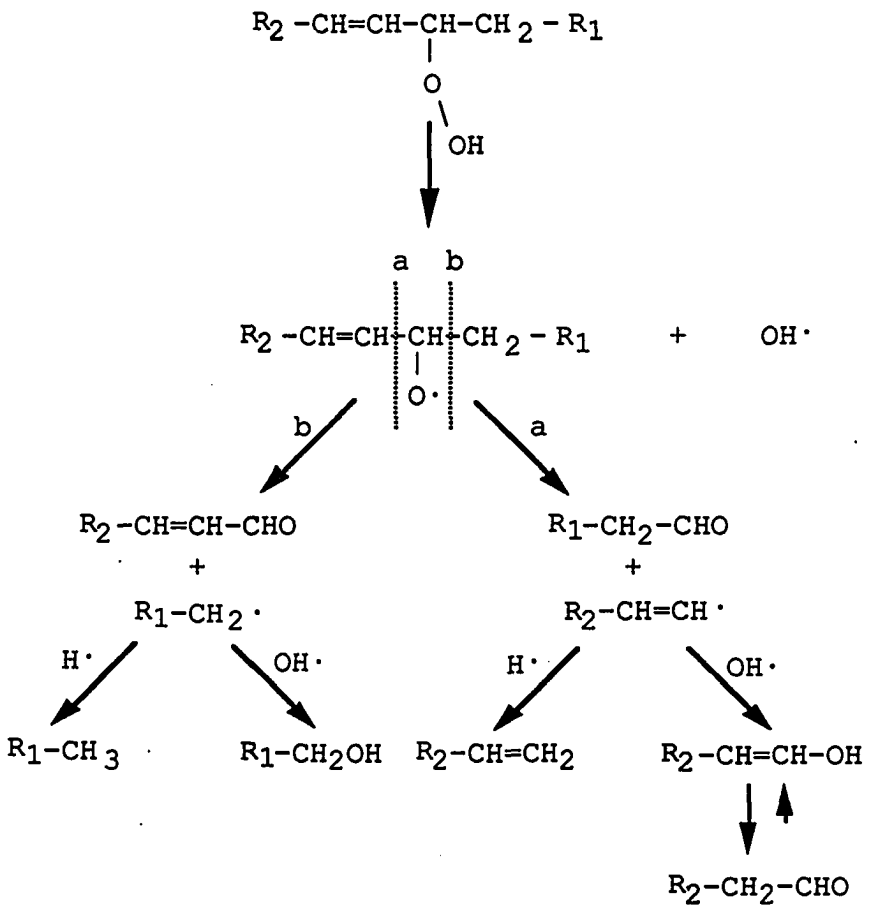

Figure 2. Reaction scheme for the homolytic cleavage of monohydroperoxides into secondary oxidation products (Frankel, 1982; Frankel, 1984)

acid test performed on muscle foods (distillate of the food, whole food followed by extraction, or extract of the food), the distillation method of Tarladgis et al. (1960) is the more popular method (Rhee, 1978).

The method of Tarladgis et al. (1960) involves blending and mixing a 10-g meat sample in distilled water and subjecting it to an acid-heat treatment (hydrochloric acid) on

distillation apparatus to collect a 50-ml distillate. TBA reagent, prepared from the addition of 2-thiobarbituric acid to 90% glacial acetic acid, is added (5 ml) to a 5-ml sample of the distillate in a capped test tube, immersed in a boiling water bath for a 35-min period and then cooled. This reaction forms a pink colored complex that is analyzed spectrophotometrically, usually at 532 nm.

Modifications to Tarladgis et al. (1960) TBA test

TBA solvent In the original procedure (Tarladgis et al., 1960), TBA reagent was prepared with 90% glacial acetic acid. Tarladgis et al. (1962, 1964) reported that substitution of water for acetic acid as the TBA solvent in the method of Tarladgis et al. (1960) resulted in greater absorbance readings at 532nm for TBA-malondialdehyde complexes and minimized absorbances at 450nm. Crackel et al. (1988) reported that use of the aqueous TBA reagent lowered the absorbance (and therefore concentration of "interfering products" at their maximum absorbance) at 450 nm. Other workers have reported the substitution of water as the TBA solvent when performing the method of Tarladgis et al. (1960) (Ang and Hamm, 1986).

Despite the fact that acetic acid is very odorous, considered to be corrosive, and skin contact may cause symptoms of mild irritation to severe tissue destruction

(Lenga, 1985), the method of Tarladgis et al. (1960) is still widely used in its unmodified form. St. Angelo et al. (1987) analyzed several variations of the TBA test for meat lipid oxidation analysis; their method of choice was the original method of Tarladgis et al. (1960) because of clarity in the distillate and its content of several aldehydes for TBA reactivity.

Antioxidant addition Researchers have reported that some malonaldehyde detected by the TBA test arises from the acid-catalyzed or thermal decomposition of hydroperoxides during the TBA analysis (Pryor et al., 1976, Kim and LaBella, 1987). Several researchers have advocated the addition of antioxidants/chelators in an attempt to control further oxidation during the TBA distillation of Tarladgis et al. (1960) (Moerck and Ball, 1974; Rhee, 1978, Yamauchi et al., 1982; Pikul et al., 1983). Rhee (1978) added propyl gallate and EDTA to meat samples prior to the blending step of the distillation TBA test; no significant effect was seen on beef, pork and chicken, but TBA reactivity was suppressed for fish. Crackel et al. (1988) added TBHQ prior to blending and heat distillation; no effect was found on beef but TBA numbers were significantly lower for fish and chicken.

TBA reactive substances (TBARS)

The TBA test was originally based on the premise that malondialdehyde (MDA), a three carbon dialdehyde [$(CHO)_2CH_2$], was a foremost secondary product of lipid oxidation. Researchers throughout the years determined that malondialdehyde and TBA form a 1:2 MDA:TBA complex (Sinnhuber et al., 1958; Nair and Turner, 1984; Yu et al., 1986). Both 1,1,3,3-tetraethoxypropane (TEP) (proposed by Sinnhuber and Yu, 1958) and 1,1,3,3-tetramethoxypropane (TMP) (Siu and Draper, 1978; Pikul et al., 1983) are hydrolyzable precursors of malondialdehyde and are used to react with TBA to derive standard concentration curves.

However, researchers concluded relatively early that MDA was only one of the TBARS (Kwon et al., 1965). Kwon et al. (1965) indicated that TBARS from oxidized lipids and foods have many properties very similar to those of malondialdehyde, and hence the reactions of malondialdehyde may be very close to those of TBARS. In heating autoxidized linoleic acid at 80°C (176°F), Pryor et al. (1976) estimated that 10% of the TBA-reactive material was malondialdehyde. Addis et al. (1983) advised that the TBA test be used to measure the general extent of lipid oxidation, and not be an attempt to quantify malondialdehyde specifically, since numerous substances are known to be involved with the TBA reaction.

More light has been shed on this question in recent years. Hexanal has been extensively utilized as an analytical tool to monitor lipid oxidation because of its relative high concentration (Wu and Sheldon, 1988; Yasuhara and Shibamoto, 1989; Hwang et al., 1990; King and Boyd, 1991). TBA values have been significantly correlated with gas chromatographic analyses of hexanal (Shahidi et al., 1987b; Wu and Sheldon, 1988). However, a revelation occurred when St. Angelo et al. (1987), utilizing gas chromatography/mass spectrometry (GC/MS), found that the aldehyde composition in TBA distillate of raw, freshly cooked, cooked and refrigerated ground meat paralleled that of aqueous meat extracts in many ways, to include relative concentrations. Therefore, a host of lipid oxidative products are available for reaction with TBA in the distillation method.

Correlations with sensory evaluation

Labuza (1971) stated that although sensory evaluation is the final test in research of lipid oxidation in food products, it could only be used qualitatively; chemical methods of rancidity measurement afford a more easily determined index of rancidity. High correlations have been calculated between sensory panel warmed-over flavor scores and the distillation TBA test (Younathan et al., 1980; Igene et al., 1985; Wu and Sheldon, 1988; Mann et al., 1989). Of the

measurements used to determine lipid oxidation in meat, the TBA test is the one that has been widely correlated with oxidative rancidity (Melton, 1985).

Advantages of the TBA test

As a comparative method, the TBA test offers numerous benefits which have contributed to its usefulness as an analytical tool: (1) equipment requirements are minimal, (2) because lipid extraction is not required, exposure to harmful (and costly) organic solvents is limited, and sample preparation is minimal, (3) time requirements for the analysis are relatively short and multiple samples can easily be analyzed simultaneously, (4) results are relatively easy to interpret and (5) it has been successfully correlated with taste panel results by several researchers.

Sodium Tripolyphosphate in Meats

Functions

Polyphosphates serve several mechanistic functions, such as: acting as buffers, serving as anionic polyelectrolytes, increasing the ionic strength of solutions and sequestering cations (Shimp, 1981). As functional ingredients in meat processing, polyphosphates increase both the amount of water bound and the strength of the meat particle-particle binding in processed meat products (Trout and Schmidt, 1983).

Antioxidative abilities of polyphosphates in meats have been known for several years (Tims and Watts, 1958); they have been considered for their antimicrobial properties as well (Sofos, 1986; Knabel et al., 1991). Polyphosphte rate of addition to meat systems is ≤0.50% w/w. Sodium tripolyphosphate (STPP) is the most widely used phosphate in the meat industry (Shimp, 1981; Anonymous, 1987).

Antioxidative effectiveness

When added to meat systems prior to cooking, STPP has consistently been observed to exhibit antioxidative effects (Sato and Hegarty, 1971; Matlock et al., 1984; Smith et al., 1984; Ang and Hamm, 1986; Shahidi et al., 1986; Huffman et al., 1987; Roozen, 1987; Shahidi et al., 1987a, 1987b; Yun et al., 1987; King and Earl, 1988; Ang and Young, 1989; Mann et al., 1989; Raharjo et al., 1989; Kolodziejska et al. 1990).

Some researchers have also reported antioxidative capabilities of STPP during storage of raw meat products (Marion and Forsythe, 1962; Lamkey et al., 1986; Choi et al., 1987; Molins et al., 1987; King and Earl, 1988; Ang and Young, 1989; Liu et al., 1991). However, other researchers have witnessed no STPP antioxidative effectiveness in uncooked meat products (Schwartz and Mandigo, 1976; Smith et al., 1984; Miller et al., 1986; McNeill et al., 1988; Patel et al., 1988; Boling et al., 1990; Decker and Crum, 1991).

Antioxidative Mechanisms

Chelation of transition metals has traditionally been accepted as the major antioxidative mechanism of polyphosphates. However, research exploring other potential mechanisms, as well as consideration of the antioxidative activity of other phosphoric acid derivatives, suggest that other mechanisms may also be involved. Some of these mechanisms are categorized below.

Chelation of transition metals In foods, the main catalysts of homolytic hydroperoxide decomposition are transition metal ions (Gardner, 1975). Metal-catalyzed hydroperoxide decomposition has been observed at room temperature (Frankel et al., 1987). Chain initiation in transition metal-catalyzed autoxidation is due to the decomposition of a complex formed between the metal ion and the lipid hydroperoxides (Betts and Uri, 1966; Black, 1978). Saidia and Hammond (1989) found Fe(II), and Cu(II) especially, to accelerate the rate of hydroperoxide decomposition. These findings have led to the conclusion that the most important suppressors of autoxidation initiation are the metal inactivators that coordinate with metal ions (Frankel, 1980).

Phosphoric acid derivatives extend the shelf life of lipid-containing foods because of their chelating properties (Gordon, 1990). In model aqueous emulsions of linoleic acid,

Allen et al. (1979) observed that metal-catalyzed oxidation was substantially slower in phosphate than in other buffers. Phytic acid ($C_6H_6[OPO(OH)_2]_6$) has been shown to form an iron chelate that inhibits iron-catalyzed radical formation lipid peroxidation (Empson et al., 1991). In a pH range typical for meat products (pH 5-6), STPP can sequester approximately 12.5%-11% of its weight in ferric (III) iron (Irani and Morgenthaler, 1963). Translated into common usage levels, at a level of 0.30% in meat, STPP theoretically could chelate a maximum of 345ppm of iron; iron content for meat has typically been reported as ≤35 ppm (Igene et al., 1979; Schricker, 1982; Tay et al., 1983).

However, chelation as a sole antioxidative mechanism has been questioned. Lee et al. (1983) concluded that metals were suspected not to be essential for the observed decomposition of hydroperoxides by phospholipids. Schmedes and Holmer (1989) added desferal, a potent iron chelator, to a solution of methyl linolenate hydroperoxide prior to heating for TBARS evaluation and observed that even at the highest concentrations, desferral could not inhibit more than approximately 65% of the hydroperoxide cleavage; their conclusions were that either the chelated iron still served as a catalyst, or a certain amount of degradation occurred even without the presence of transition metals. Recognizing that phospholipids have traditionally been considered to possess

secondary antioxidant characteristics due to their metal chelating ability, Gordon (1990) cited other work suggesting that metal complexing was not an important mechanism. Dziedzic and Hudson (1984) stated their studies have shown that the concentrations of phosphotidylethanolamine required to exhibit significant synergism are of a much higher molarity than would be needed to interact with the few parts per million at which transition metals naturally occur in fats and oils.

Though chelation is important, the antioxidative activity of phosphoric acid derivatives may potentially rely on a combination of mechanisms, as will be discussed below.

Hydroperoxide interaction In his review, Frankel (1980) stated that peroxide destroyers are a type of preventative antioxidant that react with hydroperoxides to give stable products by nonradical processes and provided two phosphoric acid derivatives, phosphites and phosphines, as examples able to reduce hydroperoxides into the more oxidatively stable alcohols (Frankel, 1980). This reduction in the amount of carbonyls from hydroperoxide decomposition by reduction of the alkoxy radicals (RO·) to alcohols was also suggested by Saidia and Hammond (1989).

Phospholipids have been shown to demonstrate anitioxidant capabilities (Lee et al., 1983; Dziedzic and Hudson 1984; Husain et al., 1986). Citing work suggesting metal

complexing was not an important phospholipid mechanism, Gordon (1990) speculated that phospholipids act by releasing protons that could bring about rapid hydroperoxide decomposition without free radical formation and/or could regenerate primary antioxidants.

Another type of hydroperoxide interaction has been proposed. Privett and Quackenbush (1954) investigated the reaction of phosphoric acid with lard at 75°C and noted a decreased recovery of unreacted phosphoric acid over time; they concluded that phosphoric acid, and its derivatives, are capable of direct reaction with hydroperoxides under heat to form products (insoluble, presumably polymeric, phosphoric-containing substances) that allow fats to absorb large amounts of oxygen without accumulating substantial titratable peroxides.

Specific polyphosphate involvement in these types of reactions in food systems has not been identified.

Free fatty acid interaction Oxidation begins while the fatty acid is still part of the intact phospholipid, but evidence indicates that there also is a breakdown of the phospholipid molecule, releasing free fatty acids (Willemot et al., 1985). Free fatty acids oxidize at a greater rate than their corresponding esters or alcohols (Miyashita and Takagi, 1986; Kim and LaBella, 1987) and subsequent hydroperoxides of free fatty acids decompose faster than those from methyl

esters (Saidia and Hammond, 1989). Free fatty acids are also responsible for accelerating the oxidation rate of esters and the decomposition rate of resulting hydroperoxides; carboxyl groups are thought to catalyze free radical formation through hydroperoxide decomposition in the initial stages of autoxidation (Miyashita and Takagi, 1986).

Researchers have observed that cooking muscle tissue results in greater concentrations of free fatty acids than in uncooked muscle; the fact that this increase corresponds with large decreases in phospholipid PUFAs has led to the conclusion that free fatty acids are important intermediates in the oxidative degradation of polar lipids (Yamauchi, 1973; Willemot et al., 1985).

Sherman (1961) put forth the idea that alkaline phosphates interact with free fatty acids to form what was termed as "soap"; this saponification reaction was said to be promoted when free fatty acids were present in high concentration. Increased "soapy" flavor has been associated and monitored with the addition of polyphosphates to processed meat products (Craig et al., 1990; Schantz and Bowers, 1990). Wanous (1990) added water-dissolved STPP to soybean oil at the same STPP/lipid ratio as 0.15% STPP would exist in ground meat containing 15% fat; after thorough mixing with a stirbar and overnight incubation at ambient temperature, a white, interfacial, soapy-appearing foam was observed between the

water and lipid phases. This is an area that warrants further investigation.

Ionic strength contribution Ang and Hamm (1986) reported that salt and STPP were more effective than STPP alone in controlling oxidation accumulation rate, suggesting a synergism between salt and STPP. Increasing ionic strength has been suggested to decrease the rate of lipid oxidation (McDonald and Hultin, 1987; Ang and Young 1989). Lowering of nonheme iron content that occurs during cooking is one benefit that has been identified with increased ionic strength (Ang and Young, 1989).

Enhanced protein and water interaction Phosphates are known to enhance protein extraction and improve water holding capacity of muscle tissue (Paterson et al., 1988).

Research has demonstrated that the volatile alkenals and alkanals associated with warmed-over flavor bind with the muscle proteins, myosin and actin (Gutheil et al., 1990). Carnosine, a skeletal muscle dipeptide, has been identified as effectively inhibiting lipid oxidation in meats (Decker and Crum, 1991).

Water holding capacity, and therefore water content, in cooked meat products is enhanced when formulated with STPP. Water has been reported to offer a protective effect on the decomposition of hydroperoxides through hydration of metal

ions and the solvation of hydroperoxides with water (Chen, 1989, 1990).

pH increase Ande (1985) concluded that the prevention of lipid oxidation in meat by STPP was linked to its ability to increase meat pH. However, Ang and Young (1989) concluded that the oxidative stability of precooked meat with STPP was due to its metal-chelating abilities more than its influence on pH.

Research Objectives

Overall goals of this research were to: (1) determine the reactivity of TBA with major meat lipid oxidation aldehyde groups at primary and alternate wavelengths, via the method of Tarladgis et al. (1960), (2) evaluate STPP for interference with the distillation TBA test of lipid oxidation assessment of raw and cooked muscle, (3) determine concentration-related effectiveness of STPP as an antioxidant in comminuted muscle systems.

Explanation of the Dissertation Format

The alternate format was followed in the organization of this dissertation. A general introduction, pertinent to the whole document is included at the beginning. Four individual manuscripts suitable for publication are enclosed, and are individually designated as Part I, Part II, Part III and Part IV; tables and figures for each manuscript are situated after

the respective results and discussion section, immediately prior to the conclusions section, and are presented in the order mentioned. A general summary, general conclusions, and a list of literature cited are at the end of the dissertation.

PART I.

EVALUATION OF ALDEHYDE CLASSES FOR REACTIVITY WITH THE 2-THIOBARBITURIC ACID TEST FOR MEAT LIPID OXIDATION ASSESSMENT

Evaluation of aldehyde classes for reactivity with the 2-thiobarbituric acid test for meat lipid oxidation assessment

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Running Title: Aldehydes and Meat TBA Test

ABSTRACT

Malonaldialdehyde, hexanal and 2,4-hexadienal were reacted with 2-thiobarbituric acid (TBA) using water or 90% glacial acetic acid, according to the methods established by Tarladgis et al. (1960, 1962, 1964) for meat lipid oxidation analysis. In addition to 532nm, absorbances were also monitored at 495nm and 455nm. TBA solvent had minimal effect on malondialdehyde absorbances which increased linearly at both 532nm and 495nm with concentration. Hexanal and 2,4-hexadienal absorbances increased at all three wavelengths with concentration. Hexanal absorbance predominated at 455nm; acid-based 2,4-hexadienal absorbed the greatest at 495nm whereas 532nm was the dominant wavelength for the water-based 2,4-alkadienal. Therefore, the TBA test for meat lipid oxidation monitors the major aldehyde groups associated with meat lipid oxidation.

INTRODUCTION

The distillation 2-thiobarbituric acid (TBA) test described by Tarladgis et al. (1960) is commonly used to assess meat lipid oxidation. Because the test was designed on the premise that malondialdehyde was a prominent secondary lipid oxidation product, it has been judged as lacking specificity because of its reactivity with other products. Addis et al. (1983) advised that the TBA test be used to measure the general extent of lipid oxidation, and not be an attempt to quantify malondialdehdye specifically.

St. Angelo et al. (1987) analyzed distillates of raw, fresh-cooked and warmed-over meat obtained from the method described by Tarladgis et al. (1960) utilizing gas chromatography and mass spectrometry (GC/MS). Their results demonstrated that major constituents of the distillate included alkanals, 2,4-alkadienals, ketones, alkenals and alkanes in similar proportions to aqueous extracts analyzed via GC/MS. Hexanal and 2,4-decadienal have been determined to be the meat lipid oxidation aldehydes present in the highest concentration (St. Angelo et al., 1987; Yasuhara and Shibamoto, 1989; Hwang et al., 1990).

Under varying combinations of TBA solvent (acid versus water), heating temperature and heating time, researchers have analyzed alkanals, 2,4-alkadienals, alkenals and

malondialdehyde for their reactivity with 2-thiobarbituric acid, and have determined their corresponding identifying wavelengths (Marcuse and Johansson, 1973; Kosugi and Kikugawa, 1985; Kosugi et al., 1988). Because of these multiwavelength absorbances, the TBA test has been regarded as a useful tool for estimating a broad range of lipid peroxidation products (Kosugi et al., 1989).

The primary goal of this work was to determine the relative reactivities of hexanal, 2,4-hexadienal and malondialdehyde with 2-thiobarbituric acid (as commonly used to assess meat lipid oxidation) at primary and secondary absorbing wavelengths, comparing acetic acid (Tarladgis et al.,1960) and deionized water (Tarladgis et al., 1962, 1964) as the TBA solvents.

MATERIALS AND METHODS

Preparation of Standards

TBA reactive substances (TBARS) standards

Malondialdehyde Malondialdehyde standard curves were derived from solutions of 1,1,3,3-tetraethyoxypropane (TEP) (Sigma, St. Louis, MO) and designed to range in concentration from 2.0×10^{-10} to 1.0×10^{-6} moles/ml; prepared samples were incubated with either water- or acid-based TBA solutions and analyzed at 532nm and 495nm. The 495nm/532nm ratios were calculated directly from absorbances at the two wavelengths for each data point.

Hexanal, 2,4-hexadienal Reactivity with TBA by other lipid oxidation products was tested with calibration curves utilizing solutions of hexanal (Sigma, St. Louis, MO) and 2,4-hexadienal (Aldrich, Milwaukee, WI) representing alkanals and 2,4-alkadienals, respectively. Solutions were prepared in dilutions from 1.0×10^{-7} to 1.0×10^{-6} moles/ml, incubated with either water- or acid-based TBA solutions and analyzed for TBARS at 532nm, 495nm and 455nm.

2-Thiobarbituric Acid (TBA) Analysis

Reaction with TBA

For comparison, solutions of 0.02M 2-thiobarbituric acid were prepared utilizing one of two different solvents: 90%

glacial acetic acid ("acid-based") as presented by Tarladgis et al. (1960) or water ("water-based") as recommended by other researchers (Tarladgis et al., 1962, 1964; Crackel et al., 1988). Aliquots of 5.0 ml of standard solution were added to 5.0 ml of either water-based or acid-based 0.02M TBA solution in test tubes and tightly capped. The capped test tubes were then incubated in a 95°C water bath for 35 minutes to promote color formation. Immediately after heating, the test tubes were cooled in tap water in preparation for spectrophotometric analysis.

Spectrophotometric analysis Samples were then analyzed for 2-thiobarbituric acid reacting substances (TBARS) on a GILFORD Response® UV-VIS Spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH) equipped with a rapid sampler accessory set for a sample time of 0.5 secs and an equilibration time of 2 secs. Wavelength scans with bandwidth set at 1.0 nm and scan increment of 0.5 nm were utilized to verify wavelength peaks. Absorbance readings were then performed at the wavelengths specified under the above descriptions for the standards preparations. Similar solvent-based (water- vs. acid-based) treatments were analyzed together. Blanks based upon the appropriate solvent were utilized at the beginning of each solvent family analysis.

Data Analysis and Presentation

Absorbance readings were plotted, and regression equations and coefficients determined, utilizing DeltaGraph, version 1.5, (DeltaPoint, Monterrey, CA) for the Apple® Macintosh™ microcomputer (Apple® Computer Inc., Cupertino, CA).

RESULTS AND DISCUSSION

Standards Evaluations

Malondialdehyde Analyses

Absorbances at 532nm and 495nm The standard curve using 1,1,3,3-TEP as a malondialdehyde (MDA) precursor is presented in Figure 1. TBARS concentration-dependent absorbances at the traditional 532nm, and at 495nm, were linear for both wavelengths with regression coefficents of 1.000 for all derived equations. As evidenced by the minimal divergence between different TBA solvent standard curves for like wavelengths, solvent choice exercised minimal influence on absorbance readings. These results are in contrast to Crackel et al. (1988) who, using fewer data points (5) and analyzing to an absorbance endpoint of 0.30, demonstrated an almost immediate divergence between the two TBA solvents utilizing tetramethoxypropane as the standard. Kosugi and Kikugawa (1985) observed water-based malondialdehyde TBARS absorbances approximately 4 times as large as the acid-based TBARS.

495nm/532nm absorbance ratios The relative importance of the 495nm absorbance to that of the 532nm was determined to detect any potential concentration interactions. The relative proportions of absorbances at 495nm to those at 532nm for each sample concentration are depicted in Figure 2. Except for

minor deviations at the low concentrations, a relatively constant relationship existed between these two wavelengths for malondialdehyde. Also interesting is the observation that the 495nm/532nm ratios were lower for the acid-based than for the water-based TBA solutions. The average ratio for water-based 495nm/532nm was 0.2563; for acid-based 495nm/532nm, 0.2229. Because malondialdehyde represents a system of unambiguous, "noise-free" TBARS reactivity relative to meat samples, monitoring of this ratio may be indicative of the amount of "pure" TBARS and/or state of oxidation.

Alkanal and 2,4-alkadienal analyses

The hexanal curves are in Figures 3 and 4; those for 2,4-hexadienal are in Figures 5 and 6. Hexanal and 2,4-hexadienal were used (as representatives of the alkanals and alkadienals) to assess their influence on primary (532nm) and secondary (495nm, 455nm) TBARS wavelengths, as influenced by TBA solvent and aldehyde concentration. Though alkanals and 2,4-alkadienals react differently with TBA, it has been confirmed that individual members of these classes behave similarly (Marcuse and Johansson, 1973).

Hexanal Many researchers have reported hexanal as being the most prevalent product arising from meat lipid oxidation (Wu and Sheldon, 1988; Yasuhara and Shibamoto, 1989; Hwang et al., 1990). As seen in Figures 3 and 4, its highest

absorbance with both water-based and acid-based TBA is at the alternate wavelength of 455nm. Absorbances measured at 455nm were similar to those obtained for raw and cooked pork (Parts II, III and IV of this dissertation). Calibration curves for hexanal were not linear; Kosugi and Kikugawa (1985) observed that the relationship between hexanal concentration and glacial acetic acid-based 455nm TBARS wasn't linear until after a 5-hour reaction period at 100°C.

Choice of TBA solvent substantially influenced absorbance yields for all three wavelengths, with the acid-based treatment yielding approximately twice the absorbance of the water-based equivalent. All TBA/hexanal solutions initially were clear, but turned to light yellow after heating. When concentrations greater than 10^{-6} moles/ml were analyzed, the water-based TBA-hexanal solution turned very cloudy, whereas acid-based solutions retained their clarity. Solvent choice had an interesting influence on wavelength absorbance predominance after 455nm. Water-based 495nm TBARS yielded higher absorbances than did those for 532nm; the converse was true for acid-based TBARS.

Hexanal was much less reactive with TBA than with MDA. In spite of hexanal concentrations equal to 50 times that of MDA, absorbances of MDA were 20 to 30 times those of hexanal. High expression at 450nm and low expression at 530nm by hexanal in water-based TBA also was reported by Marcuse and

Johansson (1973); it appears, however, that greater absorbance of alkanals at 450nm can be promoted in favor of those at 532nm using lower temperature and longer heat times (Marcuse and Johansson, 1973). Changes with autooxidation time of acetic acid-based, TBARS absorbance values at 455nm have been shown to parallel those of peroxide values (Kosugi and Kikugawa, 1985).

2,4-Hexadienal A range of concentrations identical to those for hexanal were used for the 2,4-hexadienal solutions; the absorbances are presented in Figures 5 and 6 for water-based and acid-based TBA solutions, respectively. A nonlinear, concave absorbance relationship with concentration was established. Kosugi et al. (1988) also reported 2,4-alkadienal calibration curves to be concave and not linear.

A distinguishing TBA solvent effect is the much greater expression of 532nm TBARS as the prominent wavelength in the water-based solution (Figure 5). All water-based TBA/2,4-hexadienal solutions turned yellow upon contact, but changed to red after heat application. Absorbances for 495nm and 455nm were quite similar for both solvents; 532nm absorbances were repressed by the acid-based solvent and were the lowest of the three wavelengths monitored for that solvent treatment, allowing 495nm to be the prominent wavelength.

St. Angelo et al. (1987) reported that 2,4-decadienal is a principal compound in both distillates and aqueous extracts

of meat analyzed for lipid oxidation. In evaluating 2,4-hexadienal, 2,4-nonadienal and 2,4-decadienal, Kosugi et al. (1988) noted similar patterns of absorbance at 532nm except that reactivity increased with increasing carbon number. Marcuse and Johansson (1973) reported considerable reactivity of 2,4-hexadienal in water TBA at 530nm and 450nm. Absorbance response by 2,4-alkadienals may be enhanced by the presence of other aldehydes and hydroperoxides (Kosugi et al., 1988).

Detection of absorbances by 2,4-alkadienals at all three wavelengths agrees with Kosugi and Kikugawa (1985) who reported that glacial acetic acid-based TBARS at 455nm and 532nm were lower than at 495nm; acid-based 455nm and 532nm absorbances were also reported to be much less than those for the alkanals and malondialdehyde, respectively. In water-based TBA, the 2,4-alkadienal TBARS at 455nm and 495nm contributed to absorbance but the peaks were not as well defined (Kosugi and Kikugawa, 1985).

The 2,4-hexadienal absorbances at 495nm and 532nm were as much as 20 to 40 times higher than those of hexanal. Kosugi et al. (1988) listed hexanal absorbance at 532nm to be "extremely low" relative to malondialdehyde or even the 2,4-alkadienals. The absorbance range for 2,4-hexadienal at 532nm in water-based TBA was similar to that for MDA, but at approximately a 50 times greater concentration. Kosugi et al. (1988) reported 2,4-alkadienals to yield 532nm aqueous-acetic

acid-based TBARS absorbances at the rate of 1/10-1/20 of that of malondialdehyde.

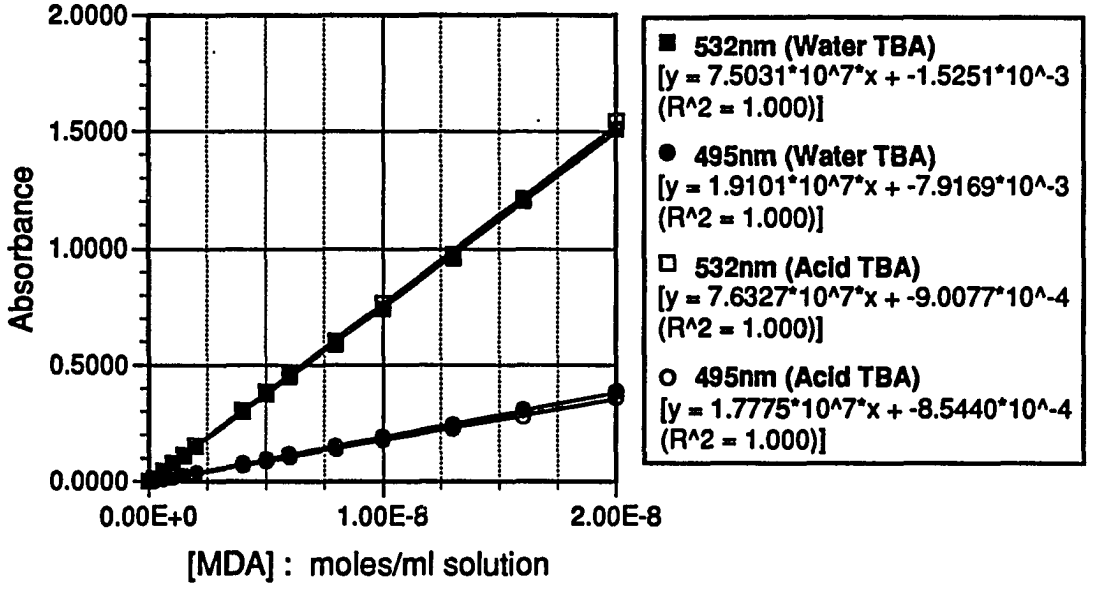

Figure 1. TBARS standard curve of 1,1,3,3-tetraethoxypropane in solution at 532nm and 495nm, utilizing water and acid as TBA solvents

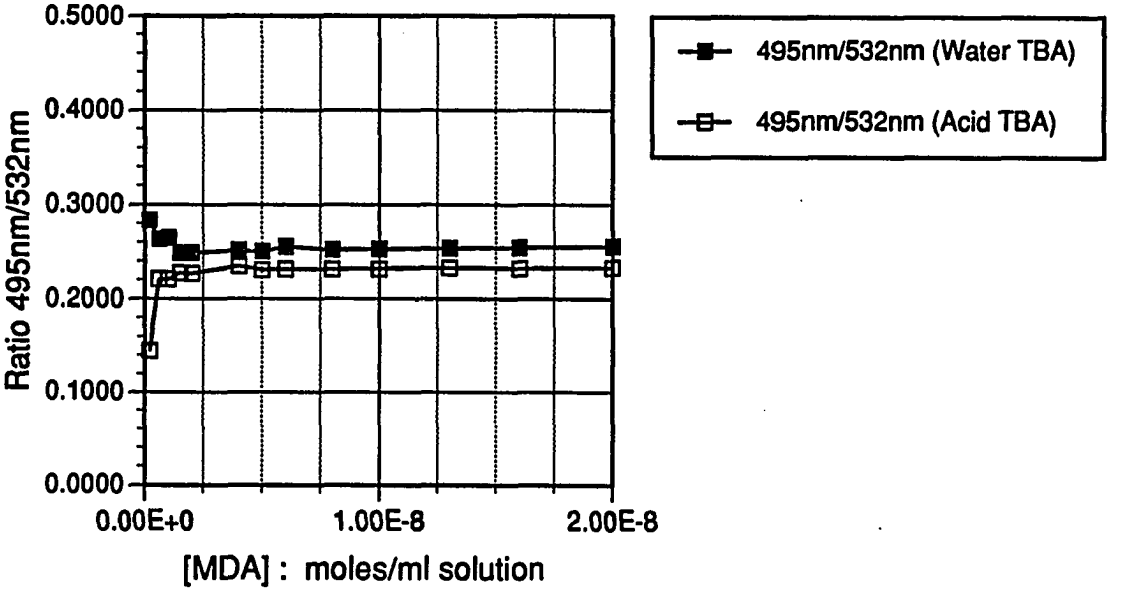

Figure 2. TBARS 495nm/532nm absorbance ratios for 1,1,3,3-tetraethoxypropane

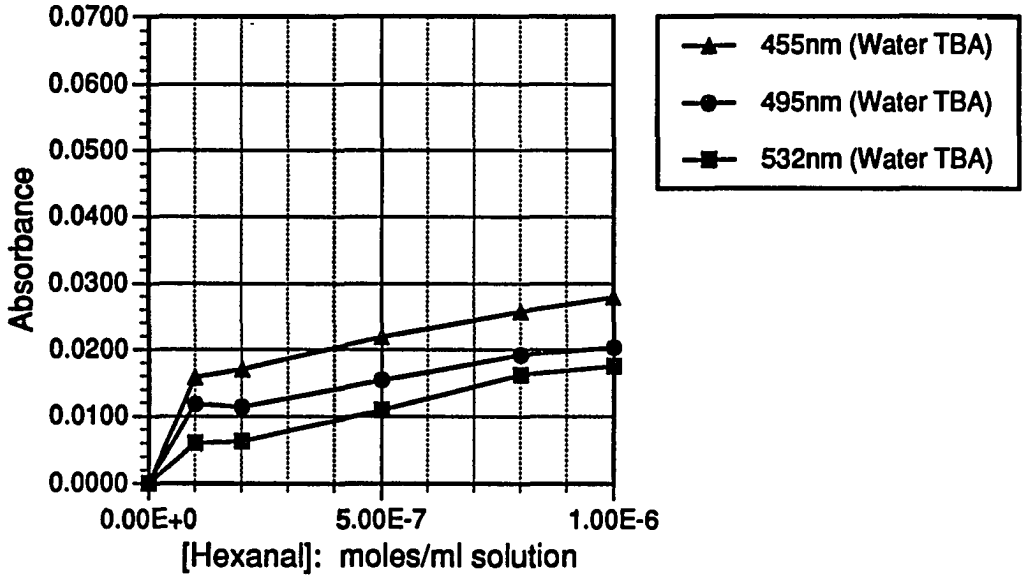

Figure 3. Concentration-related absorbance curves of hexanal *water-based* TBARS at 532nm, 495nm and 455nm

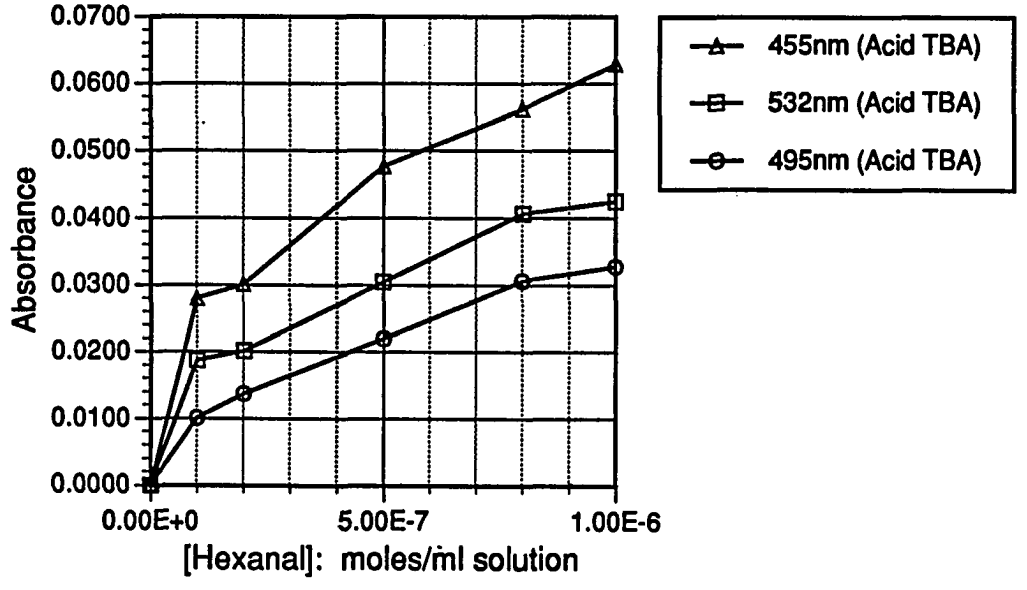

Figure 4. Concentration-related absorbance curves of hexanal *acid-based* TBARS at 532nm, 495nm and 455nm

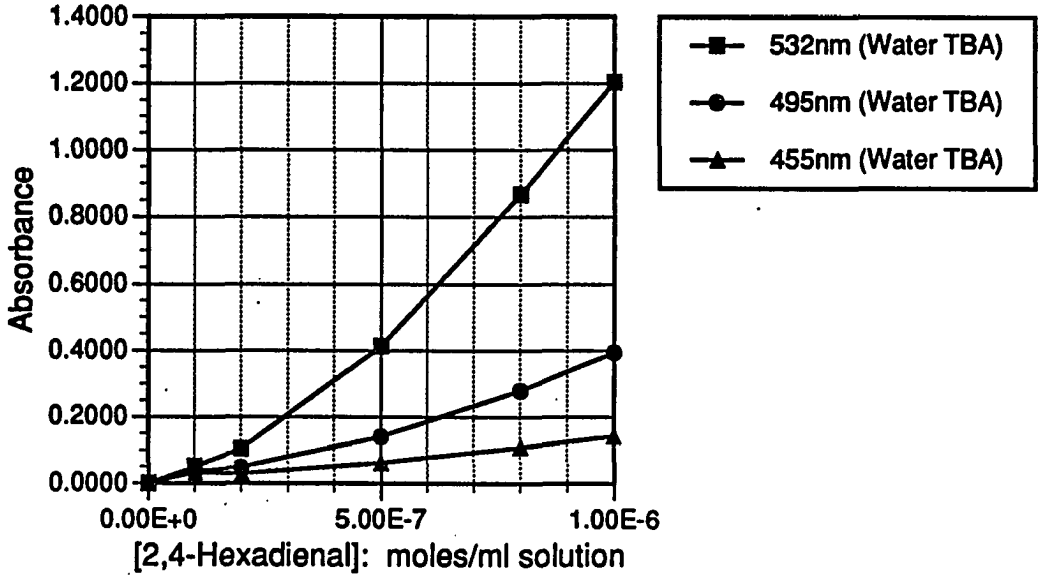

Figure 5. Concentration-related absorbance curves of 2,4-hexadienal *water-based* TBARS at 532nm, 495nm and 455nm

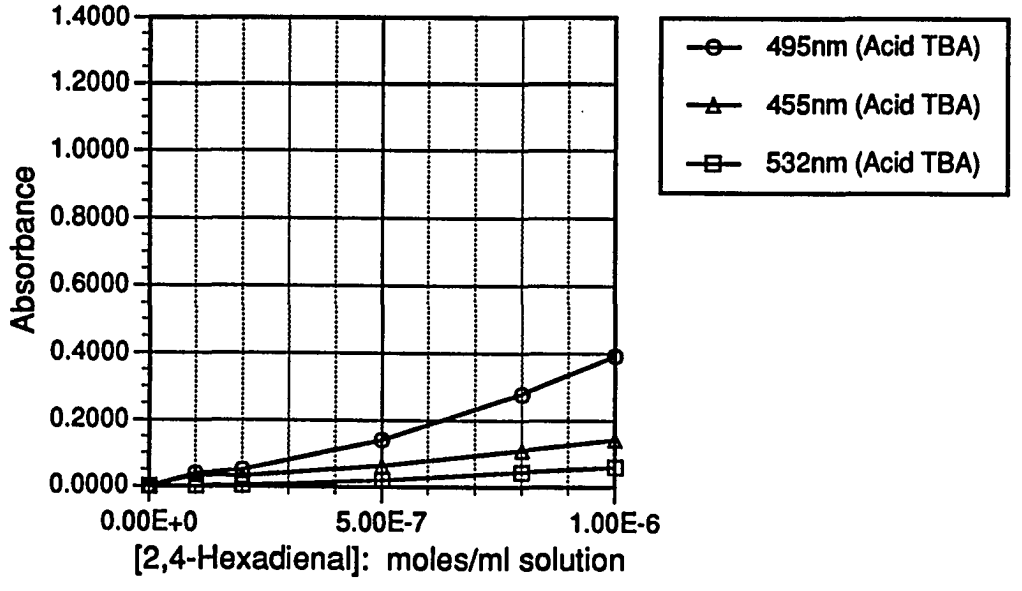

Figure 6. Concentration-related absorbance curves of 2,4-hexadienal *acid-based* TBARS at 532nm, 495nm and 455nm

CONCLUSIONS

Assessment of lipid oxidation at 455nm and 495nm, in addition to 532nm, offers much promise for monitoring the progess of specific lipid oxidation products. However, further work needs to be done to better assess their utility in meat systems. More research also needs to be done to determine optimum combinations of solvent choice, heating temperature and heating time to yield the most information on specifically desired aldehyde classes of lipid oxidation products.

REFERENCES

Addis, P. B., Csallany, A. S., and Kindom, S. E. 1983. Some lipid oxidation products as xenobiotics. In *Xenobiotics in Foods and Feeds*, D. E. Schwass and J. W. Finley (Ed.), p. 85. ACS Symp. Series No. 234. American Chemical Society, Washington, D.C.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M. and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28: 187.

Hwang, S.-Y., Bowers, J. A. and Kropf, D. H. 1990. Flavor, texture, color and hexanal and TBA values of frozen cooked beef packaged in modified atmosphere. J. Food Sci. 55(1): 27.

Kosugi, H. and Kikugawa, K. 1985. Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid. Lipids 20(12): 915.

Kosugi, H. and Kikugawa, K. 1986. Reaction of thiobarbituric acid with saturated aldehydes. Lipids 21(9): 537.

Kosugi, H., Kato, T. and Kikugawa, K. 1988. Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid oxidation. Lipids 23(11): 1024.

Kosugi, H., Kojima, T. and Kikugawa, K. 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24(10): 873.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50(10): 387.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C., Jr. and Dupuy, H. P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. J. Food Sci. 52(5): 1163.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. R., Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., Pearson, A. M. and Dugan, L. R., Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39(1): 34.

Tarladgis, B. G., Pearson, A. M. and Dugan, L. R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Wu, T. C. and Sheldon, B. W. 1988. Flavor components and factors associated with the development of off-flavors in cooked turkey rolls. J. Food Sci. 53(1): 49.

Yasuhara, A. and Shibamoto, T. 1989. Analysis of aldehydes and ketones in the headspace of heated pork fat. J. Food Sci. 54(6): 1471.

PART II.

ASSESSMENT OF SODIUM TRIPOLYPHOSPHATE INFLUENCE ON THE 2-THIOBARBITURIC ACID TEST FOR EVALUATING LIPID OXIDATION IN RAW COMMINUTED MUSCLE FOODS

Assessment of sodium tripolyphosphate influence on the 2-thiobarbituric acid test for evaluating lipid oxidation in raw comminuted muscle foods

by

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Running Title: STPP and TBA Test of Raw Meat

ABSTRACT

Sodium tripolyphosphate interference on the distillation 2-thiobarbituric acid test (TBA) was assessed during 14 days refrigerated storage (1°C) of raw ground pork. After selected periods, samples were removed and levels of sodium tripolyphosphate (0%, 0.01%, 0.05%, 0.10%, 0.15%, 0.25%, 0.35% 0.50%) were blended with the raw pork prior to distillation. Water and acetic acid were compared as TBA solvents. STPP exercised no influence ($P>0.05$) on the TBA reactive substances at 532nm, 495nm or 455nm. Highest correlations were between adjacent wavelengths. Lipid oxidation increased at a first order rate with time. The use of wavelength absorbance ratios (WARS) may be useful in estimating meat lipid oxidative status.

INTRODUCTION

Polyphosphates are utilized in the meat processing industry as multifunctional food ingredients. Their antioxidative abilities in controlling meat lipid decomposition, and therefore extending shelf-life through delay of off-flavor development, have been known for several years (Tims and Watts, 1958).

The 2-thiobarbituric Acid (TBA) test is the most widely used assay for measuring the extent of lipid oxidative deterioration in muscle foods (Melton, 1983, 1985; Hwang et al., 1990). Several researchers have reported on the antioxidative effectiveness of polyphosphates in raw meat products based upon results from the TBA method according to Tarladgis et al. (1960) (Lamkey et al., 1986; Miller et al., 1986; Molins et al., 1987; King and Earl, 1988; Patel et al., 1988; Boling et al., 1990).

When evaluating fresh meat treatments for polyphosphate antioxidative properties, some researchers have observed unexplainable differences between polyphosphate treatments and controls at "Time 0." These observations have led some researchers to speculate on potential interference by polyphosphate with the TBA test of Tarladgis et al. (1960), resulting in underestimation of lipid oxidative deterioration (Molins et al., 1987; Raharjo et al., 1989).

Hydroperoxides are primary products of lipid oxidation; the aldehydic secondary products from hydroperoxide decomposition are responsible for off-flavors associated with oxidative rancidity. Catalysts of this decomposition include transition metals, a supply of which exists endogenously in muscle systems (Igene et al., 1979; Schricker, 1982; Tay et al., 1983).

Researchers have reported that some thiobarbituric acid reactive substances (TBARS) arise from the acid-catalyzed or thermal decomposition of hydroperoxides during the actual TBA analysis (Pryor et al., 1976, Kim and LaBella, 1987). Though heat promotes this degradative reaction, metal-catalyzed hydroperoxide decomposition has been experimentally conducted at room temperature (Frankel et al., 1987).

The metal-chelating properties of polyphosphates, and other phosphoric acid derivatives, is well known (Irani and Morgenthaler, 1963; Gordon, 1990). Researchers have reported the repressive effects chelators such as desferal (Schmedes and Holmer, 1989) and EDTA (Kosugi et al., 1991) have demonstrated on TBA results.

In a typical lipid oxidation cycle, a buildup of hydroperoxides occurs as polyunsaturated fatty acids decompose; hydroperoxides decompose in turn, leading to the buildup of the volatile and non-volatile oxidative end products (Gardner 1983, 1987). Metal catalysis and metal

chelation would be expected to exercise varying influences according to which point in the oxidative cycle that stress (such as heat associated with distillation for the TBA test) was exerted.

In the original TBA method of Tarladgis et al. (1960), 90% glacial acetic acid was recommended as the TBA solvent. Tarladgis et al. (1962, 1964) recommended substitution of deionized water for acetic acid as the solvent to promote greater absorbance readings at the predominant wavelength of 532nm and simultaneously minimize "interfering" absorbances at 450-455nm; this recommendation has received recent attention and endorsement by Crackel et al. (1988).

Distillate from the TBA procedure has been analyzed utilizing gas chromatography/mass spectrometry (GC/MS); its constituents included hexanal and 2,4-decadienal as the major aldehydes, though other alkanals, 2,4-alkadienals, ketones, alkenals and alkanes existed in relative proportions similar to aqueous GC/MS extracts (St. Angelo et al., 1987). Under varying combinations of TBA solvent (acid versus water), heating temperature and heating time, researchers have analyzed alkanals, 2,4-alkadienals, alkenals and malondialdehyde for their reactivity with 2-thiobarbituric acid, and have determined their corresponding identifying wavelengths (Marcuse and Johansson, 1973; Kosugi and Kikugawa, 1985; Kosugi et al., 1988). Because of these multiwavelength

absorbances, the TBA test has been regarded as a potentially useful tool for estimating a broad range of lipid peroxidation products (Kosugi et al., 1989).

Objectives of this research were to: (1) analyze raw comminuted pork during a 2-week refrigerated storage period to assess sodium tripolyphosphate (STPP) effects on the TBA test at various points of the lipid oxidation cycle, (2) monitor TBARS mixture as affected by storage and/or STPP by investigating TBARS absorbances at the primary wavelength of 532nm, as well as at 495nm and 455nm, utilizing the commonly used TBA procedures for assessment of meat lipid oxidation, (3) analyze effects of both TBA solvents (water and acid) on TBARS, and (4) calculate the rate of lipid oxidation for fresh pork during 2 weeks of refrigerated storage as measured by the TBA test.

MATERIALS AND METHODS

Sample Preparation

Raw material selection

Fresh, boneless pork picnic shoulders obtained from the Iowa State University (ISU) Meats Laboratory were closely trimmed of external fat, cut into strips for grinding, vacuum-packaged to limit oxygen exposure and stored in a covered box kept in a cooler at 1°C for 1-2 days prior to grinding.

Meat handling and processing

Comminution On Day 0 of experimentation, the meat strips were removed from storage and subjected to two grindings, first through a 9.52-mm (3/8-in) plate followed by a 3.18-mm (1/8-in) plate. Hand-mixing was performed after the first grinding, and again after the second grinding to promote homogeneity. Half of the ground meat was then formed into approximately 2-cm-thick patties and subsequently placed into oxygen-permeable plastic bags which were then sealed; the bags were placed in covered cardboard boxes and stored in a cooler maintained at 1°C. (The other half of the sample was utilized the same day for a companion study involving cooked pork.)

STPP addition On the day of experimentation (Day 0), and at Day 4, Day 7 and Day 14 of storage, sealed bags of patties were arbitrarily selected and removed (without

replacement) from storage for sodium tripolyphosphate (STPP) addition and subsequent lipid oxidation analysis.

Representative samples of 10 g each were selected from randomly chosen pattie locations and weighed into small plastic weighing dishes which were placed on trays of crushed ice to minimize pre-analysis temperature abuse. Preweighed amounts of sodium tripolyphosphate (STPP), designed for addition to the 10-g meat samples at the rate of 0.00%, 0.01%, 0.05%, 0.10%, 0.15%, 0.25%, 0.35%, and 0.50% (w/w) were dissolved in 1.0 ml of deionized distilled water and added in random order to the meat samples. Thorough mixing of the STPP solutions and meat samples in the weighing dishes was performed for 1 min using stainless steel laboratory weighing spatulas.

Analyses

Product Composition

Representative meat samples were analyzed for fat and moisture utilizing a CEM Moisture/Solids Analyzer, Model AVC 80 equipped with 100-g balance capacity and automatic extraction system (Matthews, NC). A representative meat sample from each replication was first thoroughly comminuted to a paste-like texture utilizing a Cuisinart Basic Food Processor (Greenwich, CT). A sample size of 4 g was used for the combination moisture/fat analyses. The CEM analyzer was

programmed selecting "Mode M=3" for alternating meat moisture/fat analysis. CEM analyzer moisture parameters were power=90% and time=4:00 min; fat parameters were power=100% and time=3:30 min.

2-Thiobarbituric acid (TBA) analysis

Sample distillation After mixing with STPP, the samples were prepared as described by Tarladgis et al. (1960) for distillation, with the exception that the mixed 10-g samples were transferred into 250-ml round-bottomed flasks, 96.5 ml deionized distilled water were added, followed by 2.5 ml 4N HCl. The 250-ml round-bottomed flasks were then transferred for homogenization to a Kinematica Polytron PT 10/35 equipped with a PTA 20 shaft assembly (Brinkman Instruments Co., Westbury, NY) at a speed setting of "6" for 30 sec. After homogenization, a few drops of antifoam C and glass boiling beads were added prior to randomized placement of the flasks onto distillation units. Heat was applied stepwise uniformly to all treatment samples. Approximately 25-30 min were required for 50 ml of distillate which was collected into 50-ml Erlenmeyer flasks, capped with parafilm, and stored in the dark at 1°C until analyzed within 24 hr.

Reaction with TBA For comparison, solutions of 0.02M 2-Thiobarbituric acid were prepared utilizing one of two different solvents: 90% glacial acetic acid ("acid-based") as

presented by Tarladgis et al. (1960) or deionized water ("water-based") as recommended by other researchers (Tarladgis et al., 1962, 1964; Crackel et al., 1988). After removal from ≤24 hr storage and prior to uncapping, the 50-ml Erlenmeyer flasks were shaken/swirled to ensure homogenization of distillate prior to sample distillate extraction. Two aliquots of 5.0 ml of distillate were extracted; 1 aliquot was added to 5.0 ml of water-based and the other to 5.0 ml of acid-based 0.02M TBA solutions in test tubes and tightly capped. The capped test tubes were then incubated in a 95°C water bath for 35 min to promote color formation. Immediately after water bath removal, the test tubes were subjected to a cold water rinse in preparation for spectrophotometric analysis.

Spectrophotometric analysis Samples were analyzed for 2-thiobarbituric acid reacting substances (TBARS) on a GILFORD Response® UV-VIS Spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH) equipped with a rapid sampler accessory set for a sample time of 0.5 secs and an equilibration time of 2 secs. Wavelength scans with bandwidth set at 1.0 nm and scan increment of 0.5 nm were utilized to verify wavelength peaks. Absorbance readings were then performed at the selected wavelengths of 455nm, 495nm and 532nm. Similar solvent-based (water- vs. acid-based) STPP treatments were analyzed together; order of solvent-based analyses varied with

each replication. Blanks based upon the appropriate solvent were utilized at the beginning of each solvent family analysis.

Statistical analysis

Experimental design was based on a split,split-plot format (Cochran and Cox, 1957; Steel and Torrie, 1980). Main plots were the storage periods (in days), subdivided into the different STPP levels which, in turn, were subdivided into type of solvent-based TBA reagent (water vs. acid). Three replications were performed; duplicates were taken for every reading and averaged to yield one data figure used for analysis. TBARS absorbance readings were statistically analyzed without any mathematical conversion. Data analysis was performed utilizing the Statistical Analysis System, version 6.06.01, (SAS Institute Inc., Cary, NC). Significance between treatment and subtreatment means was tested using the least significant difference (LSD) procedure, but recognized only if a treatment significant F test resulted [Fisher's (protected) lsd] (Steel and Torrie, 1980). Standard errors and coefficients of variability were calculated from SAS results (Cochran and Cox, 1957;Steel and Torrie, 1980). All statistics for absorbance ratios (495nm/532nm, 455nm/532nm and 455nm/495nm) were calculated from the total model (versus data point wavelength averages); regression equations and

coefficients derived for TBARS formation rates presented in the legend tables of plotted averages were calculated from the total model.

RESULTS AND DISCUSSION

Raw Material Composition

Average fat and water content of the ground pork utilized in this study was 8.05% and 70.01%, respectively.

STPP Effect on 532nm TBARS

A primary objective was to explore potential STPP (sodium tripolyposphate) interference upon the 532nm TBARS (2-thiobarbituric acid reactive substances) measurement as speculated in the literature for raw comminuted muscle. Evaluation of potential STPP interactions with level of oxidation (affected by refrigerated storage) and with TBA solvent [deionized water vs. 90% glacial acetic acid; Tarladgis et al. (1960, 1962, 1964)] were also of interest.

Figures 1 and 2 graphically depict this data for the water-based and acid-based 532nm measurements, respectively, for the range of STPP concentrations at each day of analysis throughout the 14-day refrigerated storage period. Results are presented for the individual solvent classes; no significant STPP level*TBA solvent interaction ($P>0.05$) was detected but solvent differences existed and will be discussed later. Storage effects will also be specifically addressed in a later section.

Water-based 532nm STPP had no significant effect ($P>0.05$) on water-based, 532nm TBARS absorbances during the

analyzed oxidation period (Table 1). Minimal variation between STPP levels was demonstrated for Day 0 and Day 4 (Figure 1). No significant STPP level*storage interaction ($P>0.05$) existed, but the repressive trend on 532nm TBARS by STPP concentrations ≥0.15% at Day 14 (also present but less pronounced at Day 7) warrants further investigation into potential interference at higher oxidation levels of raw meats.

Acid-based 532nm. STPP did not significantly ($P>0.05$) alter acid-based, 532nm TBARS absorbances (Table 2). All acid-based absorbances throughout the tested oxidation period were lower than their water-based counterparts (Table 1). No significant STPP level*storage interaction ($P>0.05$) existed, but graphical depiction of the data (Figure 2) again indicated a slight pattern of absorbance decreases at later stages of oxidation with greater STPP levels.

TBARS Evaluations at 532nm, 495nm & 455nm

A variety of aldehydic lipid oxidation products exists in the distillate obtained via the TBA method of Tarladgis et al. (1960) (St. Angelo et al., 1987). Specific analysis conditions for their detection (TBA solvent, incubation time and temperature, wavelength) have been investigated (Marcuse and Johansson, 1973; Kosugi and Kikugawa, 1985, 1986; Kosugi et al., 1988).

TBA reactivity of the major distillate-containing aldehyde classes under conditions used for meat lipid oxidation assessment (Tarladgis et al., 1960), has been demonstrated at 455nm, 495nm and 532nm (Part I of this dissertation). These wavelengths were monitored in the present study. However, because of contributions by aldehyde classes to all three wavelengths under the conditions of this evaluation (Part I of this dissertation), it is difficult to attribute wavelength absorbance fluctuations to a single class. Therefore, absorbance comparisons among wavelengths were derived and analyzed from individual sample averages (as opposed to overall average absorbances) as 495nm/532nm, 455nm/532nm and 455nm/495nm wavelength absorbance ratios (WARS) and were monitored for changes under the various experimental conditions. A related approach was taken by Igene et al. (1985), who added together total absorbances of oxidized meat samples at 532nm and 450nm, and then presented individual wavelength absorbance percentages.

STPP Effects

STPP concentration effects on all wavelengths and absorbance ratios, averaged over all storage periods, are presented for water-based TBARS (Table 3, Figure 3) and for acid-based TBARS (Table 4, Figure 4). No STPP level*TBA solvent interactions existed ($P>0.05$), but solvent-related

differences did exist (Table 3, 4); differences will be specifically addressed in the section on solvent effects.

Water-based TBARS As stated earlier, no STPP effects were noted on the water-based 532nm absorbances (P>0.05). A slight trend at 532nm with increasing STPP level was probably influenced by the higher oxidation levels at Day 14 (Figure 1) where a similar trend was observed.

STPP exhibited no significant effect (P>0.05) on water-based 495nm or 455nm TBARS, nor did it affect the 455nm/532nm absorbance ratios (P>0.05) (Table 3). Changes in the 495nm/532nm and 455nm/495nm ratios with STPP level approached significance (P=0.08 & P=0.07, respectively); changes in the 495nm/532nm ratios appeared to be concentration related, but those for 455nm/495nm did not. Of the three wavelengths monitored, the coefficient of variability (C.V.; standard deviation relative to its average) was slightly higher for the 455nm absorbances.

Acid-based TBARS STPP level exhibited no influence on acid-based TBARS absorbances at any of the wavelengths (P>0.05) (Table 4, Figure 4), though STPP effects on the 495nm/532nm ratio did approach significance (P=0.06).

A significant STPP level*storage interaction (P≤0.05) did exist for the 495nm/532nm ratios at Day 0, demonstrating a trend in which the ratio at 0.50% STPP was higher than at the

other STPP levels. This trend was TBA solvent-dependent and was not seen in the water-based equivalent.

Graphic representation of the data (Figure 4) suggests an effect by STPP of lowered absorbances at 532nm with increased STPP levels, though this effect was not significant ($P>0.05$).

The coefficient of variability (C.V.) was slightly higher for the 455nm absorbance; all acid-based C.V.s were greater than the water-based data catagories, indicating more variability among the acid-based TBARS values.

Refrigerated storage effects

Wavelength absorbances Most dramatic were the significant increases for absorbance values at both 532nm and 495nm between Day 7 and Day 14 (Tables 5 and 6, Figures 5 and 6). Though peroxide values were not specifically determined, this observation would theoretically signify an increased rate of hydroperoxide formation and subsequent decomposition into secondary lipid oxidation products.

532nm Length of storage had significant effects on absorbances at 532nm ($P<0.05$; water- and acid-based) (Tables 5 and 6). Results for the two TBA solvents paralleled each other (Figure 5). Analysis of these values over the entire experimental model (versus storage day averages) yielded a significant ($P<0.0001$) first order logarithmic increase

(Labuza, 1982) in lipid oxidation over time, with regression coefficients of approximately 0.82 (Figure 6).

495nm Storage significantly increased water-based 495nm absorbances ($P<0.05$); the acid-based counterparts approached significance ($P=0.06$) (Tables 5 and 6, Figure 7). Analysis of values from the entire experimental determined a significant logarithmic effect ($P<0.0001$) of 495nm absorbance increases with time, with regression coefficients approximately of ≥ 0.62 (Figure 8). The solvent-related values approximately paralleled each other throughout the experiment except for an unexplainable aberration at Day 4.

455nm A nonsignificant ($P>0.05$) increase with time occurred for both water- and acid-based 455nm TBARS.

Overall comparison Relative absorbance increases among the three wavelengths over time are presented both in a linear format (Figure 9) and semilogarithmic plot (Figure 10). First order log relationships are typical for some oxidative rancidity reactions (Labuza, 1982). This semilogarithmic relationship agrees with the findings of Ozilgen and Ozilgen (1990), who calculated a logarithmic dependence of lipid oxidation value on linear storage time for several reports on lipid oxidation data in the literature.

Absorbance increases at 532nm, the primary wavelength, overshadowed those at 495nm, and especially those at 455nm in the linear plot (Figure 9). In the semilogarithmic plot

(Figure 10), however, the lines for the 532nm and 495nm wavelengths nearly paralleled each other.

Table 7 includes relative rates of formation based on slopes of the rate-forming equations shown in Figures 10 and 13. For both 532nm and 495nm, acid-based values during storage generally were less than the water-based equivalents (Table 8); however, the actual rate of formation of the acid-based TBARS was slightly greater than for the water-based TBARS at both wavelengths (Table 7).

Wavelength absorbance ratios (WARS) WARS are based on the differential at which the wavelength absorbances increase with advancing lipid oxidation (i.e., 532nm>495nm>455nm).

495nm/532nm Time-related increases with the acid-based absorbance ratio approached significance ($P=0.06$, Table 6, Figure 11). Decreases in this ratio are indicative of the faster rate of 532nm TBARS accumulation relative to those at 495nm (Figures 9 and 10).

455nm/532nm Storage time had a significant effect on the decrease of 455nm/532nm absorbance ratios for both solvent types ($P<0.01$, water-based; $P<0.001$, acid-based) (Tables 5 and 6, Figure 12). A regression procedure performed on the total model yielded a significant logarithmic decrease ($P<0.0001$, $R^2 \geq 0.750$) of these values over storage time (Figure 13). This decrease was due to the rapid increase of 532nm TBARS relative to 455nm.

455nm/495nm Decreases of this ratio due to length of storage were highly significant ($P<0.001$, water-based; $P<0.01$, acid-based) (Tables 5 and 6, Figure 11). A semilogarithmic decrease over time was also calculated for this ratio (Figure 14). Storage-related changes of this ratio are compared with those of 495nm/532nm (Figure 11) and 455nm/532nm (Figure 12); the 455nm/532nm curve closely parallels the 455nm/495nm curve but 495nm/532nm converges with it after extended storage/oxidation, especially for the water-based ratios (Figure 11). Of all the WARS from both solvent groups (Tables 5 and 6), the water-based 455nm/495nm ratio had the lowest standard error and coefficient of variability.

TBA solvent, TBA solvent*Day effects

532nm, 495nm & 455nm Water as the TBA solvent resulted in significantly greater average absorbances at 532nm ($P<0.0001$) and 495nm ($P<0.0001$) (Table 8). Acid TBA solvent favored greater 455nm absorbances ($P<0.01$) (Table 8) and is in agreement with results reported by Crackel et al. (1988).

Significant TBA solvent*Day of storage interactions occurred for 532nm ($P<0.0001$), 495nm ($P<0.0001$) and 455nm ($P<0.01$) (Table 8; Figures 5, 7 and 9). In general, the greatest day-related differences between solvent types for 532nm and 495nm absorbances existed at Day 7 and Day 14 as lipid oxidation was significantly increasing. The proportion

of distillate constituents affected by solvent choice probably remained similar but their quantities increased with oxidation, causing greater disparity between the different solvent-related absorbances.

Wavelength absorbance ratios (WARS) As a direct consequence of significant solvent effects on the wavelength absorbances, all three WARS were subject to differences attributed to TBA solvent (Table 9). As a general rule, all ratios decreased with increased oxidation (Table 9, Figures 11 and 12). The reason for this is apparent from Figure 9; relative rates of absorbance increases were 532nm>495nm>455nm and the numerator in each ratio was the lower wavelength of the two.

Significant TBA solvent*Day of storage effects also were observed for each of the WARS. The greatest difference due to solvent consistently was for Day 0, with the differences generally decreasing with advancing oxidation (Table 9, Figures 11 and 12).

495nm/532nm Water-based 495nm/532nm WARS were significantly greater than those for acid-based ($P<0.0001$). The TBA solvent*Day of storage interaction was significant at $P<0.0001$. Average water-based 495nm absorbances were approximately 40% as great as those for 532nm whereas this figure was approximately 37% for the acid-based ratio (Table 9).

Water TBA solvent boosts the 532nm absorbances for 2,4-alkadienals (Part I of this dissertation); apparently, relatively greater amounts of substances favoring the water solvent absorb at 495nm to allow the margin of the water-based 495nm/532nm ratio to occur between the two solvent choices.

455/532nm Acid-based 455nm/532nm absorbance ratios were significantly greater than were water-based ratios ($P<0.01$) (Table 9, Figure 12). The TBA solvent*Day of storage interaction was significant at $P<0.01$. Average water-based 455nm absorbances were approximately 30% as great as those for 532nm; those for acid-based TBARS were approximately 39% as great (Table 9).

455nm/495nm Acid-based TBA yielded significantly higher average ratios ($P<0.0001$) (Table 9, Figure 11). The TBA solvent*Day of storage interaction was significant at $P<0.0001$. Water-based absorbances for 455nm were approximately 70% as great as those for 495nm, but were about 101% in the acid TBA solvent.

One reason for the largest solvent differences at the onset for ratios with 455nm as the numerator is that early in oxidation, 495nm and 532nm were less prominent relative to 455nm, which has been reported to have higher absorbances in acid solvent (Crackel et al., 1988). Much higher absorbance readings for acid-based hexanal at 455nm also have been observed (Part I of this dissertation). As oxidation

progressed, 495nm and 532nm absorbances increased at a faster rate, decreasing the prominence of the 455nm absorbance in the ratio.

An interesting observation is the convergence of the 455nm/495nm and 495nm/532nm WARS with the progress of lipid oxidation. This convergence appears to be greater for the water-based ratios than for the acid-based ones (Table 9, Figure 11). The 455nm/495nm also appears to be very sensitive to changes in oxidative status; the 495nm/532nm curve was fairly stable with time, a characteristic which enhances its utility as a reference for comparison with the 455nm/495nm WARS. Comparison of these two ratios (455nm/495nm and 495nm/532nm) could be useful in determining if the lipids under analysis were at a rate of rapid increase in oxidation (ratios converged) or at a beginning phase of slower increase (ratios diverged).

Correlations of Wavelengths and Ratios

Correlations were calculated between the wavelengths and wavelength absorbance ratios to further evaluate relationships between these in raw meat lipid oxidation.

Water-based correlations

All correlations between water-based wavelengths and ratios were at least very highly significant at $P \leq 0.001$; the majority were significant at $P < 0.0001$ (Table 10). Correlations

were highest between adjacent wavelengths; the highest correlations existed between 532nm and 495nm ($r=0.9834$, $P<0.0001$), followed by that between 495nm and 455nm ($r=0.6513$, $P<0.0001$).

The observation that 455nm/495nm WARS had the highest ratio correlations with all three wavelengths, and was the only one that significantly correlated with 455nm (Table 10) bodes well for its use as a potential oxidation cycle indicator.

The 495nm/532nm WARS were the least correlated ratios with the wavelengths. Refering to Figure 11, this low correlation is verified by the stability of the 495nm/532nm curve with time.

Acid-based correlations

Most correlations between acid-based wavelengths and ratios were significant at least at $P\leq0.05$; the majority were very highly significant at $P<0.0001$ (Table 11). As with the water-based correlations, the highest correlations existed between 532nm and 495nm ($r=0.9834$, $P<0.0001$) and between 495nm and 455nm ($r=0.7492$, $P<0.0001$). The acid-based 495nm and 455nm correlation was higher than the corresponding water-based correlation (0.7492 vs. 0.6513).

Again, the 455nm/495nm WARS were the highest correlated ratios with 495nm and 455nm. The 495nm/532nm WARS were the least correlated ratios with the wavelengths. Though

relatively small, 455nm was correlated with 455nm/532nm ($P<0.05$, $R=|0.22|$), whereas it was not with the water-based TBARS.

Water-based vs. acid-based wavelength correlations

All wavelength absorbances were correlated and all were very highly statistically significant ($P<0.0001$) (Table 12). Correlations between 532nm acid and water approached 1.00; all combinations between solvent versions of 532nm and 495nm were also very correlated ($R\geq|0.91|$). An interesting observation is the relatively low correlation between the two 455nm versions ($r=0.66$), reflecting the strong TBA solvent effects at this wavelength.

Table 1. Effect of STPP addition and refrigerated storage on the measurement of 532nm *water-based* TBARS absorbance of raw comminuted pork[1,2,3]

STPP Level	Day 0	Day 4	Day 7	Day 14	Average
0.00%	0.0304**a**	0.0843**a**	0.1153**a**	0.3210**a**	0.1378**a**
0.01%	0.0304**a**	0.0894**a**	0.1253**a**	0.3317**a**	0.1442**a**
0.05%	0.0302**a**	0.0818**a**	0.1451**a**	0.3364**a**	0.1484**a**
0.10%	0.0296**a**	0.0890**a**	0.1358**a**	0.3287**a**	0.1458**a**
0.15%	0.0308**a**	0.0807**a**	0.1292**a**	0.3326**a**	0.1433**a**
0.25%	0.0353**a**	0.0820**a**	0.1239**a**	0.3074**a**	0.1372**a**
0.35%	0.0332**a**	0.0809**a**	0.1111**a**	0.3047**a**	0.1325**a**
0.50%	0.0345**a**	0.0794**a**	0.1235**a**	0.3094**a**	0.1367**a**
Avg.	0.0318	0.0834	0.1262	0.3215	0.1407
S.E.	0.0124	0.0124	0.0124	0.0124	0.0062
C.V.	47.58%	18.13%	11.99%	4.71%	10.75%

[1]N=3 observations per mean; N=12 per "Average" mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

[3]Abbreviations: Avg.= average, S.E.= standard error, C.V.= coefficient of variability.

Table 2. Effect of STPP addition and refrigerated storage on the measurement of 532nm *acid-based* TBARS absorbance of raw comminuted pork[1,2]

STPP Level	Day 0	Day 4	Day 7	Day 14	Average
0.00%	0.0244**a**	0.0763**a**	0.0938**a**	0.2788**a**	0.1183**a**
0.01%	0.0221**a**	0.0785**a**	0.1050**a**	0.2881**a**	0.1234**a**
0.05%	0.0219**a**	0.0778**a**	0.1208**a**	0.2929**a**	0.1284**a**
0.10%	0.0231**a**	0.0818**a**	0.1131**a**	0.2862**a**	0.1261**a**
0.15%	0.0226**a**	0.0738**a**	0.1088**a**	0.2886**a**	0.1235**a**
0.25%	0.0264**a**	0.0764**a**	0.1016**a**	0.2692**a**	0.1184**a**
0.35%	0.0256**a**	0.0745**a**	0.0938**a**	0.2646**a**	0.1146**a**
0.50%	0.0231**a**	0.0742**a**	0.1041**a**	0.2682**a**	0.1174**a**
Avg.	0.0237	0.0767	0.1051	0.2796	0.1213
S.E.	0.0110	0.0110	0.0110	0.0110	0.0055
C.V.	57.20%	17.65%	12.87%	4.84%	11.16%

[1]N=3 observations per mean; N=12 per "Average" mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

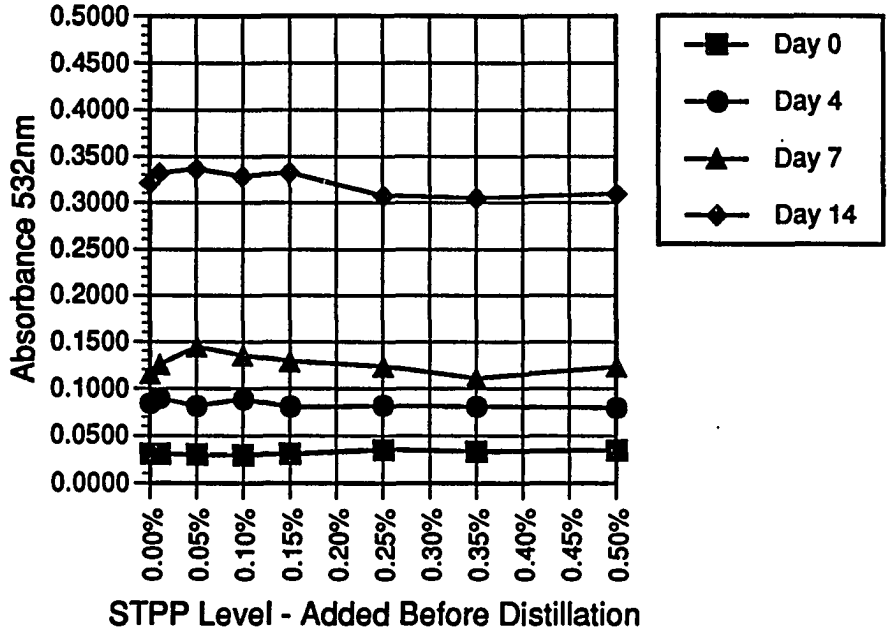

Figure 1. Effects of added STPP and refrigerated storage on 532nm *water-based* TBARS of raw comminuted pork .

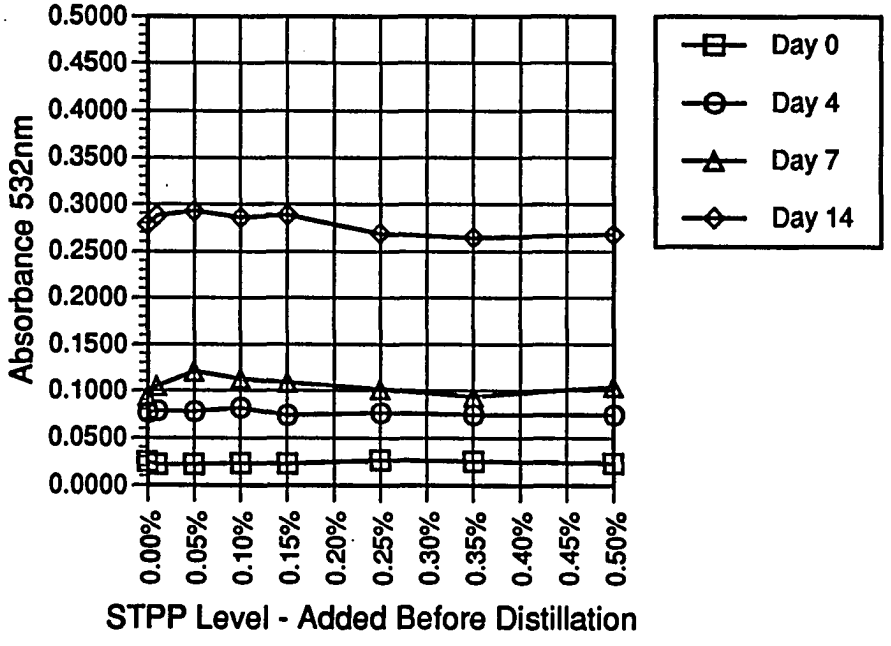

Figure 2. Effects of added STPP and refrigerated storage on 532nm *acid-based* TBARS of raw comminuted pork

Table 3. STPP effect on measurement of *water-based* TBARS absorbances and ratios of raw comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	0.1378a	0.0446a	0.0242a	0.3757a	0.2794a	0.6893a
0.01%	0.1442a	0.0477a	0.0248a	0.3960a	0.3118a	0.7123a
0.05%	0.1484a	0.0485a	0.0252a	0.3878a	0.2849a	0.6840a
0.10%	0.1458a	0.0486a	0.0222a	0.3949a	0.2904a	0.6769a
0.15%	0.1433a	0.0495a	0.0268a	0.4222a	0.3316a	0.7134a
0.25%	0.1372a	0.0479a	0.0248a	0.4159a	0.3022a	0.6720a
0.35%	0.1325a	0.0459a	0.0263a	0.4031a	0.3104a	0.7230a
0.50%	0.1367a	0.0473a	0.0258a	0.4131a	0.3119a	0.6965a
Avg.	0.1407	0.0475	0.0250	0.4011	0.3028	0.6965
S.E.	0.0062	0.0020	0.0017	0.0157	0.0202	0.0268
C.V.	10.75%	10.32%	16.98%	9.60%	16.34%	9.42%
Sig.				*(P=0.08)*		*(P=0.07)*

[1]N=12 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 4. STPP effect on measurement of *acid-based* TBARS absorbances and ratios of raw comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	0.1183**a**	0.0376**a**	0.0264**a**	0.3882**a**	0.3603**a**	0.8732**a**
0.01%	0.1234**a**	0.0368**a**	0.0284**a**	0.3414**a**	0.3691**a**	1.0708**a**
0.05%	0.1284**a**	0.0392**a**	0.0294**a**	0.3626**a**	0.3665**a**	0.9664**a**
0.10%	0.1261**a**	0.0399**a**	0.0254**a**	0.3739**a**	0.3746**a**	0.9515**a**
0.15%	0.1235**a**	0.0383**a**	0.0300**a**	0.3662**a**	0.4373**a**	1.1921**a**
0.25%	0.1184**a**	0.0375**a**	0.0272**a**	0.3681**a**	0.3877**a**	1.0250**a**
0.35%	0.1146**a**	0.0360**a**	0.0287**a**	0.3805**a**	0.4153**a**	1.0250**a**
0.50%	0.1174**a**	0.0381**a**	0.0269**a**	0.3995**a**	0.4226**a**	0.9762**a**
Avg.	0.1213	0.0379	0.0278	0.3725	0.3916	1.0094
S.E.	0.0055	0.0019	0.0025	0.0173	0.0418	0.0942
C.V.	11.16%	12.09%	22.47%	11.38%	26.13%	22.82%
Sig.				*(P=0.06)*		

[1]N=12 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

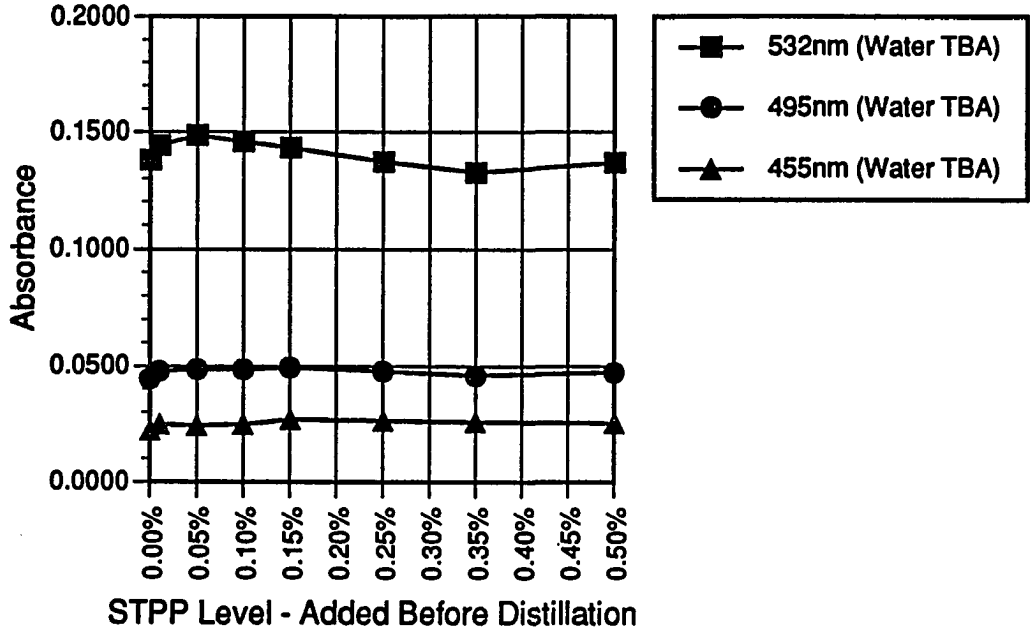

Figure 3. Average STPP level effects on 532nm, 495nm and 455nm *water-based* TBARS of raw comminuted pork

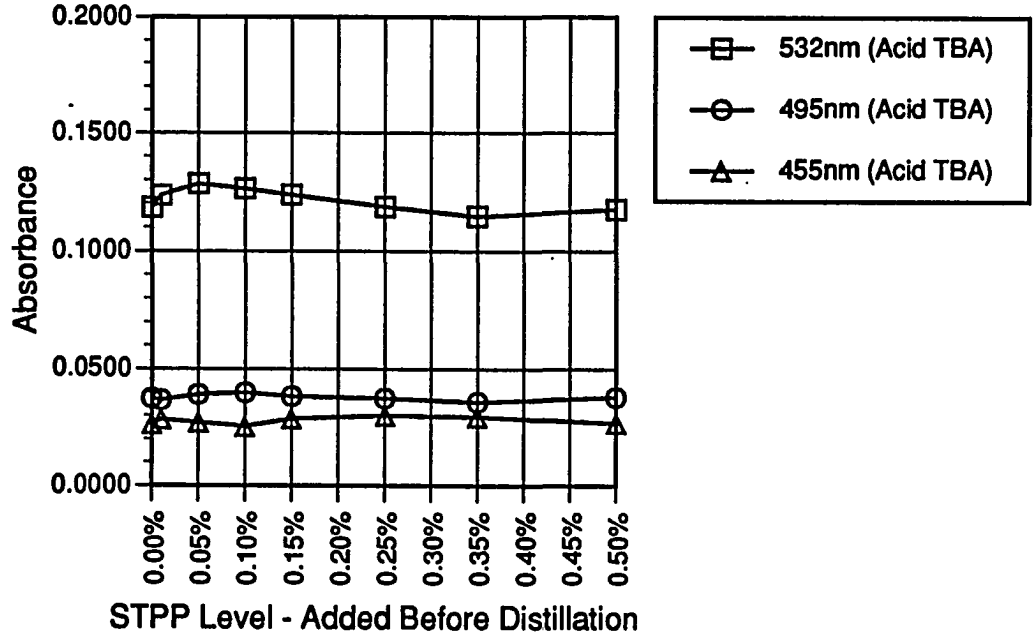

Figure 4. Average STPP level effects on 532nm, 495nm and 455nm *acid-based* TBARS of raw comminuted pork

Table 5. Refrigerated storage effect on *water-based* TBARS absorbances and ratios of raw comminuted pork[1,2]

Storage Day #	Wavelength 532nm	Wavelength 495nm	Wavelength 455nm	Wavelength Ratio 495nm/532nm	Wavelength Ratio 455nm/532nm	Wavelength Ratio 455nm/495nm
Day 0	0.0318**b**	0.0176**b**	0.0185**a**	0.5264**a**	0.5662**a**	1.1069**a**
Day 4	0.0834**b**	0.0340**b**	0.0272**a**	0.4184**a**	0.3482**b**	0.8054**b**
Day 7	0.1262**b**	0.0436**b**	0.0226**a**	0.3463**a**	0.1812**bc**	0.5146**c**
Day 14	0.3215**a**	0.0947**a**	0.0316**a**	0.3132**a**	0.1157**c**	0.3592**c**
Avg.	0.1407	0.0475	0.0250	0.4011	0.3028	0.6965
S.E.	0.0768	0.0189	0.0068	0.0860	0.0746	0.0725
C.V.	66.83%	48.65%	33.34%	26.27%	30.15%	12.76%
Sig.	(P<0.05)	(P<0.05)			(P<0.01)	(P<0.001)

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 6. Refrigerated storage effect on *acid-based* TBARS absorbances and ratios of raw comminuted pork[1,2]

Storage Day #	Wavelength 532nm	Wavelength 495nm	Wavelength 455nm	Wavelength Ratio 495nm/ 532nm	Wavelength Ratio 455nm/ 532nm	Wavelength Ratio 455nm/ 495nm
Day 0	0.0237**b**	0.0104**a**	0.0162**a**	0.4282**a**	0.7144**a**	1.7723**a**
Day 4	0.0767**b**	0.0374**a**	0.0357**a**	0.4876**a**	0.4785**b**	0.9803**b**
Day 7	0.1051**b**	0.0324**a**	0.0233**a**	0.3095**a**	0.2326**c**	0.7545**b**
Day 14	0.2796**a**	0.0715**a**	0.0360**a**	0.2650**a**	0.1411**c**	0.5304**b**
Avg.	0.1213	0.0379	0.0278	0.3725	0.3916	1.0094
S.E.	0.0685	0.0175	0.0077	0.0692	0.0697	0.0377
C.V.	69.19%	56.47%	33.97%	22.74%	21.81%	26.77%
Sig.	(P<0.05)	*(P=0.06)*		*(P=0.06)*	(P<0.001)	(P<0.01)

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

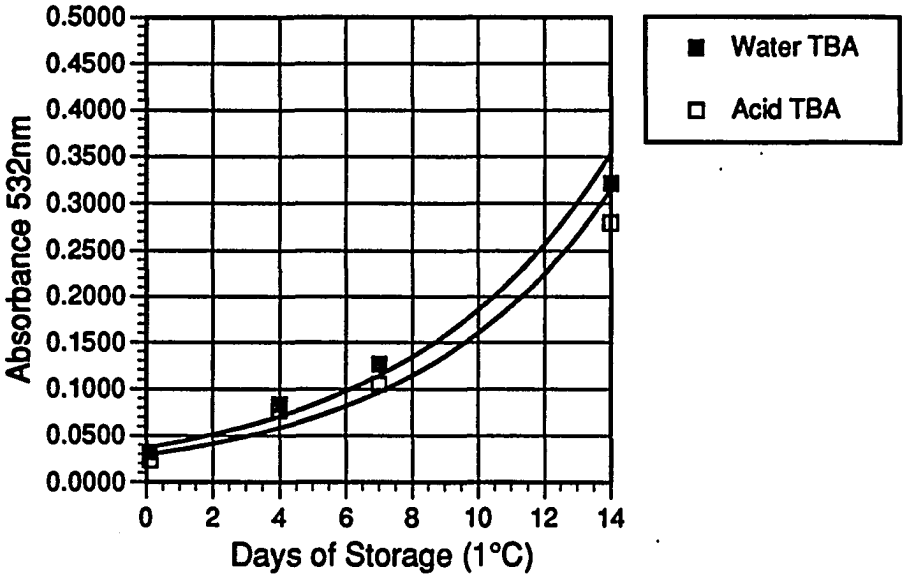

Figure 5. Effects of refrigerated storage and TBA solvent on 532nm TBARS of raw comminuted pork

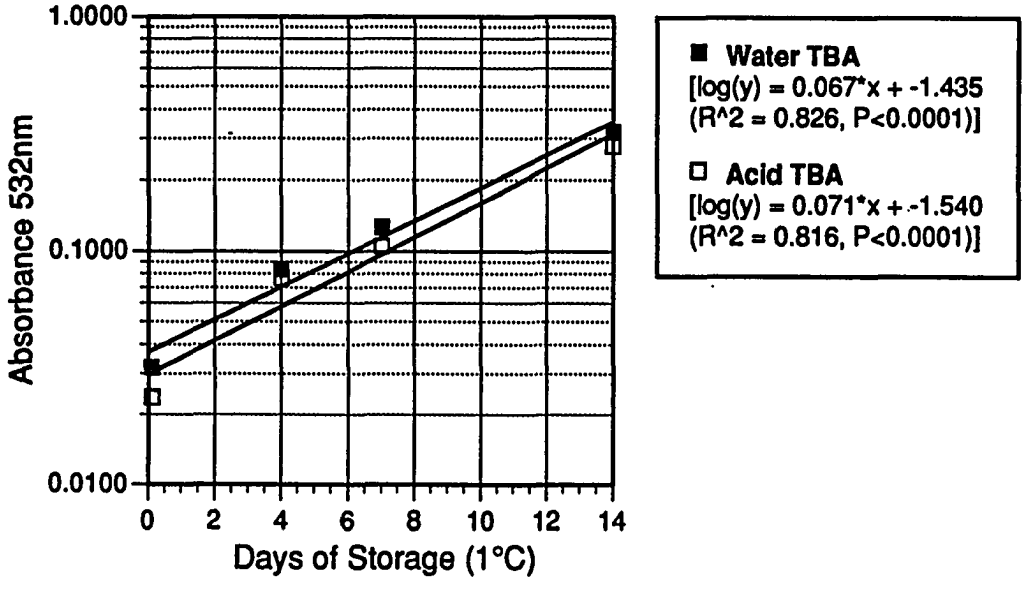

Figure 6. Semilogarithmic plot of refrigerated storage effect on 532nm *water- and acid-based* TBARS of raw comminuted pork

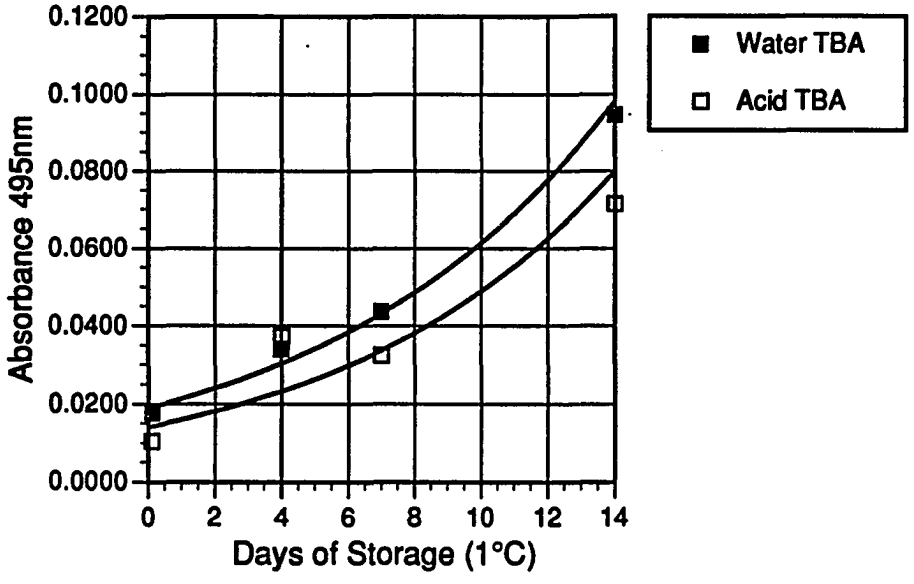

Figure 7. Effects of refrigerated storage and TBA solvent on 495nm TBARS of raw comminuted pork

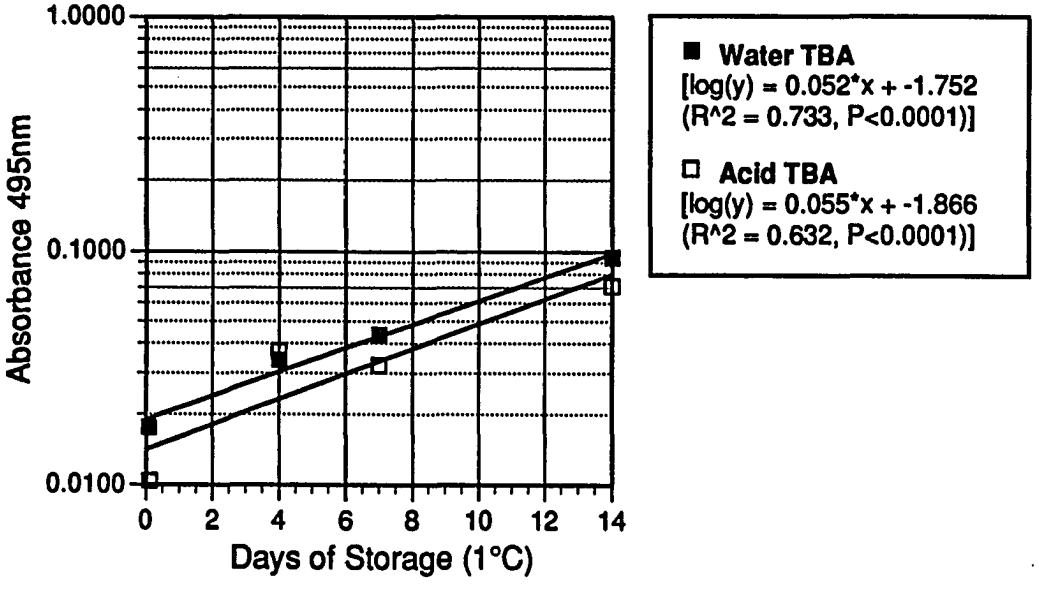

Figure 8. Semilogarithmic plot of refrigerated storage effect on 495nm *water- and acid-based* TBARS of raw comminuted pork

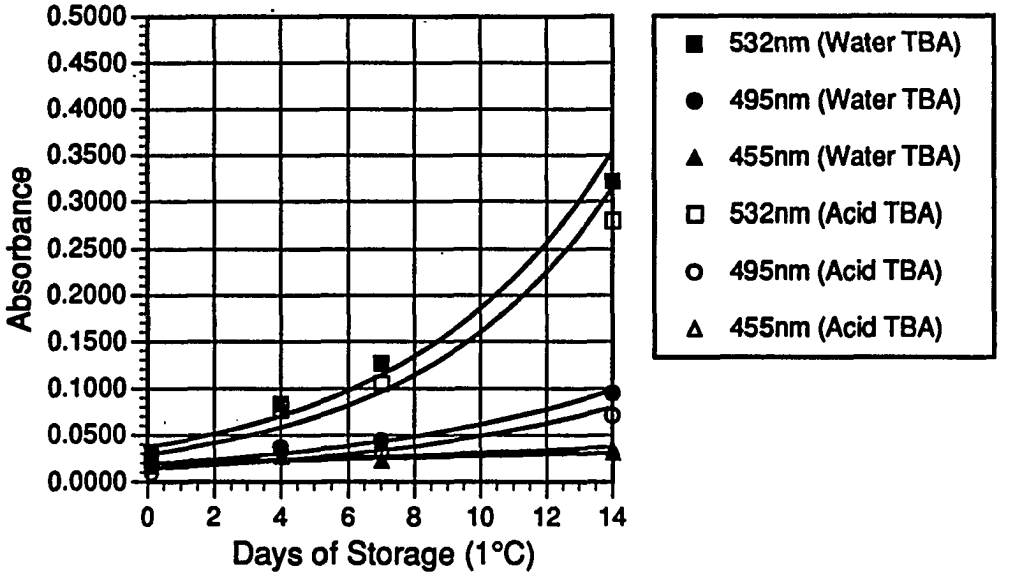

Figure 9. Linear comparison of 532nm, 495nm and 455nm TBARS of raw comminuted pork as affected by refrigerated storage and TBA solvent

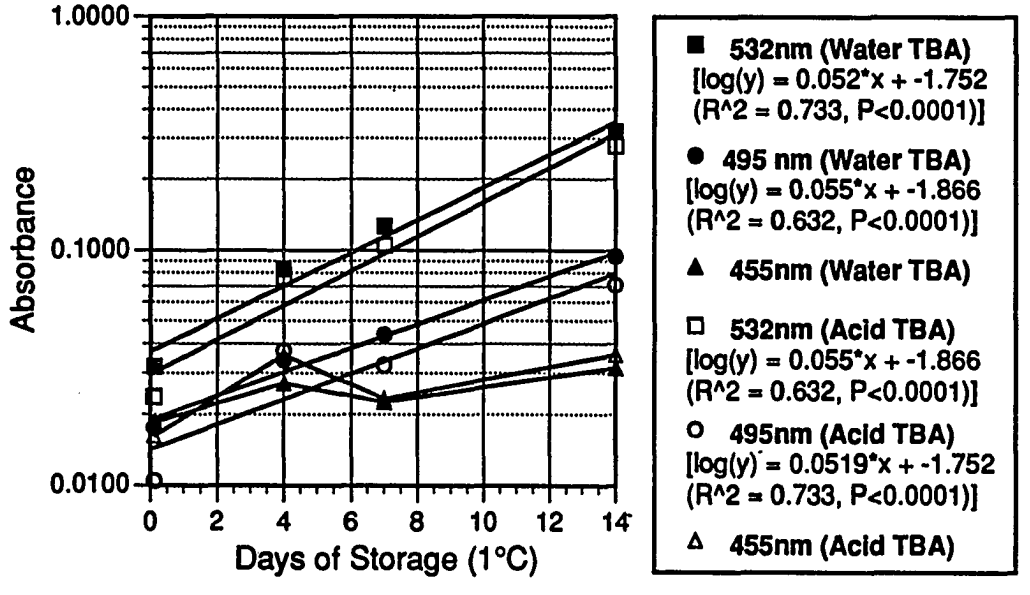

Figure 10. Semilogarithmic comparison of 532nm, 495nm and 455nm TBARS of raw comminuted pork as affected by refrigerated storage and TBA solvent

Table 7. Comparison of *water- and acid-based* TBARS formation rates of raw comminuted pork during refrigerated storage[1]

Comparison	Water	Acid	Water/Acid
532nm	0.067	0.071	0.941
495nm	0.052	0.055	0.950
532nn:495nm	1.293	1.306	0.990
455nm/532nm	-0.052	-0.052	0.990
455nm/495nm	-0.037	-0.036	1.025

[1]Calculated from slopes of equations presented in Figures 6, 8, 10, and 13.

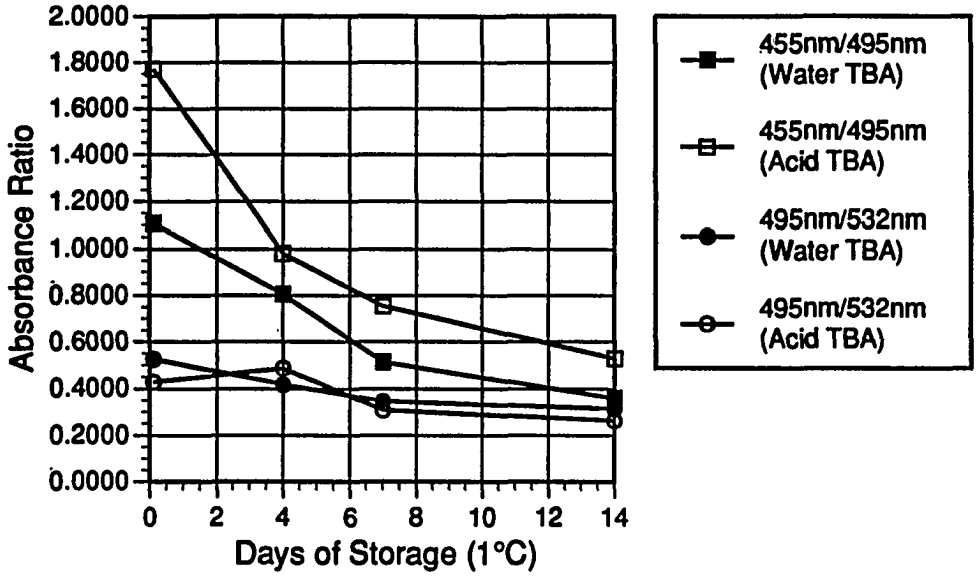

Figure 11. Refrigerated storage and TBA solvent effects on average 455nm/495nm and 495nm/532nm TBARS absorbance ratios of raw comminuted pork

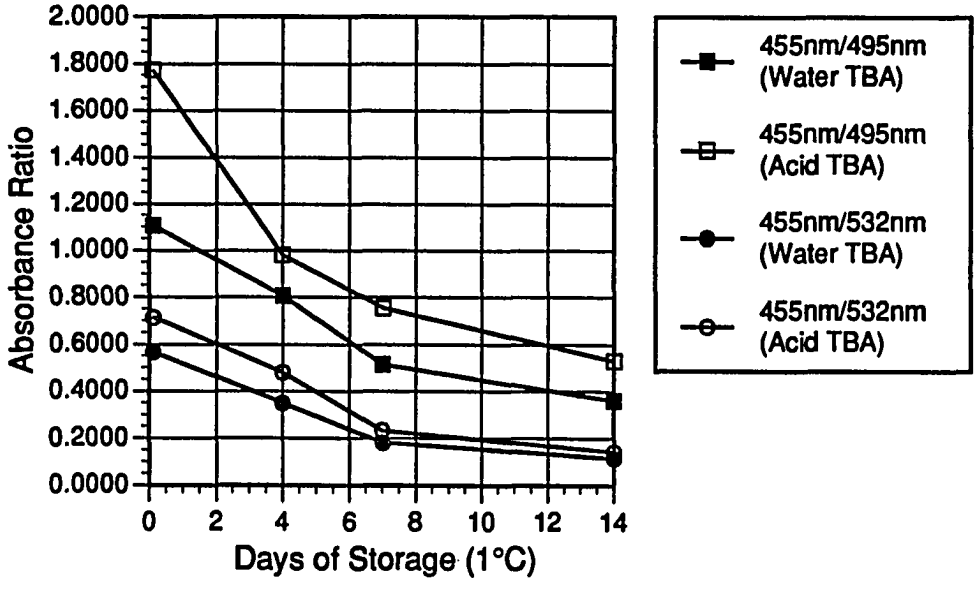

Figure 12. Refrigerated storage and TBA solvent effects on average 455nm/532nm TBARS absorbance ratios (compared to 455nm/495nm) of raw comminuted pork

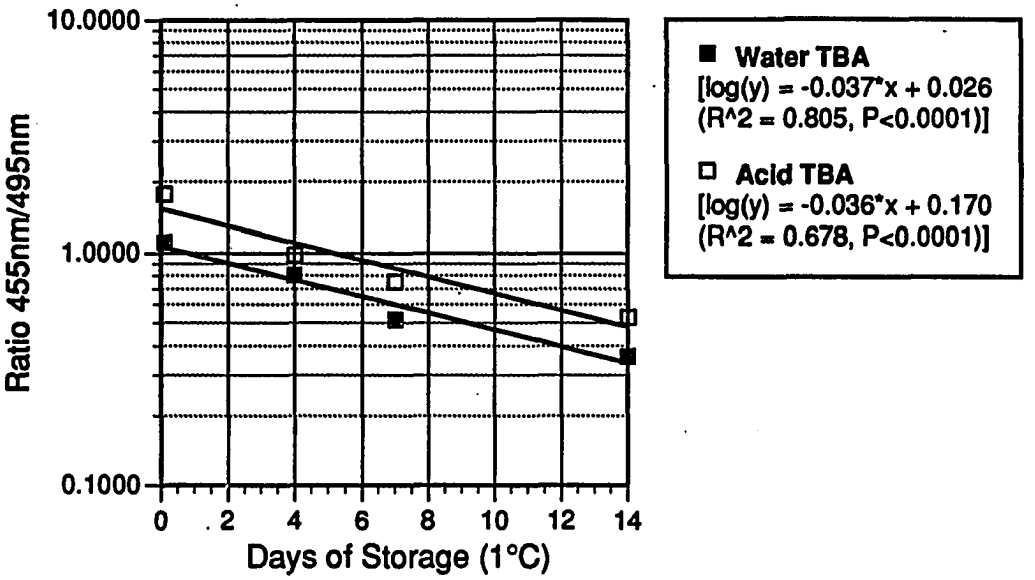

Figure 13. Semilogarithmic plot of refrigerated storage effect on *water- and acid-based* 455nm/495nm TBARS absorbance ratios of raw comminuted pork

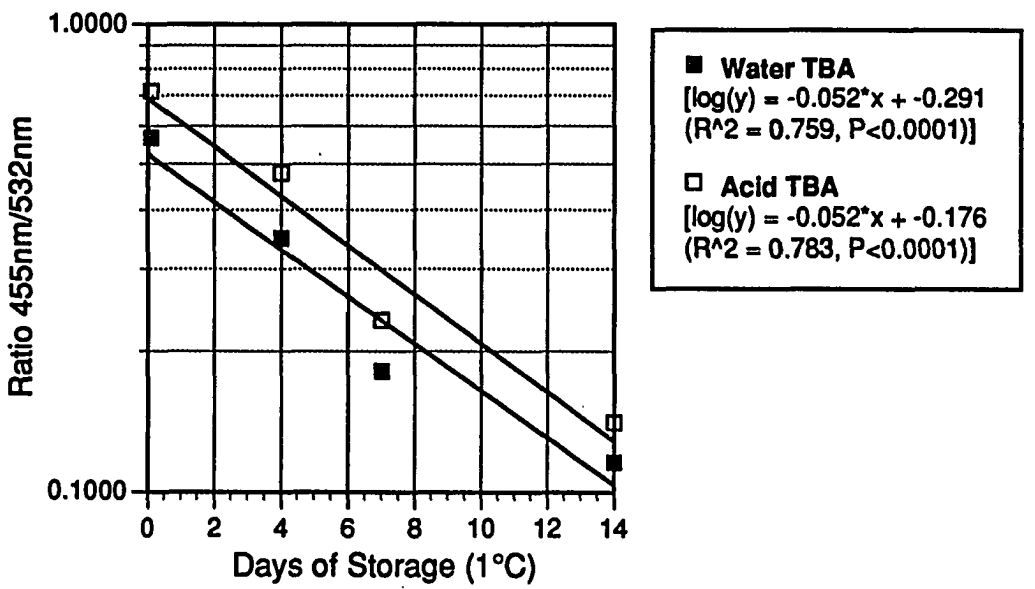

Figure 14. Semilogarithmic plot of refrigerated storage effect on *water- and acid-based* 455nm/532nm TBARS absorbance ratios of raw comminuted pork

Table 8. TBA solvent effects on TBARS absorbances during refrigerated storage of raw comminuted pork[1,2]

Solvent	Day 0	Day 4	Day 7	Day 14	Average
532nm[3]					
Water	0.0318**a**	0.0834**a**	0.1262**a**	0.3215**a**	0.1407**a**
Acid	0.0237**a**	0.0767**a**	0.1051**b**	0.2796**b**	0.1213**b**
Avg.	0.0277	0.0801	0.1156	0.3005	0.1310
S.E.	0.0046	0.0046	0.0046	0.0046	0.0023
(W-A)[4]	0.0082	0.0068	0.0210	0.0419	
495nm[3]					
Water	0.0176**a**	0.0340**a**	0.0436**a**	0.0947**a**	0.0475**a**
Acid	0.0104**b**	0.0374**a**	0.0324**b**	0.0715**b**	0.0379**b**
Avg.	0.0140	0.0357	0.0380	0.0831	0.0427
S.E.	0.0028	0.0028	0.0028	0.0028	0.0014
(W-A)[4]	0.0072	-0.0034	0.0112	0.0233	
455nm[5]					
Water	0.0185**a**	0.0272**b**	0.0226**a**	0.0316**b**	0.0250**b**
Acid	0.0162**a**	0.0357**a**	0.0233**a**	0.0360**a**	0.0278**a**
Avg.	0.0174	0.0314	0.0230	0.0338	0.0264
S.E.	0.0019	0.0019	0.0019	0.0019	0.0010
(W-A)[4]	0.0022	-0.0084	-0.0007	-0.0044	

[1]N=24 observations per mean; N=96 per "Average" mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

[3]TBA solvent effect and TBA solvent*Day interaction effects both significant at ($P < 0.0001$).

[4](W-A) = Water minus Acid for significant TBA solvent*Day interaction.

[5]TBA solvent effect and TBA solvent*Day interaction effects both significant at ($P < 0.01$).

Table 9. TBA solvent effects on absorbance ratios during refrigerated storage of raw comminuted pork[1,2]

Solvent	Day 0	Day 4	Day 7	Day 14	Average
495nm/532nm[3]					
Water	0.5264**a**	0.4184**b**	0.3463**a**	0.3132**a**	0.4015**a**
Acid	0.4282**b**	0.4876**a**	0.3095**a**	0.2650**b**	0.3725**b**
Avg.	0.4773	0.4530	0.3279	0.2891	0.3870
S.E.	0.0226	0.0226	0.0226	0.0226	0.0113
(W-A)[4]	0.0983	-0.0692	0.0368	0.0483	
455nm/532nm[5]					
Water	0.5662**b**	0.3482**b**	0.1812**a**	0.1157**a**	0.3028**b**
Acid	0.7144**a**	0.4785**a**	0.2326**a**	0.1411**a**	0.3917**a**
Avg.	0.6403	0.4134	0.2069	0.1284	0.3473
S.E.	0.0281	0.0281	0.0281	0.0281	0.0141
(W-A)[4]	-0.1482	-0.1303	-0.0514	-0.0254	
455nm/495nm[5]					
Water	1.1069**b**	0.8054**b**	0.5146**b**	0.3592**b**	0.6965**b**
Acid	1.7723**a**	0.9803**a**	0.7545**a**	0.5304**a**	1.0094**a**
Avg.	1.4396	0.8929	0.6346	0.4448	0.8530
S.E.	0.0654	0.0654	0.0654	0.0654	0.0327
(W-A)[4]	-0.6654	-0.1749	-0.2399	-0.1712	-0.3129

[1]N=24 observations per mean; N=96 per "Average" mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

[3]TBA solvent effect significant ($P \leq .01$); TBA solvent*Day interaction significant ($P \leq 0.0001$).

[4](W-A) = Water minus Acid for significant TBA solvent*Day interaction.

[5]TBA solvent effect significant ($P \leq 0.0001$); TBA solvent*Day interaction significant ($P \leq 0.01$).

Table 10. Correlations of *water-based* TBARS absorbances and ratios of raw comminuted pork throughout refrigerated storage[1]

	495nm	455nm	495nm/532nm	455nm/532nm	455nm/495nm
532nm	0.9834 (P<0.0001)	0.5258 (P<0.0001)	-0.5658 (P<0.0001)	-0.6732 (P<0.0001)	-0.7446 (P<0.0001)
495nm		0.6513 (P<0.0001)	-0.4822 (P<0.0001)	-0.6439 (P<0.0001)	-0.7584 (P<0.0001)
455nm					-0.3647 (P<0.0001)

[1]N=96 observations.

Table 11. Correlations of *acid-based* TBARS absorbances and ratios of raw comminuted pork throughout refrigerated storage[1]

	495nm	455nm	495nm/532nm	455nm/532nm	455nm/495nm
532nm	0.9544 (P<0.0001)	0.5868 (P<0.0001)	-0.4925 (P<0.0001)	-0.6556 (P<0.0001)	-0.5956 (P<0.0001)
495nm		0.7492 (P<0.0001)	-0.2726 (P<0.01)	-0.6073 (P<0.0001)	-0.6616 (P<0.0001)
455nm				-0.2191 (P<0.05)	-0.3840 (P<0.0001)

[1]N=96 observations.

Table 12. Correlations of *water-based* and *acid-based* TBARS absorbances of raw comminuted pork throughout refrigerated storage[1,2]

	532nm(A)	495nm(A)	455nm(A)
532nm(W)	0.9929 (P<0.0001)	0.9223 (P<0.0001)	0.5522 (P<0.0001)
495nm(W)	0.9721 (P<0.0001)	0.9077 (P<0.0001)	0.5862 (P<0.0001)
455nm(W)	0.5012 (P<0.0001)	0.5300 (P<0.0001)	0.6622 (P<0.0001)

[1]N=96 observations.

[2]Acid-based TBARS absorbances designated by "(A)"; water-based TBARS by "(W)".

CONCLUSIONS

STPP did not significantly ($P>0.05$) affect 2-thiobarbituric acid absorbance values at 532nm, 495nm or 455nm absorbances, in either water- or acetic acid-based TBA solution, during the two weeks of refrigerated storage of raw comminuted pork. Species verification of these findings should be performed on poultry and seafood which possess greater potential for higher rates of lipid oxidation due to their greater polyunsaturated fatty acid content.

Rate of TBARS increase during refrigeration was uniform for the first 7 days, but dramatically increased during the second week, demonstrating a first order logarithmic relationship over the entire period. Order of increase in TBARS formation for the wavelengths was 532nm>495nm>455nm.

Water as the TBA solvent allowed for greater absorbances at 532nm and 495nm; these differences generally increased with level of oxidation. Acetic acid-based TBA solvent favored greater absorbances at 455nm.

Because of contributions by the various aldehyde classes to all three wavelengths under the conditions of this research, it is difficult to attribute wavelength absorbance fluctuations to a single aldehyde group. Wavelength absorbance ratios (WARS) are based on the differential at which the wavelength absorbances increase with advancing lipid

oxidation. Monitoring relative changes in the convergence of 455nm/495nm and 495nm/532nm WARS at the higher rates of thiobarbituric acid reactive substances (TBARS) formation after advanced storage may be a useful indicator in addition to wavelength absorbance(s) for assessing oxidative status and warrants additional research.

The greatest correlations existed between absorbances of adjacent wavelengths (i.e., 532nm and 495nm, 495nm and 455nm). The 455nm/495nm WARS was the most highly correlated ratio with the wavelength absorbances, further supporting its utility in monitoring the progress of meat lipid oxidation.

REFERENCES

Boling, C. S., Graham, P. P., Marriot, N. G. and Kelly, R. F. 1990. Effect of butylated hydroxyanisole and ascorbic acid on storage stability of restructured pork. J. Anim. Sci. 68(1): 108.

Cochran, W. G. and Cox, G. M. 1957. *Experimental Designs*, 2nd ed. John Wiley & Sons, New York, NY.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M. and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28: 187.

Frankel, E. N., Neff, W. E., Selke, E., and Brooks, D. D. 1987. Thermal and metal-catalyzed decomposition of methyl linolenate hydroperoxides. Lipids 22(5): 322.

Gardner, H. W. 1983. Effects of lipid hydroperoxides on food components. In *Xenobiotics in Foods and Feeds*, D. E. Schwass and J. W. Finley (Ed.), p. 63. ACS Symp. Series No. 234. American Chemical Society, Washington, D.C.

Gardner, H. W. 1987. Reactions of hydroperoxides-products of high molecular weight. Ch. 3. In *Autoxidation of Unsaturated Lipids*, H. W.-S. Chan (Ed.), p. 51. Academic Press, Inc., London.

Gordon, M. H. 1990. The mechnism of antioxidant action *in vitro*. Ch. 1. In *Food Antioxidants*, B. J. F Hudson (Ed.), p. 1. Elsevier Science, Publishers Ltd, Essex, England.

Hwang, S.-Y., Bowers, J. A. and Kropf, D. H. 1990. Flavor, texture, color and hexanal and TBA values of frozen cooked beef packaged in modified atmosphere. J. Food Sci. 55(1): 27.

Igene, J. O., King, J. A., Pearson, A. M. and Gray, I. J. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27(4): 838.

Igene, J. O., Yamauchi, K., Pearson, A. M., Gray, J. I. and Aust, S. D. 1985. Evaluation of 2-thiobarbituric acid reactive substances (TBRS) in relation to warmed-over flavor (WOF) development in cooked chicken. J. Agric. Food Chem. 33: 364.

Irani, R. R. and Morgenthaler, W. W. 1963. Iron sequestration by polyphosphates. J. Am. Oil. Chem. Soc. 40(7): 283.

Kim, R. S. and LaBella, F. S. 1987. Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. J. Lipid Res. 28: 1110.

King, A. J. and Earl, L. A. 1988. Effect of selected sodium and potassium salts on TBA values of dark meat turkey patties. J. Food Sci. 53(3): 723.

Kosugi, H. and Kikugawa, K. 1986. Reaction of thiobarbituric acid with saturated aldehydes. Lipids 21(9): 537.

Kosugi, H., Kato, T. and Kikugawa, K. 1987. Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. Anal. Biochem. 165: 456.

Kosugi, H., Kato, T. and Kikugawa, K. 1988. Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid oxidation. Lipids 23(11): 1024.

Kosugi, H., Kojima, T. and Kikugawa, K. 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24(10): 873.

Kosugi, H., Kojima, T. and Kikugawa, K. 1991. Characteristics of the thiobarbituric acid reactivity of oxidized fats and oils. INFORM 2(2): 154.

Labuza, T. P. 1982. *Shelf-life dating of foods*. Food & Nutrition Press, Inc. Westport, CT.

Lamkey, J. W., Mandigo, R. W. and Calkins, C. R. 1986. Effect of salt and phosphate on the texture and color stability of restructured beef steaks. J. Food Sci. 51(4): 873.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50(10): 387.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7): 105.

Melton, S. L. 1985. Methods of assessing oxidation. In *Proceedings of the Meat Industry Research Conference*, Chicago, IL, October 18-19. American Meat Institute, Washington, DC.

Miller, M. F., Davis, G. W., Seideman, S. C., Ramsey, C. B. and Rolan, T. L. 1986. Effects of various phosphates on the palatability, appearance and storage traits of flaked and formed restructured beef steaks. J. Food Sci.51(6): 1435.

Molins, R. A., Kraft, A. A., Walker, H. W., Rust, R. E., Olson, D. G. and Merkenich, K. 1987. Effect of inorganic polyphosphates on ground beef characteristics: some chemical, physical and sensory effects on frozen beef patties. J. Food Sci. 52(1): 50.

Ozilgen, S. and Ozilgen, M. 1990. Kinetic model of lipid oxidation in foods. J. Food Sci. 55(2): 498.

Patel, S. B., King, A. J. and Lee, Y. B. 1988. Effect of sodium salts and added water on physical, chemical and sensory properties of dark turkey meat subjected to long-term frozen storage. J. Food Sci. 53(6): 1610.

Pryor, W. A., Stanley, J. P. and Blair, E. 1976. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids 11(5): 370.

Raharjo, S., Sofos, J. N., Maga, J. A. and Schmidt, G. R. 1989. Influence of meat restructuring systems on lipid oxidation in beef. Lebensm. Wiss. Technol. 22: 199.

Schmedes, A. and Holmer, G. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Am. Oil Chem. Soc. 66(6): 813.

Schricker, B. R., Miller, D. D. and Stouffer, J. R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47: 740.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C., Jr. and Dupuy, H. P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. J. Food Sci. 52(5): 1163.

Steel, R. G. D. and Torrie, J. H. 1980. *Principles and Procedures of Statistics*, 2nd ed. McGraw-Hill Book Company, New York, NY.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. R., Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39(1): 34.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Tay, H. C., Aberle, E. D. and Judge, M. D. 1983. Iron catalyzed oxidative rancidity in prerigor ground pork. J. Food Sci. 48: 1328.

Tims, M. J. and Watts, B. M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

PART III.

ASSESSMENT OF SODIUM TRIPOLYPHOSPHATE INFLUENCE ON THE 2-THIOBARBITURIC ACID TEST FOR EVALUATING LIPID OXIDATION IN COOKED COMMINUTED MUSCLE FOODS

Assessment of sodium tripolyphosphate influence on the 2-thiobarbituric acid test for evaluating lipid oxidation in cooked comminuted muscle foods

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ABSTRACT

Sodium tripolyphosphate interference on the distillation 2-thiobarbituric acid test (TBA) was assessed during 14 days refrigerated storage (1°C) of cooked ground pork. After selected periods, pork samples and sodium tripolyphosphate (0%, 0.01%, 0.05%, 0.10%, 0.15%, 0.25%, 0.35% 0.50%) were blended prior to distillation. STPP exercised a significant but minor repression ($P<0.01$) of TBA reactive substances (TBARS) at 532nm and 495nm but not at 455nm. Water as the TBA solvent yielded higher TBARS than acetic acid at all three wavelengths ($P<0.0001$). All wavelengths were highly correlated ($r\geq0.96$, $P<0.0001$). TBARS formation rate peaked at the onset; the rate of TBARS formation decreased logarithmically with refrigerated storage. Values for 455nm/495nm and 495nm/532nm wavelength absorbance ratios (WARS) coincided at high levels of oxidation.

INTRODUCTION

The effectiveness of polyphosphates in repressing cooked meat lipid oxidation has been reported in the literature (Tims and Watts, 1958; Sato and Hegarty, 1971; Igene et al., 1985; Huffman et al., 1987; Roozen, 1987; Mann et al., 1989; Raharjo et al., 1989; Kolodziejska et al., 1990). The analytical tool used in all of these specific studies was the 2-thiobarbituric acid (TBA) test as reported by Tarladgis et al. (1960), which is the most widely used assay for monitoring meat lipid oxidation (Melton, 1983, 1985).

In some of these papers, significant oxidative advantages at "Time 0" of storage for cooked meat treatments formulated with polyphosphate have been observed (Tims and Watts, 1958; Igene et al.1985; Huffman et al., 1987, Raharjo et al., 1989). Possible polyphosphate interference with the TBA test of Tarladgis et al. (1960) has been an explanation for such observations (Koniecko, 1979; Diosady, 1988; Raharjo et al., 1989).

Researchers have suggested that some thiobarbituric acid reactive substances (TBARS) arise from the acid-catalyzed or thermal decomposition of hydroperoxides during the actual TBA analysis (Pryor et al., 1976, Kim and LaBella, 1987). In fact, variations of the TBA test have been designed to specifically analyze hydroperoxides (Ohkawa et al., 1979;

Asakawa and Matsushita, 1979, 1980; Janero and Burghardt, 1989). In foods, the main catalysts of hydroperoxide decomposition are transition metal ions (Gardner, 1975). Polyphosphates have been demonstrated to possess metal-chelating properties (Irani and Morgenthaler, 1963); the repressive effects of chelators such as EDTA on TBA results have been reported (Kosugi et al., 1991).

Hydroperoxides accumulate in the propagation phase of the lipid oxidation cycle; these primary products decompose into secondary products such as the volatile aldehydes responsible for off-flavor development (Gardner, 1983, 1987). Metal catalysis and metal chelation influence on the TBA test results theoretically would vary according to which point of the oxidation cycle that oxidative stress (such as heat associated with distillation for the TBA test) was exerted.

Meat distillate prepared for reaction with TBA according to the method of Tarladgis et al. (1960) has been verified to contain a range of aldehydes (St. Angelo et al., 1987). Utilizing the traditional wavelength of 532nm, along with 495nm and 455nm, researchers have concluded that the TBA test has the potential for monitoring a broad range of lipid oxidation products (Kosugi et al., 1989). However, TBA solvent choice (90% glacial acetic acid vs. water) in the method of Tarladgis et al. (1960) has been known to affect the relative mix of TBA reactive substances (TBARS) at different

wavelengths (Tarladgis et al., 1962, 1964; Crackel et al., 1988) Earlier work has shown that monitoring the absorbances at these alternate wavelengths and conversion into wavelength absorbance ratios (WARS) has potential utility in monitoring lipid oxidative stability (Part II of this dissertation).

Objectives of this study were to: (1) analyze cooked comminuted pork during a 2-week refrigerated storage period to assess sodium tripolyphosphate (STPP) effects on the TBA test at various points of the lipid oxidation cycle, (2) monitor TBARS absorbances at the primary wavelength of 532nm, and at 495nm and 455nm, utilizing the commonly used TBA procedures for assessment of meat lipid oxidation, (3) analyze the effects of both TBA solvents (water and acid) on TBARS, and (4) calculate the rate of lipid oxidation increase for cooked pork during 2 weeks of refrigerated storage as measured by the TBA test.

MATERIALS AND METHODS

Sample Preparation

Raw material selection

Fresh, boneless pork picnic shoulders obtained from the Iowa State University (ISU) Meats Laboratory were closely trimmed of external fat, cut into strips for grinding, vacuum-packaged to limit oxygen exposure and stored in a covered box kept in a cooler at 1°C for 1-2 days prior to grinding.

Meat handling and processing

Comminution On Day 0, the meat strips were removed from storage and subjected to two grindings, first through a 9.52-mm (3/8-in) plate followed by a 3.18-mm (1/8-in) plate. Hand mixing was performed after the first grinding, and again after the second grinding to promote homogeneity. Half of the comminuted meat was then divided into 450-g lots and placed into 1-L beakers for cooking. (The other half was for a companion study involving raw pork.)

Cooking The 1-L beakers of ground meat were transferred to a PRECISION shaking water bath (GCA Corp., Chicago, IL) with the temperature set at approximately 97°C and shaker speed at 15 oscillations/min. The ground meat was stirred every 5 min using a stainless steel spoon. Meat temperature was monitored via copper/constantin probes

connected to an OMEGA Trendicator °C (OMEGA Engineering, Inc., Stamford, CT) digital temperature readout. Heat was applied to an endpoint of 70°C, upon which the beakers were removed and immediately placed into an icewater bath.

Storage preparation After cooling down to approximately 5°C, the cooked ground meat was removed from the beakers, collected into a plastic tub and hand-mixed for homogeneity. The ground meat was placed into oxygen-permeable plastic bags and formed into approximately 2-cm-thick patties. The bags were sealed, placed in covered cardboard boxes and stored in a cooler maintained at 1°C.

STPP addition On the day of preparation (Day 0), and at Day 4, Day 7 and Day 14 of storage, sealed bags of patties were removed from storage (without replacement) for sodium tripolyphosphate (STPP) addition and subsequent lipid oxidation analysis.

Representative samples of 10 g each were selected from randomly chosen pattie locations, weighed into small plastic weighing dishes which were subsequently placed on trays of crushed ice to minimize pre-analysis temperature abuse. Preweighed amounts of sodium tripolyphosphate (STPP), designed for addition to the 10-g meat samples at the rate of 0.00%, 0.01%, 0.05%, 0.10%, 0.15%, 0.25%, 0.35%, and 0.50% (w/w) were dissolved in 1.0 ml of deionized distilled water and added in random order to the meat samples. Thorough mixing of the STPP

solutions and meat samples in the weighing dishes was performed for 1 min using stainless steel laboratory weighing spatulas.

Analyses

Product Composition

Representative meat samples were analyzed for fat and moisture utilizing a CEM Moisture/Solids Analyzer, Model AVC 80 equipped with 100-g balance capacity and automatic extraction system (Matthews, NC). A representative meat sample from each replication was first thoroughly comminuted to a paste-like texture utilizing a Cuisinart Basic Food Processor (Greenwich, CT). A sample size of 4 g was used for the combination moisture/fat analyses. The CEM analyzer was programmed selecting "Mode M=3" for alternating meat moisture/fat analysis. CEM analyzer moisture parameters were power at 90% and time for 4:00 min; fat parameters were power at 100% and time for 3:30 min.

2-Thiobarbituric acid (TBA) analysis

Sample distillation After mixing with STPP, the samples were prepared as described by Tarladgis et al. (1960) for distillation, with the exception that the mixed 10-g samples were transferred into 250-ml round-bottomed flasks, 96.5 ml deionized distilled water were added, followed by 2.5

ml 4N HCl. The 250-ml round-bottomed flasks were then transferred for homogenization to a Kinematica Polytron PT 10/35 equipped with a PTA 20 shaft assembly (Brinkman Instruments Co., Westbury, NY) at a speed setting of "6" for 30 sec. After homogenization, a few drops of antifoam C and glass boiling beads were added prior to randomized placement of the flasks onto distillation units. Heat was applied stepwise uniformly to all treatment samples. Approximately 25-30 min were required for 50 ml of distillate which was collected into 50-ml Erlenmeyer flasks, capped with parafilm, and stored in the dark at 1°C until analyzed within 24 hr.

Reaction with TBA For comparison, solutions of 0.02M 2-thiobarbituric acid were prepared utilizing one of two different solvents: 90% glacial acetic acid ("acid-based") as presented by Tarladgis et al. (1960) or water ("water-based") as recommended by other researchers (Tarladgis et al., 1962, 1964; Crackel et al., 1988). After removal from ≤24 hr storage and prior to uncapping, the 50-ml Erlenmeyer flasks were shaken/swirled to ensure homogenization of distillate prior to sample distillate extraction. Two aliquots of 5.0 ml of distillate were extracted; 1 aliquot was added to 5.0 ml of water-based and the other to 5.0 ml of acid-based 0.02M TBA solutions in test tubes and tightly capped. The capped test tubes were then incubated in a 95°C water bath for 35 minutes to promote color formation. Immediately after water bath

removal, the test tubes were subjected to a cold water rinse in preparation for spectrophotometric analysis.

Spectrophotometric analysis Samples were analyzed for 2-thiobarbituric acid reacting substances (TBARS) on a GILFORD Response® UV-VIS Spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH) equipped with a rapid sampler accessory set for a sample time of 0.5 sec and an equilibration time of 2 sec. Wavelength scans with bandwidth set at 1.0 nm and scan increment of 0.5 nm were utilized to verify wavelength peaks. Absorbance readings were then performed at the selected wavelengths of 455nm, 495nm and 532nm. Similar solvent-based (water- vs. acid-based) STPP treatments were analyzed together; order of solvent-based analyses was varied with each replication. Blanks based upon the appropriate solvent were utilized at the beginning of each solvent family analysis.

Statistical analysis

Experimental design was based on a split,split-plot format (Cochran and Cox, 1957; Steel and Torrie, 1980). Main plots were the storage periods (in days), subdivided into the different STPP levels which, in turn, were subdivided into type of solvent-based TBA reagent (water vs. acid). Three replications were performed; duplicates were taken for every reading and then averaged to yield one data figure used for analysis. TBARS absorbance readings were directly

statistically analyzed without any mathematical conversion. Data analysis was performed utilizing the Statistical Analysis System, version 6.06.01, (SAS Institute Inc., Cary, NC). Significance between treatment and subtreatment means was tested using the least significant difference (lsd) procedure, but recognized only if a treatment significant F test resulted [Fisher's (protected) lsd] (Steel and Torrie, 1980). Standard errors and coefficients of variability were calculated from SAS results (Cochran and Cox, 1957; Steel and Torrie, 1980). All statistics for absorbance ratios (495nm/532nm, 455nm/532nm and 455nm/495nm) were calculated from the total model (versus data point wavelength averages); regression equations and coefficients derived for TBARS formation rates presented in the legend tables of plotted averages were calculated from the total model as well.

RESULTS AND DISCUSSION

Product Attributes and Preparation

Composition

Average fat and water content of the ground pork utilized in this study was 8.05% and 70.01%, respectively. A companion study assessing raw ground pork (Part II of this dissertation) was based upon the same raw materials.

Cooked preparation

Cooking of the ground pork to an internal temperature of 70°C required an average of 22 min in a 97°C water bath.

STPP Effect on 532nm TBARS

Reported earlier for raw muscle systems (Part II of this dissertation), a major objective of this study was to assess sodium tripolyphosphate (STPP) interference upon the commonly reported 532nm 2-thiobarbituric acid measurement in cooked muscle products. Evaluation of potential STPP interactions with increasing oxidation (during refrigerated storage) as well as with TBA solvent [water vs. 90% glacial acetic acid; Tarladgis et al. (1960, 1962, 1964)] were also of interest.

Figures 1 and 2 graphically depict this data for the water-based and acid-based 532nm measurements, respectively, for the range of STPP concentrations at each day of analysis throughout the 14-day refrigerated storage period. Results

are presented for the individual solvent classes; no significant STPP level*TBA solvent interaction ($P>0.05$) was detected but solvent differences existed and will be discussed later. Storage effects will also be specifically addressed in a later section.

Water-based 532nm STPP addition prior to TBA analysis significantly ($P<0.001$) lowered water-based 532nm TBARS absorbances (Table 1, Figure 1). STPP influence was not significant ($P>0.05$) at Day 0, but was significant after each succeeding storage period ($P<0.05$); no significant STPP level*storage interaction existed ($P>0.05$).

In practical terms, these statistical differences appear to be of minimal relative significance (Figure 1), and do not fully explain STPP treatment differences reported in the literature.

Acid-based 532nm STPP addition prior to TBA analysis significantly ($P<0.01$) lowered acid-based 532nm TBARS absorbances (Table 2, Figure 2). Again, STPP influence was not significant ($P>0.05$) at Day 0, but was significant after each succeeding storage period ($P<0.05$); no significant STPP level*storage interaction existed, however ($P>0.05$). All acid-based absorbances throughout the tested oxidation period were lower than their water-based counterparts; this will be addressed later in the analysis of TBA solvent influence. As

with the water-based 532nm evaluations, these statistical differences appear to be of minimal relative significance.

Standard errors for both TBA solvents were similar to those in another study involving raw pork (Part II of this dissertation); however, because all absorbances were greater for cooked pork, this translated into lower coefficient of variabilities in this study.

TBARS Evaluations at 532nm, 495nm & 455nm

A variety of aldehydic lipid oxidation products exist in distillate obtained via the TBA method of Tarladgis et al. (1960) (St. Angelo et al., 1987). TBA reactivity of the major distillate-containing aldehyde classes under conditions used for meat lipid oxidation assessment (Tarladgis et al., 1960), has been demonstrated at 455nm, 495nm and 532nm (Part I of this dissertation).

It was of interest to monitor these wavelengths as had been done previously with raw pork (Part II of this dissertation). Absorbance comparisons among wavelengths were derived and analyzed from individual readings as 495nm/532nm, 455nm/532nm and 455nm/495nm wavelength absorbance ratios (WARS) and were monitored for changes under the various experimental conditions.

These comparisons were also performed in research that monitored lipid oxidation of raw pork (Part II of this

dissertation). Igene et al. (1985) added together total absorbances of oxidized meat samples at 532nm and 450nm, and then presented individual wavelength absorbance percentages.

STPP Effects

STPP concentration effects on all wavelengths, averaged over all storage periods, are presented for water-based TBARS (Table 3 and Figure 3) and for acid-based TBARS (Table 4 and Figure 4). No significant STPP level*storage interactions existed ($P>0.05$) at any of the wavelengths or for any of the WARS. No STPP level*TBA solvent interactions existed ($P>0.05$), but solvent-related differences did exist (Tables 3 and 4) and will be discussed in the solvent effects section.

Water-based TBARS STPP effects were noted on the water-based 532nm absorbances ($P<0.001$); significant absorbance lowering effects were also calculated for the water-based TBARS absorbing at 495nm ($P<0.01$) (Table 3, Figure 3). These differences appeared to have minimal practical significance. No STPP effects were calculated for 455nm or the wavelength ratios ($P<0.05$).

Coefficients of variability, indicating an attribute's standard deviation relative to its average, were similar for the wavelength absorbances and the 495nm/532nm absorbance ratios, but were slightly higher for the 455nm/532nm absorbance ratios.

Acid-based TBARS STPP level lowered acid-based 532nm absorbances ($P<0.01$); significant absorbance lowering effects were also calculated for 495nm ($P<0.01$) (Table 4, Figure 4). As with its water-based comparison, STPP level exhibited no significant ($P>0.05$) repressive effects on 455nm absorbances or on the wavelength absorbance ratios ($P>0.05$).

Coefficients of variability (C.V.s) were similar to the water-based values, with the exceptions of 455nm and 495nm/532nm, which were somewhat higher for the acid-based figures. Again, C.V.s were lower than what was observed in TBA evaluation of raw pork during storage (Part II of this dissertation).

Because of the magnitude of the absorbance values with cooked pork, STPP exercised no effects of practical significance on TBARS measurements. Ande (1985) concluded that STPP interfered with TBARS color development with a non-distillation method, but this interference was not observed when the distillation procedure was used.

Refrigerated storage effects

Wavelength absorbances Lipid oxidation products rapidly accumulated between Day 0 to Day 7; less TBARS accumulation occurred at all three wavelengths after Day 7 (Tables 5 and 6; Figures 5, 7 and 9). This contrasts with the oxidation rate previously observed in refrigerated raw pork

(Part II of this dissertation), in which the rate gradually increased during a 2-week refrigerated period to levels similar to starting values (Day 0) of this study.

Coefficients of variance were greater for acid-based 532nm and 495nm absorbances than for their water-based equivalents. Coefficients of variance were higher for water-based 455nm absorbances, 495nm/532nm absorbance ratios, and particularly for 455nm/532nm absorbance ratios (Tables 5 and 6).

532nm Length of storage significantly increased absorbances ($P<0.0001$) with time (Tables 5 and 6, Figure 5), indicating increases in lipid oxidation. Results for the two TBA solvents paralleled each other. Graphic depiction by Empson et al. (1991) of TBARS accumulation in cooked, minced chicken breasts analyzed during a 3-week period at 4°C showed a similar trend.

Net 532nm absorbance increases between Day 0 and the days of analysis (i.e., 4, 7 and 14) were calculated, and then divided by days of storage, to determine average rates of net oxidation increase per days of storage. Net oxidation formation rates demonstrated a highly correlated, highly significant logarithmic decrease with time ($R^2 \geq 0.94$, $P<0.0001$; Figure 6). Though oxidation increased with time, the rate of net increase actually decreased over time; maximum

oxidation rates occurred at the onset of storage and decreased with storage of cooked pork muscle.

495nm Storage effects significantly increased both solvent-based 495nm absorbances ($P<0.0001$) (Tables 5 and 6; Figure 7). As with 532nm absorbances, rates of 495nm TBARS formation steadily decreased in a logarithmic fashion ($P<0.0001$, $R^2 \geq 0.90$; Figure 8).

455nm Absorbances associated with 455nm also increased with refrigerated storage ($P<0.001$, water-based; $P<0.01$, acid-based) (Tables 5 and 6, Figure 9). Increases in 455nm TBARS were not seen previously in raw ground pork during refrigerated storage (Part II of this dissertation). A significant ($P<0.0001$) logarithmic decrease in rates of net 455nm TBARS formation occurred with time. Curves for the different solvent types (water and acetic acid) nearly coincided for the 455nm absorbances (Figure 10).

Overall comparison Absorbance increases of the three wavelengths, relative to each other over time, are presented in a linear format (Figure 11); changes in net TBARS formation rates are in a semilogarithmic plot (Figure 12). In the linear representation (Figure 11), overall absorbance accumulation at 532nm overshadowed that at 495nm, and especially that at 455nm. In the semilogarithmic plot, TBARS formation rates were 532nm>495nm>455nm, as indicated by distance between the parallel curves. Rates of TBARS

formation were similar between the two solvent types at all three wavelengths (Table 7). TBARS formation rates decreased in a like fashion at all three wavelengths, as evidenced by the comparison of slopes of the curves (Table 7; Figure 12). This similar response among the wavelength absorbances resulted in high correlations (Tables 10 and 11), which will be discussed in the correlations section.

Coefficients of variability (Tables 5 and 6) were less for water-based 532nm and 495nm; acid-based 455nm had a C.V. lower than the water-based equivalents.

Wavelength absorbance ratios (WARS) WARS are based on the differential at which the wavelength absorbances increase with advancing lipid oxidation (i.e., 532nm>495nm>455nm). Refrigerated storage effects on these ratios are shown in Tables 5 and 6, and in Figure 13.

495nm/532nm This wavelength absorbance ratio demonstrated no significant response to refrigerated storage ($P>0.05$), indicating a consistent relationship between the TBARS of these wavelengths.

An interesting observation is that the values closely approximate those determined between 532nm and 495nm for malondialdehyde standard curves (Part I of this dissertation) which maintained a constant value of approximately 0.2500 for water-based TBA and 0.2200 for acid-based; the values in Table 5 and 6 approach these values and Figure 13 depicts a

relatively horizontal line throughout storage. Malondialdehyde solutions represented a "pure" TBARS reactivity (Part I of this dissertation); the high absorbances affiliated with TBARS of cooked ground pork are probably indicative of an overwhelming concentration of short-chained TBARS.

The relative unresponsiveness of this ratio to storage of cooked ground pork, indicative of a constant relationship between these two wavelengths, is shown in Figure 13.

This ratio did not display a significant response to storage for raw ground pork (Part II of this dissertation); as reported previously, this stability makes it a good candidate for ratio comparison with the responsive 455nm/495nm WARS.

455nm/532nm Storage significantly affected this ratio for both the water-based ($P<0.05$) and acid-based ($P<0.001$) combinations. Day 0 was highest for both, and after the dramatic increase in lipid oxidation between Day 0 and Day 4, this ratio remained constant (Tables 5 and 6, Figure 13), possibly indicating that oxidation had progressed to a maximum. As had been indicated with raw pork (Part II of this dissertation), the curve of this ratio roughly paralleled that for 455nm/495nm.

455nm/495nm Storage effects with concomitant increases in oxidation significantly affected this ratio ($P<0.01$, water-based; $P<0.001$, acid-based) (Tables 5 and 6,

Figure 13), though this influence mostly occurred between Day 0 and Day 4. As observed with stored raw pork (Part II of this dissertation), this curve converged with the 495nm/532nm curve as oxidation increased; the acid-based equivalents coincided while the water-based paralled one another in close proximity.

Coefficients of variation for all three WARS were unexplainably higher for the water-based than for the acid-based TBARS.

TBA solution, TBA solvent*Day effects

532nm, 495nm & 455nm Water as the TBA solvent resulted in significantly greater average absorbances at 532nm ($P<0.0001$), 495nm ($P<0.0001$) and 455nm ($P<0.0001$) (Table 8, Figures 5, 7 and 9). The higher average absorbance is difficult to explain; water-based TBA is expected to yield lower values than acid-based at 455nm (Crackel et al., 1988).

Significant TBA solvent*storage interactions occurred for 532nm ($P<0.0001$) and 495nm ($P<0.0001$). In general, the greatest day-related differences between solvent types existed after Day 4, during periods of decreasing rates of TBARS formation. These results agree with those presented by Crackel et al. (1985) for cooked meat.

Wavelength absorbance ratios (WARS) Because WARS are derived from wavelength absorbances, TBA solvent effects for

the wavelength absorbances translated into differences between their ratios (Table 9, Figure 13).

495nm/532nm For the 495nm/532nm absorbance ratios, water-based TBA yielded significantly greater values than acid-based ($P<0.0001$) (Table 9, Figure 13). A significant TBA solvent*storage interaction ($P<0.05$) was manifested by the difference at Day 0 (Table 9).

455nm/532nm No difference attributable to TBA solvent type during storage was realized ($P>0.05$)(Table 9).

455nm/495nm As with the 495nm/532nm WARS, water-based TBA yielded significantly greater values than acid-based at each day analyzed during the storage period ($P\leq0.05$) (Table 9, Figure 13).

Correlations of Wavelengths and Ratios

Correlations were calculated between the wavelengths and wavelength absorbance ratios to further evaluate relationships between them in cooked meat lipid oxidation.

Water-based correlations

All correlations among water-based wavelengths were very highly significant ($P<0.0001$) and quite substantial ($R>0.97$). In raw pork (Part II of this dissertation), the highest correlations were between 495nm and 532nm, followed by 455nm and 495nm; this also was the case for cooked pork, except that due to the high level of oxidation and increase in aldehydes

absorbing at all three wavelengths, these differences weren't as obvious (Table 10).

Correlations between the ratios and wavelengths were all significant ($P \leq 0.05$). The 455nm/495nm WARS demonstrated the highest correlations with 532nm, 495nm and 455nm ($R \geq |0.71|$); 495nm/532nm WARS had the lowest correlations ($R \geq |0.20|$). This observation again was indicative of 455nm/495nm WARS responsiveness to increased oxidation, and the relative stability of the 495nm/532nm ratio (accounted for by high correlation between the two individual wavelengths).

Acid-based correlations

No correlations existed between the acid-based 495nm/532nm wavelength absorbance ratio and any of the wavelengths ($P > 0.05$); this ratio remained relatively constant throughout the entire storage period for the cooked pork (Table 6; Figure 13). As previously mentioned, this is further testimony to the stability of this ratio.

All other correlations among acid-based wavelengths, and between the wavelengths and WARS were very highly significant ($P < 0.0001$) (Table 11). Correlations between the acid-based wavelengths and 455nm/495nm WARS were only slightly greater than those between 455nm/532nm WARS and the wavelengths.

Correlations between acid-based and water-based

All wavelength absorbances were very highly correlated (approaching 1.00) and all were very highly statistically significant ($P<0.0001$) (Table 12).

This is in contrast to stored raw pork (Part II of this dissertation) in which all of the 455nm-affiliated correlations were less than those involving 532nm and 495nm for raw pork. This difference was most likely due to the high aldehyde levels from cooking-induced lipid instability.

Table 1. Effects of STPP addition and refrigerated storage on measurement of 532nm *water-based* TBARS absorbances of cooked comminuted pork[1,2,3]

STPP Level	Day 0	Day 4	Day 7	Day 14	Average
0.00%	0.3494**a**	1.8743**a**	1.9677**ab**	2.1958**a**	1.5968**a**
0.01%	0.3037**a**	1.7637**b**	2.0037**a**	2.1947**a**	1.5665**ab**
0.05%	0.2972**a**	1.7576**b**	1.9000**b**	2.1244**ab**	1.5198**cd**
0.10%	0.3014**a**	1.7876**b**	1.9516**ab**	2.1514**ab**	1.5480**bc**
0.15%	0.2858**a**	1.7523**b**	1.9384**ab**	2.1467**ab**	1.5308**bcd**
0.25%	0.3073**a**	1.7136**bc**	1.9897**a**	2.1380**ab**	1.5372**bcd**
0.35%	0.2983**a**	1.7101**bc**	1.9394**ab**	2.1413**ab**	1.5223**cd**
0.50%	0.2974**a**	1.6589**c**	1.9465**ab**	2.0972**b**	1.5000**d**
Avg.	0.3051	1.7523	1.9546	2.1487	1.5402
S.E.	0.0397	0.0397	0.0397	0.0397	0.0198
C.V.	15.94%	2.77%	2.49%	2.26%	3.16%
Sig.					(P<0.001)

[1]N=3 observations per mean, N=12 per "Average" mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

[3]Abbreviations: Avg.= average, S.E.= standard error, C.V.= coefficient of variability, Sig.= level of statistical significance of STPP level effect.

Table 2. Effects of STPP addition and refrigerated storage on measurement of 532nm *acid-based* TBARS absorbances of cooked comminuted pork[1,2,3]

STPP Level	Day 0	Day 4	Day 7	Day 14	Average
0.00%	0.2643**a**	1.6318**a**	1.7429**ab**	2.0676**a**	1.4267**a**
0.01%	0.2527**a**	1.5511**b**	1.8034**a**	2.0662**a**	1.4183**a**
0.05%	0.2471**a**	1.5399**b**	1.7228**b**	1.9957**ab**	1.3764**bc**
0.10%	0.2490**a**	1.5620**ab**	1.7603**ab**	2.0193**ab**	1.3977**ab**
0.15%	0.2373**a**	1.5217**bc**	1.7455**ab**	1.9984**ab**	1.3757**bc**
0.25%	0.2554**a**	1.5167**bc**	1.8026**a**	2.0086**ab**	1.3958**ab**
0.35%	0.2467**a**	1.5057**bc**	1.7517**ab**	2.0059**ab**	1.3775**bc**
0.50%	0.2403**a**	1.4564**c**	1.7479**ab**	1.9598**b**	1.3511**c**
Avg.	0.2491	1.5357	1.7596	2.0152	1.3899
S.E.	0.0385	0.0385	0.0385	0.0385	0.0195
C.V.	18.95%	3.07%	2.68%	2.34%	3.43%
Sig.					(P<0.01)

[1]N=3 observations per mean; N=12 per "Average" mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

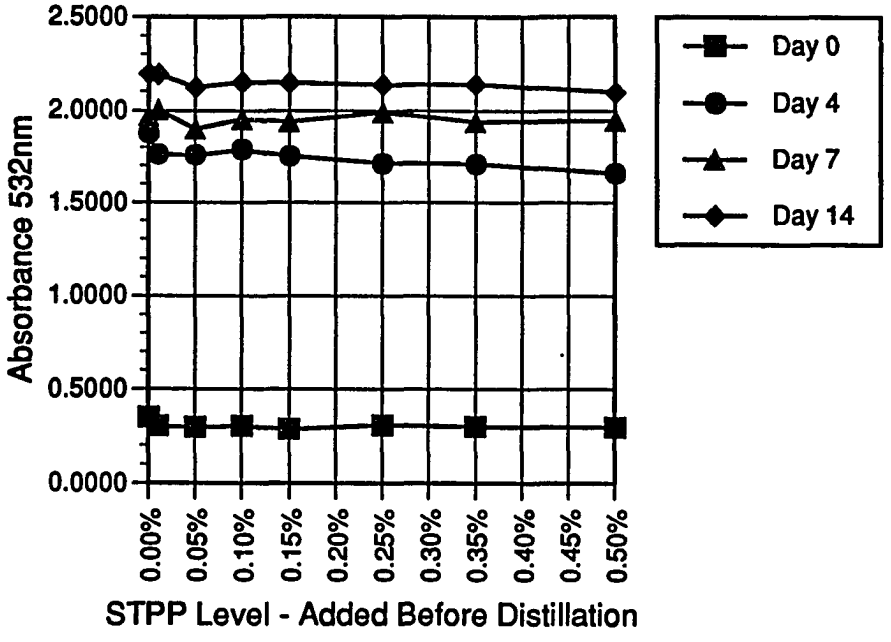

Figure 1. Effects of added STPP and refrigerated storage on 532nm *water-based* TBARS of cooked comminuted pork

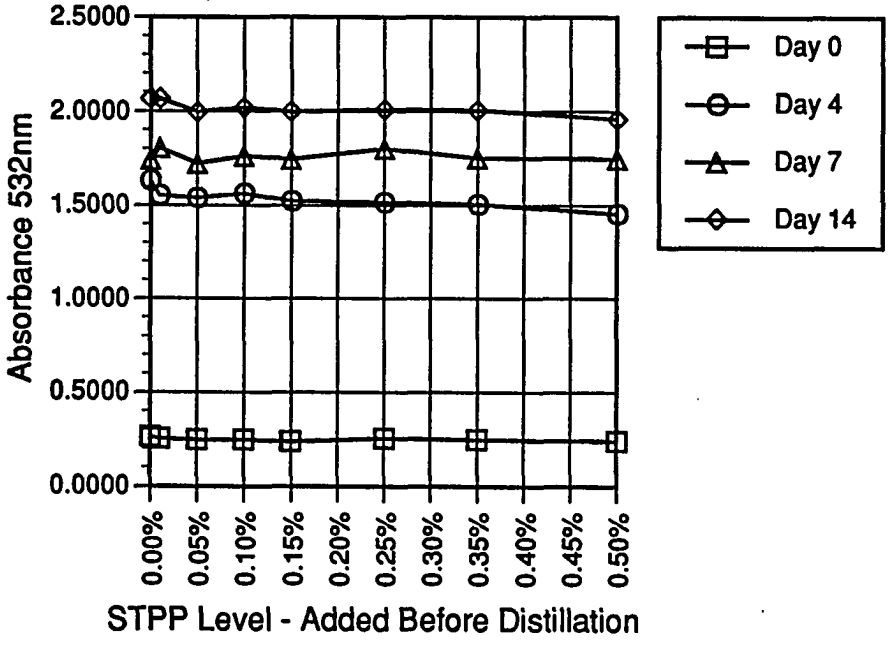

Figure 2. Effects of added STPP and refrigerated storage 532nm *acid-based* TBARS of cooked comminuted pork

Table 3. STPP effect on measurement of *water-based* TBARS absorbances and ratios of cooked comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	1.5968**a**	0.4425**a**	0.0994**a**	0.2772**a**	0.0698**a**	0.2508**a**
0.01%	1.5665**ab**	0.4354**ab**	0.0997**a**	0.2820**a**	0.0730**a**	0.2563**a**
0.05%	1.5198**cd**	0.4185**cd**	0.0967**a**	0.2778**a**	0.0749**a**	0.2663**a**
0.10%	1.5480**bc**	0.4301**abc**	0.1005**a**	0.2806**a**	0.0764**a**	0.2690**a**
0.15%	1.5308**bcd**	0.4249**bcd**	0.0991**a**	0.2822**a**	0.0781**a**	0.2710**a**
0.25%	1.5372**bcd**	0.4271**bcd**	0.0994**a**	0.2813**a**	0.0753**a**	0.2628**a**
0.35%	1.5223**cd**	0.4208**cd**	0.0978**a**	0.2803**a**	0.0765**a**	0.2671**a**
0.50%	1.5000**d**	0.4153**d**	0.0974**a**	0.2820**a**	0.0775**a**	0.2676**a**
Avg.	1.5402	0.4208	0.0987	0.2804	0.0752	0.2639
S.E.	0.0198	0.0065	0.0019	0.0026	0.0034	0.0122
C.V.	3.16%	3.76%	4.75%	2.28%	10.94%	11.29%
Sig.	(P<0.001)	(P<0.01)				

[1]N=12 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 4. STPP effect on measurement of *acid-based* TBARS absorbances and ratios of cooked comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	1.4267**a**	0.3645**a**	0.0948**a**	0.2635**a**	0.0781**a**	0.2932**a**
0.01%	1.4183**a**	0.3598**ab**	0.0938**a**	0.2527**a**	0.0732**a**	0.2896**a**
0.05%	1.3764**bc**	0.3476**cd**	0.0897**a**	0.2529**a**	0.0777**a**	0.3068**a**
0.10%	1.3977**ab**	0.3538**abc**	0.0915**a**	0.2520**a**	0.0745**a**	0.2961**a**
0.15%	1.3757**bc**	0.3496**bcd**	0.0897**a**	0.2531**a**	0.0733**a**	0.2900**a**
0.25%	1.3958**ab**	0.3533**abc**	0.0923**a**	0.2528**a**	0.0767**a**	0.3031**a**
0.35%	1.3775**bc**	0.3483**cd**	0.0910**a**	0.2512**a**	0.0727**a**	0.2900**a**
0.50%	1.3511**c**	0.3399**d**	0.0853**a**	0.2528**a**	0.0745**a**	0.2931**a**
Avg.	1.3899	0.3521	0.0910	0.2539	0.0751	0.2952
S.E.	0.0195	0.0056	0.0037	0.0056	0.0034	0.0122
C.V.	3.43%	3.90%	9.83%	5.44%	11.15%	10.09%
Sig.	($P<0.01$)	($P<0.01$)				

[1]N=12 observations per mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

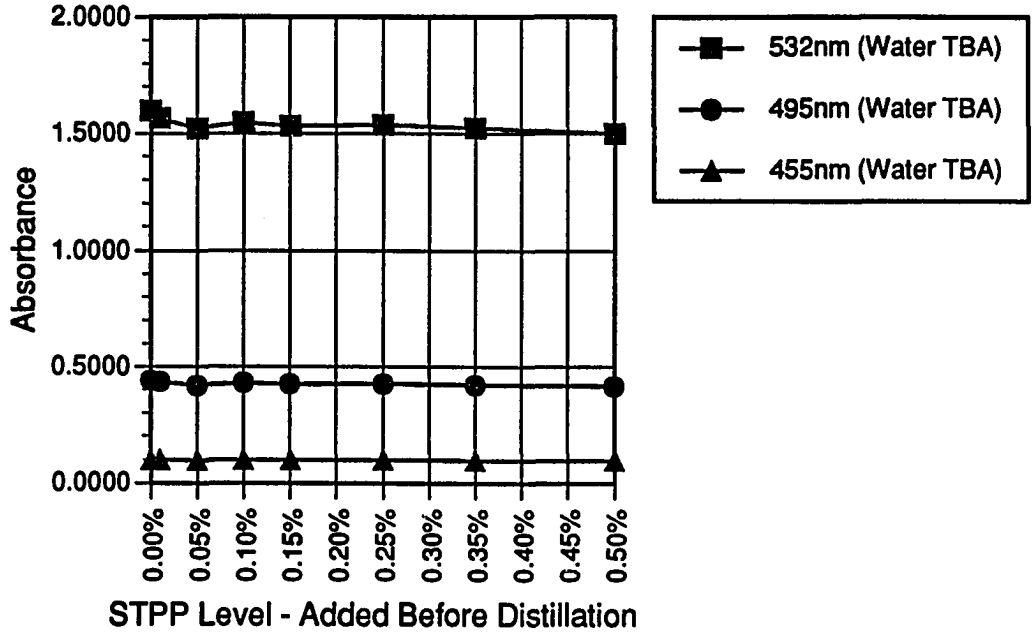

Figure 3. Effects of added STPP on 532nm, 495nm and 455nm *water-based* TBARS of cooked comminuted pork

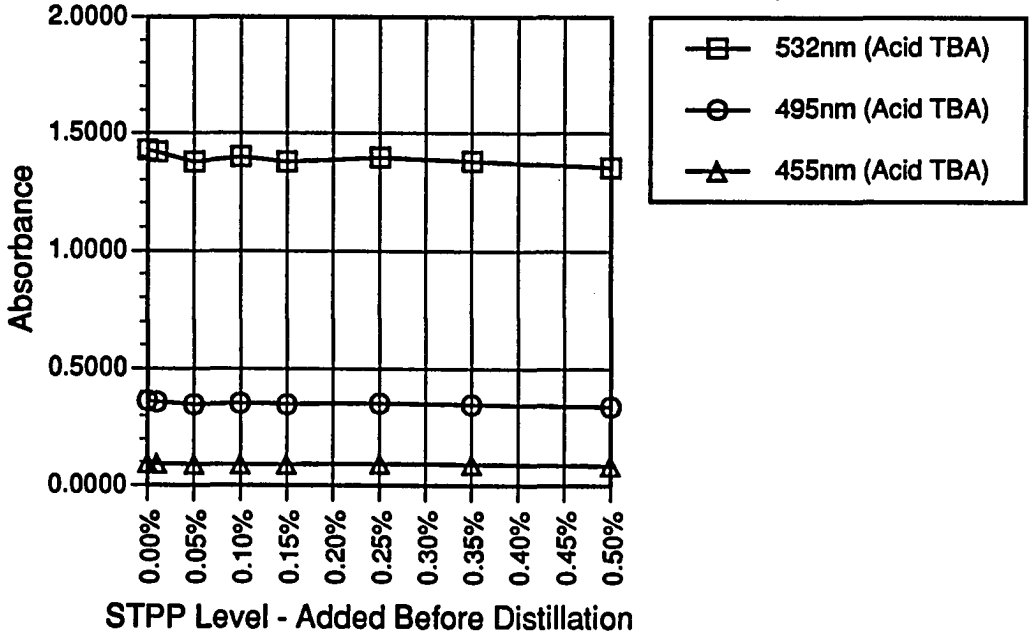

Figure 4. Effects of added STPP on 532nm, 495nm and 455nm *acid-based* TBARS of cooked comminuted pork

Table 5. Refrigerated storage effect on *water-based* TBARS absorbances and ratios of cooked comminuted pork[1,2]

Storage Day #	Wavelength 532nm	Wavelength 495nm	Wavelength 455nm	Wavelength Ratio 495nm/532nm	Wavelength Ratio 455nm/532nm	Wavelength Ratio 455nm/495nm
Day 0	0.3051**d**	0.0899**d**	0.0354**d**	0.2952**a**	0.1167**a**	0.3882**a**
Day 4	1.7523**c**	0.4669**c**	0.1049**b**	0.2663**a**	0.0598**b**	0.2245**b**
Day 7	1.9546**b**	0.5356**b**	0.1181**ab**	0.2740**a**	0.0606**b**	0.2206**b**
Day 14	2.1487**a**	0.6148**a**	0.1365**a**	0.2861**a**	0.0636**b**	0.2223**b**
Avg.	1.5402	0.4268	0.0987	0.2804	0.0752	0.2639
S.E.	0.0460	0.0150	0.0096	0.0131	0.0161	0.0377
C.V.	3.66%	4.31%	11.91%	5.74%	26.16%	17.53%
Sig.	(P<0.0001)	(P<0.0001)	(P<0.001)		(P<0.05)	(P<0.01)

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 6. Refrigerated storage effect on *acid-based* TBARS absorbances and ratios of cooked comminuted pork[1,2]

Storage Day #	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
Day 0	0.2491**c**	0.0637**c**	0.0271**c**	0.2578**a**	0.1098**a**	0.4261**a**
Day 4	1.5357**b**	0.3840**b**	0.0985**b**	0.2500**a**	0.0640**b**	0.2555**b**
Day 7	1.7596**ab**	0.4414**ab**	0.1076**b**	0.2508**a**	0.0610**b**	0.2433**b**
Day 14	2.0152**a**	0.5192**a**	0.1309**a**	0.2568**a**	0.0655**b**	0.2558**b**
Avg.	1.3899	0.3521	0.0910	0.2539	0.0751	0.2952
S.E.	0.1079	0.0356	0.0068	0.0064	0.0050	0.0189
C.V.	9.50%	12.39%	9.15%	3.09%	8.24%	7.86%
Sig.	(P<0.0001) (P<0.001)	(P<0.0001)	(P<0.0001)		(P<0.001)	

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

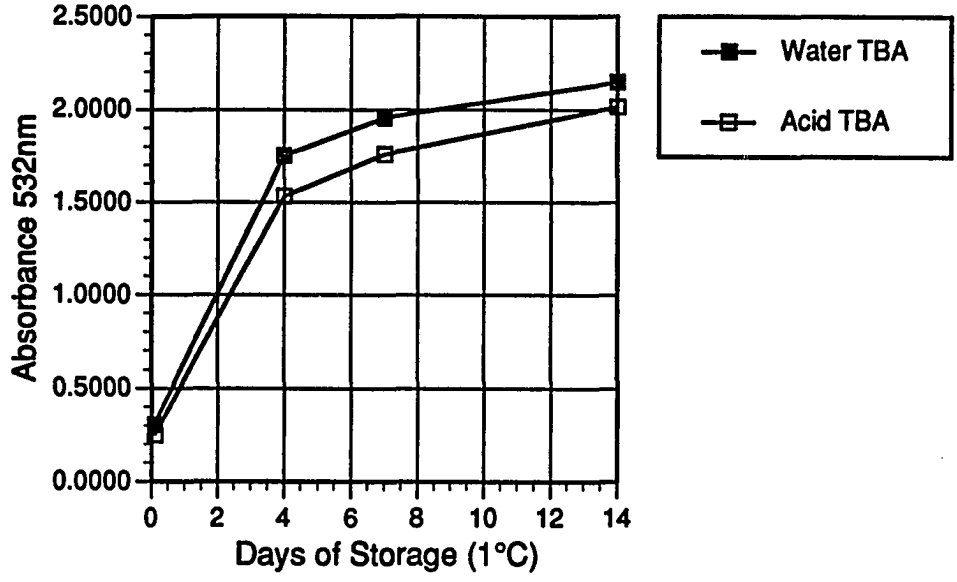

Figure 5. Effects of refrigerated storage and TBA solution on 532nm TBARS of cooked comminuted pork

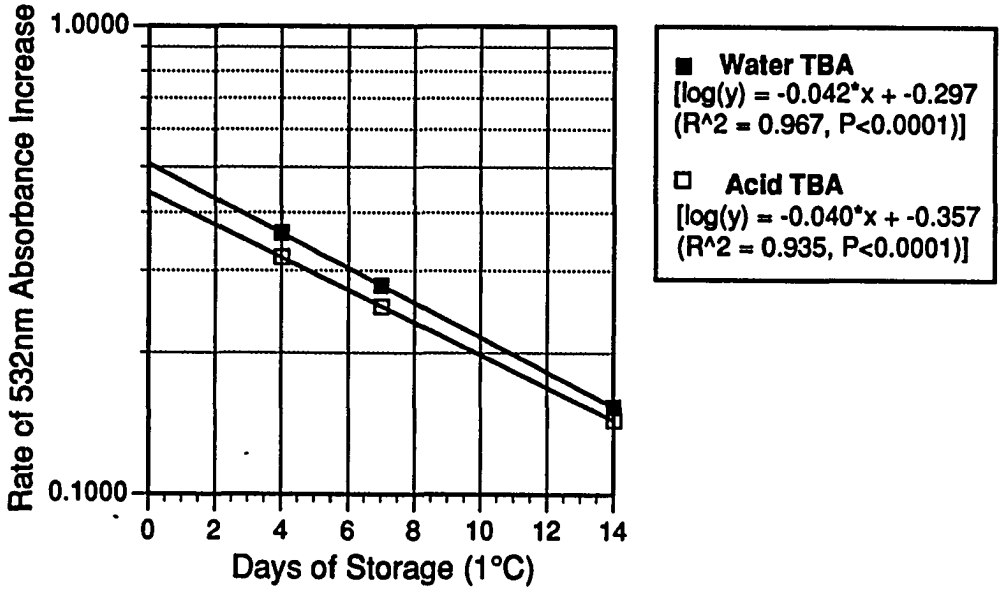

Figure 6. Semilogarithmic plot of refrigerated storage effect on rate of 532nm *water- and acid-based* TBARS increases of cooked comminuted pork

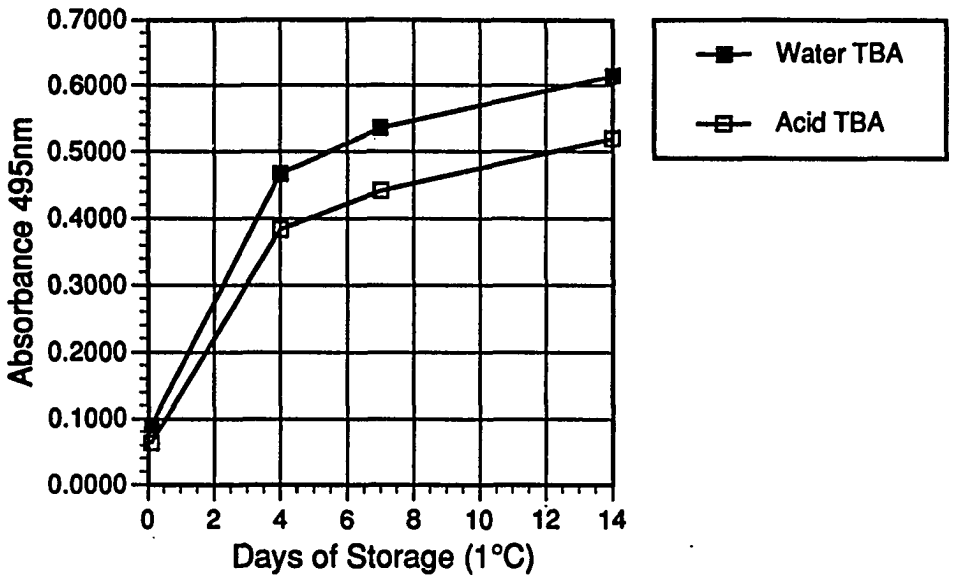

Figure 7. Effects of refrigerated storage and TBA solution on 495nm TBARS of cooked comminuted pork

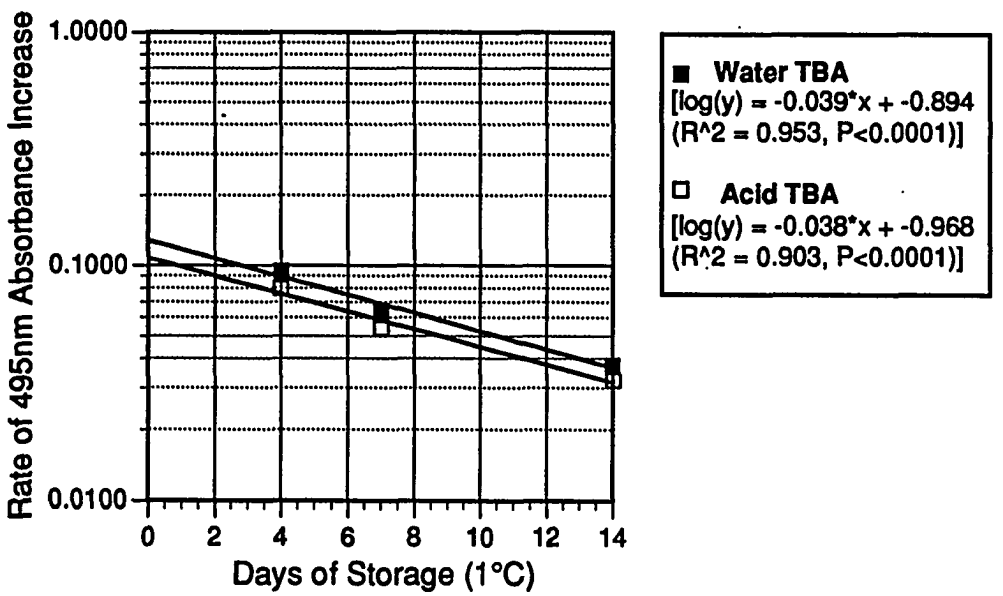

Figure 8. Semilogarithmic plot of refrigerated storage effect on rate of 495nm *water- and acid-based* TBARS increases of cooked comminuted pork

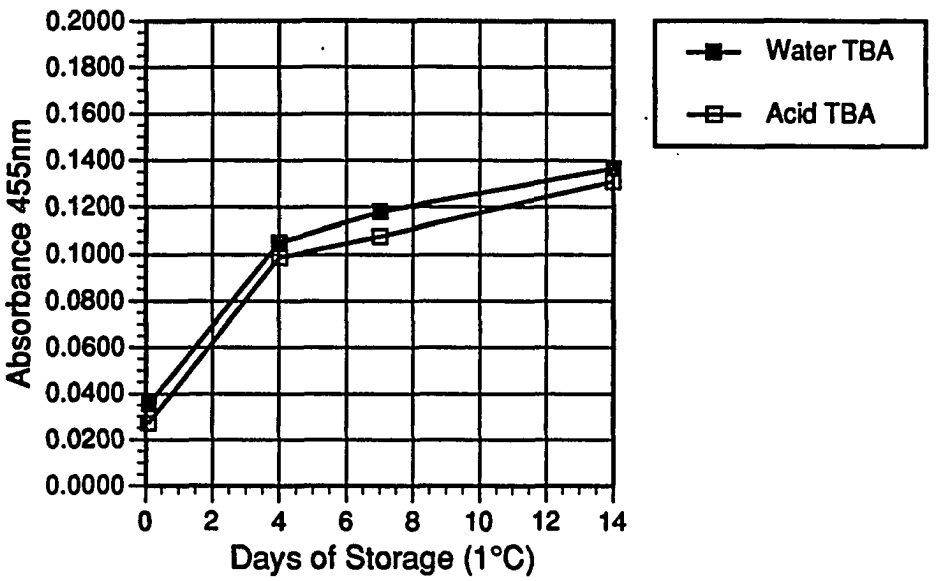

Figure 9. Effects of refrigerated storage and TBA solution on 455nm TBARS of cooked comminuted pork

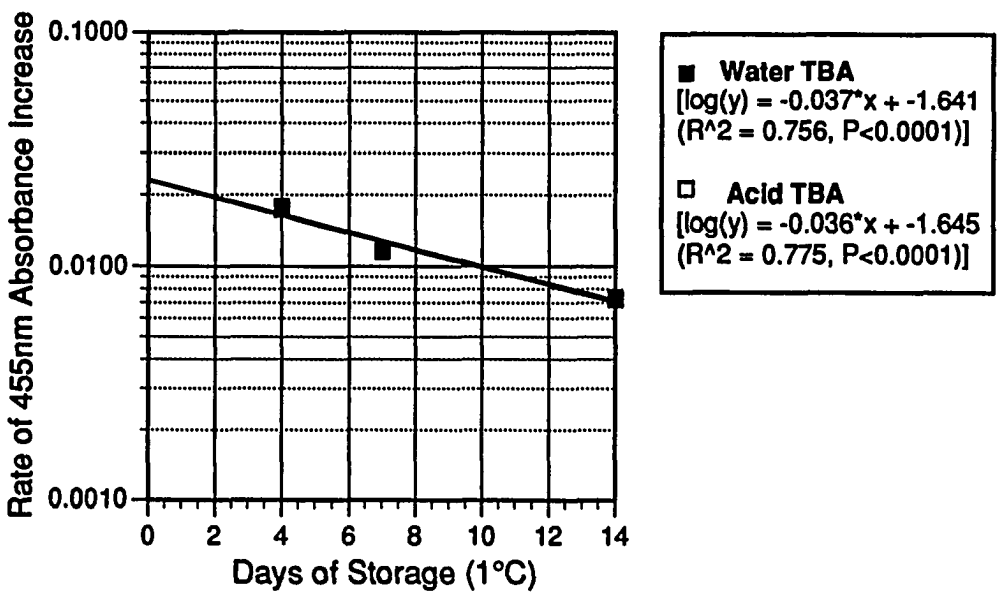

Figure 10. Semilogarithmic plot of refrigerated storage effect on rate of 455nm *water- and acid-based* TBARS increases of cooked comminuted pork

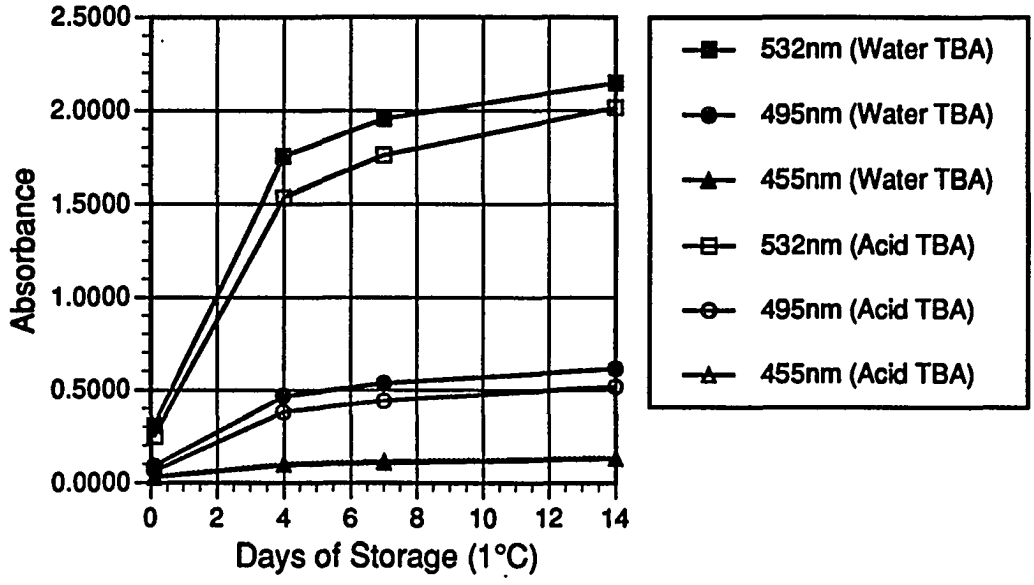

Figure 11. Linear comparison of 532nm, 495nm and 455nm TBARS of cooked comminuted pork as affected by refrigerated storage and TBA solution

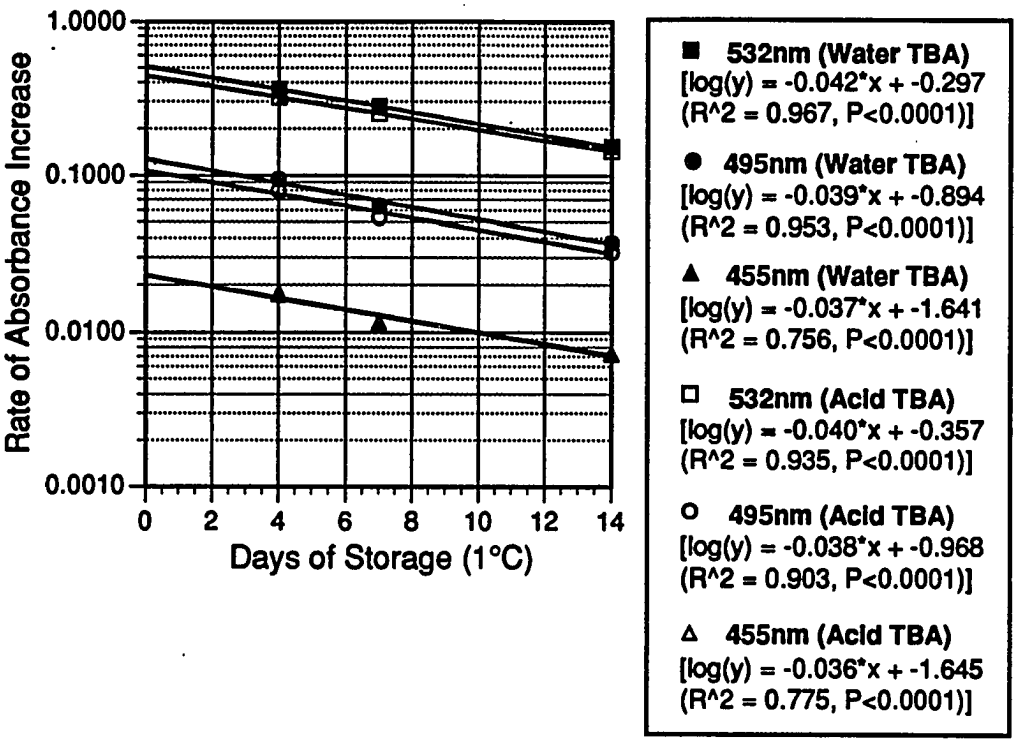

Figure 12. Semilogarithmic comparison of rate of 532nm, 495nm and 455nm TBARS increases of cooked comminuted pork as affected by refrigerated storage and TBA solution

Table 7. Comparison of 532nm, 495nm & 455nm (*water- and acid-based*) changes in TBARS formation rates (Abs./day) of cooked comminuted pork during refrigerated storage[1]

Relative Rate	Water	Acid	Water/Acid
532nm	-0.042	-0.040	1.050
495nm	-0.039	-0.038	1.026
455nm	-0.037	-0.036	1.028
532nm:495nm	1.077	1.053	1.023
532nm:455nm	1.135	1.111	1.022
495nm:455nm	1.054	1.056	0.998

[1]Calculated from slopes of equations presented in Figures 6, 8, 10 and 12.

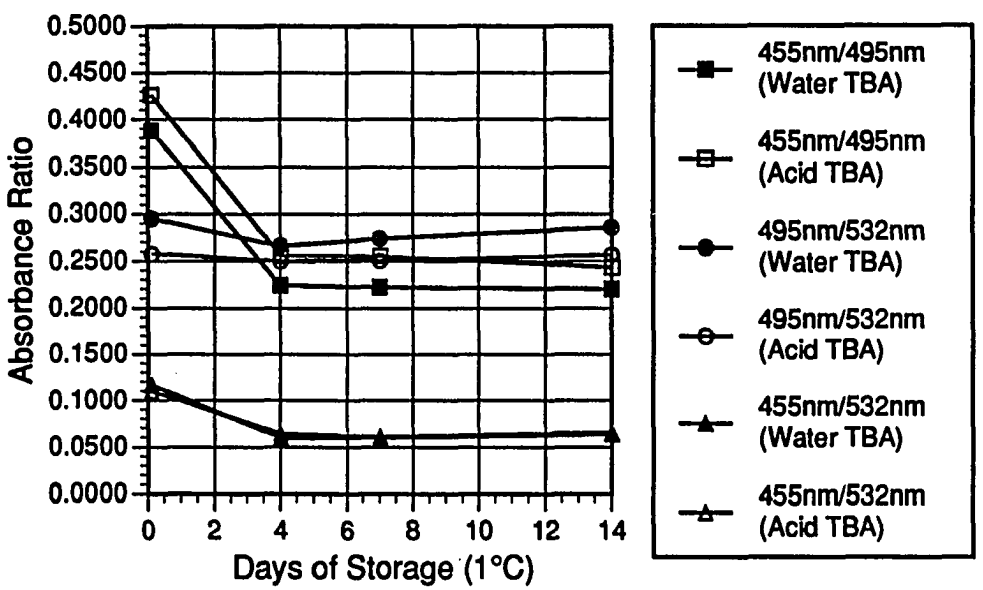

Figure 13. Refrigerated storage and TBA solution effects on average 455nm/495nm, 495nm/532nm and 455nm/532nm TBARS absorbance ratios of cooked comminuted pork

Table 8. TBA solvent effects on TBARS absorbances during refrigerated storage of cooked comminuted pork[1,2]

Solvent	Day 0	Day 4	Day 7	Day 14	Average
532nm[3,4]					
Water	0.3051**a**	1.7523**a**	1.9546**a**	2.1487**a**	1.5402**a**
Acid	0.2491**b**	1.5357**b**	1.7596**b**	2.0152**b**	1.3899**b**
Avg.	0.2771	1.6440	1.8571	2.0819	1.4650
S.E.	0.0244	0.0244	0.0244	0.0244	0.0122
(W-A)[5]	0.0560	0.2166	0.1950	0.1335	
495nm[3,4]					
Water	0.0899**a**	0.4669**a**	0.5356**a**	0.6148**a**	0.4268**a**
Acid	0.0637**a**	0.3840**b**	0.4414**b**	0.5192**b**	0.3521**b**
Avg.	0.0768	0.4255	0.4885	0.5670	0.3894
S.E.	0.0244	0.0244	0.0244	0.0244	0.0042
(W-A)[5]	0.0262	0.0829	0.0943	0.0956	
455nm[3]					
Water	0.0354**a**	0.1049**a**	0.1181**a**	0.1365**a**	0.0987**a**
Acid	0.0271**b**	0.0985**b**	0.1076**b**	0.1309**b**	0.0910**b**
Avg.	0.0312	0.1017	0.1129	0.1337	0.3894
S.E.	0.0024	0.0024	0.0024	0.0024	0.0012

[1]N=24 observations per mean; N=96 per "Average" mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

[3]Significant TBA solvent effect (P≤0.0001).

[4]Significant TBA solvent*Day interaction (P≤0.0001).

[5](W-A) = Water minus Acid for wavelengths demonstrating significant TBA solvent*Day interaction.

Table 9. TBA solvent effects on TBARS absorbance ratios during refrigerated storage of cooked comminuted pork[1,2]

Solvent	Day 0	Day 4	Day 7	Day 14	Average
495nm/532nm[3,4]					
Water	0.2952**a**	0.2663**a**	0.2740**a**	0.2861**a**	0.2804**a**
Acid	0.2578**b**	0.2500**b**	0.2508**b**	0.2568**b**	0.2538**b**
Avg.	0.2765	0.2582	0.2624	0.2715	0.2671
S.E.	0.0047	0.0047	0.0047	0.0047	0.0024
(W-A)[5]	0.0374	0.0163	0.0232	0.0293	
455nm/532nm					
Water	0.1167**a**	0.0598**a**	0.0606**a**	0.0636**a**	0.0751**a**
Acid	0.1098**a**	0.0640**a**	0.0610**a**	0.0655**a**	0.0751**a**
Avg.	0.1133	0.0619	0.0608	0.0646	0.0751
S.E.	0.0039	0.0039	0.0039	0.0039	0.0020
455nm/495nm[3]					
Water	0.3882**b**	0.2245**b**	0.2206**b**	0.2223**b**	0.2639**b**
Acid	0.4261**a**	0.2557**a**	0.2433**a**	0.2558**a**	0.2952**a**
Avg.	0.4071	0.2401	0.2320	0.2391	0.2796
S.E.	0.0092	0.0092	0.0092	0.0092	0.0046

[1]N=24 observations per mean; N=96 per "Average" mean.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

[3]Significant TBA solvent effect ($P < 0.0001$).

[4]Significant TBA solvent*Day interaction ($P \leq 0.05$).

[5](W-A) = Water minus Acid for wavelengths demonstrating significant TBA solvent*Day interaction.

Table 10. Correlations of *water-based* TBARS absorbances and ratios of cooked comminuted pork throughout refrigerated storage[1]

	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
532nm	0.9971 (P<0.0001)	0.9684 (P<0.0001)	-0.3822 (P<0.0001)	-0.7765 (P<0.0001)	-0.8388 (P<0.0001)
495nm		0.9782 (P<0.0001)	-0.3272 (P<0.001)	-0.7458 (P<0.0001)	-0.8136 (P<0.0001)
455nm			-0.2038 (P<0.05)	-0.6307 (P<0.0001)	-0.7064 (P<0.0001)

[1]N=96 observations.

Table 11. Correlations of *acid-based* TBARS absorbances and ratios of cooked comminuted pork throughout refrigerated storage[1]

	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
532nm	10.9979 (P<0.0001)	0.9661 (P<0.0001)		-0.8628 (P<0.0001)	-0.8860 (P<0.0001)
495nm		0.9646 (P<0.0001)		-0.8454 (P<0.0001)	-0.8749 (P<0.0001)
455nm				-0.7544 (P<0.0001)	-0.7756 (P<0.0001)

[1]N=96 observations.

Table 12. Correlations of *water-based* and *acid-based* TBARS absorbances of cooked comminuted pork throughout refrigerated storage[1,2]

	532nm(A)	495nm(A)	455nm(A)
532nm(W)	0.9880 (P<0.0001)	0.9776 (P<0.0001)	0.9616 (P<0.0001)
495nm(W)	0.9892 (P<0.0001)	0.9809 (P<0.0001)	0.9694 (P<0.0001)
455nm(W)	0.9556 (P<0.0001)	0.9464 (P<0.0001)	0.9661 (P<0.0001)

[1]N=96 observations.

[2]Acid-based TBARS absorbances designated by "(A)"; water-based TBARS by "(W)".

CONCLUSIONS

Increased STPP level significantly ($P<0.01$) decreased 2-thiobarbituric acid absorbance values at 532nm and 495nm, with both water- and acid-based TBA. However, these differences were minimal and do not explain the lipid oxidative stability conferred by STPP on comminuted muscle foods immediately after cooking.

Absorbance values increased throughout refrigerated storage. However, the rate of net oxidation increases from Day 0 decreased logarithmically with time.

Water TBA solvent resulted in significantly greater values for absorbances at 532nm, 495nm and 455nm. These differences became greater with higher oxidation levels.

Based on the differential at which the wavelength absorbances increase with advancing lipid oxidation, the 455nm/495nm and 495nm/532nm wavelength absorbance ratios (WARS) converged with higher levels of oxidation. Therefore as lipid oxidation advances to a maximum, the secondary oxidation product mix becomes such that a similar absorbance relationship is achieved between adjacent wavelengths (i.e., 455nm/495nm = 495nm/532nm). This relationship has also been observed in raw pork muscle (Part II of this dissertation), and may be indicative of a maximum oxidation status from readily oxidizable lipids.

REFERENCES

Ande, C. F. 1985. The influence of polyphosphate on meat pH and its relationship to lipid and myoglobin oxidation. Ph.D. dissertation, Auburn University.

Asakawa, T., and Matsushita, S. 1979. Thiobarbituric acid test for detecting lipid peroxides Lipids 14(4): 401.

Asakawa, T., and Matsushita, S. 1980. Coloring conditions of thobarbituric acid test for detecting lipid hydroperoxides. Lipids 15(3): 137.

Cochran, W. G. and Cox, G. M. 1957. *Experimental Designs*, 2nd ed. John Wiley & Sons, New York, NY.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M. and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28: 187.

Diosady, L. L. 1988. Private communication. Dept. of Chem. Engineering and Appl. Chem., Univ. of Toronto.

Empson, K. L., Labuza, T. P. and Graf, E. 1991. Phytic acid as a food antioxidant. J. Food Sci. 56(2): 560.

Gardner, H. W. 1975. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J Agric. Food Chem. 23(2): 129.

Gardner, H. W. 1983. Effects of lipid hydroperoxides on food components. In *Xenobiotics in Foods and Feeds*, D. E. Schwass and J. W. Finley (Ed.), p. 63. ACS Symp. Series No. 234, American Chemical Society, Washington, DC.

Gardner, H. W. 1987. Reactions of hydroperoxides-products of high molecular weight. Ch. 3. In *Autoxidation of Unsaturated Lipids*, H. W.-S. Chan (Ed.), p. 51. Academic Press, Inc., London.

Huffman, D. L., Ande, C. F., Cordray, J. C., Stanley, M. H. and Egbert, W. R. 1987. Influence of polyphosphate on storage stability of restructured beef and pork nuggets. J. Food Sci. 52(2): 275.

Igene, J. O., Yamauchi, K., Pearson, A. M., Gray, J. I. and Aust, S. D. 1985. Evaluation of 2-thiobarbituric acid reactive substances (TBRS) in relation to warmed-over flavor (WOF) development in cooked chicken. J. Agric. Food Chem. 33: 364.

Irani, R. R. and Morgenthaler, W. W. 1963. Iron sequestration by polyphosphates. J. Am. Oil. Chem. Soc. 40(7): 283.

Janero, D. R. and Burghardt, B. 1989. Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed lipid peroxidation: influence of peroxidtion conditions. Lipids 24(2): 125.

Kim, R. S. and LaBella, F. S. 1987. Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. J. Lipid Res. 28: 1110.

Kolodziejska, I., Skonieczny, St. and Rubin, L. J. 1990. Malondialdehyde-nitrite interactions in meat and model systems. J. Food Sci. 55(4): 925.

Koniecko, E. S. 1979. *Handbook for Meat Chemists*. Avery Publishing Group, Inc., Wayne, NJ.

Kosugi, H., Kojima, T. and Kikugawa, K. 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24(10): 873.

Kosugi, H., Kojima, T. and Kikugawa, K. 1991. Characteristics of the thiobarbituric acid reactivity of oxidized fats and oils. INFORM 2(2): 154.

Mann, T. F., Reagan, J. O., Lillard, D. A., Campion, D. R., Lyon, C, E. and Miller, M. F. 1989. Effects of phosphate in combination with nitrite or Maillard reaction products upon warmed-over flavor in precooked, restructured beef chuck roasts. J. Food Sci. 54(6): 1431.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7): 105.

Melton, S. L. 1985. Methods of assessing oxidation. In *Proceedings of the Meat Industry Research Conference*, Chicago, IL, October 18-19. American Meat Institute, Washington, DC.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351.

Pryor, W. A., Stanley, J. P. and Blair, E. 1976. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids 11(5): 370.

Raharjo, S., Sofos, J. N., Maga, J. A. and Schmidt, G. R. 1989. Influence of meat restructuring systems on lipid oxidation in beef. Lebensm. Wiss. Technol. 22: 199.

Roozen, J. P. 1987. Effects of Types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavour. Food Chem. 24: 167.

Sato, K. and Hegarty, G. R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36: 1098.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C., Jr. and Dupuy, H. P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. J. Food Sci. 52(5): 1163.

Steel, R. G. D. and Torrie, J. H. 1980. *Principles and Procedures of Statistics*, 2nd ed. McGraw-Hill Book Company, New York, NY.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. R., Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39(1): 34.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Tims, M. J. and Watts, B. M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

PART IV.

SODIUM TRIPOLYPHOSPHATE INTERACTION WITH LIPID OXIDATION OF COOKED COMMINUTED PORK MUSCLE

Sodium tripolyphosphate interaction with lipid oxidation of cooked comminuted pork muscle

by

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Running Title: STPP and Cooked Pork Lipid Oxidation

ABSTRACT

Comminuted pork muscle was treated with various levels of sodium tripolyphosphate (STPP) (0.00%-0.35%), cooked to an internal temperature of 70°C, and immediately analyzed for total carbonyls and 2-thiobarbituric acid reactive substances (TBARS) at 532nm, 495nm and 455nm. No significant effects were detected by the total carbonyl method ($P>0.05$). Cooking caused significant increases in TBARS formation ($P<0.05$). A threshold STPP level of >0.10% was required for oxidative protection during cooking. Regression analysis for STPP levels of 0.10%-0.35% revealed a very highly significant ($P<0.0001$; $R^2 \geq 0.95$) dependence of linear TBARS absorbance decreases to logarithmic STPP increases. Lipid oxidative assessment of raw meats should include pre-analysis cooking.

INTRODUCTION

The antioxidative activity of sodium tripolyphosphate (STPP) during storage of cooked processed meat products has consistently been observed when added prior to cooking (Sato and Hegarty, 1971; Matlock et al., 1984; Smith et al., 1984; Ang and Hamm, 1986; Shahidi et al., 1986; Huffman et al., 1987; Rooẓen, 1987; Shahidi et al., 1987a, 1987b; Yun et al., 1987; King and Earl, 1988; Ang and Young, 1989; Mann et al., 1989; Raharjo et al., 1989; Kolodziejska et al. 1990).

Some researchers have suggested, either through graphical presentation of data or direct discussion, that significant oxidative advantages have been realized at "Time 0", immediately at the onset of storage, for cooked meat treatments formulated with polyphosphates (Tims and Watts, 1958; Igene et al.1985; Huffman et al., 1987, Raharjo et al., 1989). A plausible explanation offered for these observations has been polyphosphate interference with the 2-thiobarbituric acid (TBA) test of Tarladgis et al. (1960) (Koniecko, 1979; Diosady, 1988; Raharjo et al., 1989).

Potential TBA interference has been analyzed via postcook addition of sodium tripolyphosphate (STPP) to refrigerated comminuted muscle immediately prior to TBA analysis; no interaction of practical significance occurred (Part II of this dissertation).

Huffman et al. (1987) speculated that an observed difference in initial TBA values (at Time 0) between a polyphosphate treatment and control was due to polyphosphate antioxidative activity during cooking or very shortly thereafter. Heat and oxygen exposure during the meat cooking process would be expected to affect the oxidative stability of constituent lipids. Rapidly increasing lipid oxidation development (with no lag period) in cooked comminuted muscle has been observed from the onset of refrigerated storage (1°C) (Part II of this dissertation). Raharjo et al. (1989) reported significantly higher TBA values for freshly cooked meat samples relative to those in the raw state. The investigation of polyphosphates in controlling lipid oxidation typically has been aimed at demonstrating oxidative advantages after postcook storage; evaluation of this effectiveness in controlling cooking-originated lipid oxidation has not been reported.

Distillate obtained from meat products for lipid oxidation assessment via the TBA analysis according to Tarladgis et al. (1960) has been reported to contain aldehydes of several classes (St. Angelo et al., 1987). These aldehydes have been shown to react with TBA and contribute absorbance at the wavelengths of 532nm, 495nm and 455nm but their expression at the different wavelengths is affected by choice of TBA solvent (water vs. acetic acid) (Part I of this dissertation).

Utilization of these absorbances in wavelength absorbance ratios (WARS) has been shown to provide additional information in determining relative progress of lipid oxidation in meats (Parts II and III of this dissertation).

Carbonyl determinations measure potentially volatile oxidation products, as well as nonvolatile carbonyl compounds that are probable precursors (Gray, 1978). Melton (1983) concluded that the method reported by Lawrence (1965) or its modification (Keller and Kinsella, 1973) was the most accurate method for assessment of total carbonyl compounds in muscle foods (Melton, 1985). Utilization of this relatively convenient assay to corroborate lipid oxidative determinations obtained via the TBA test would be of interest.

The objectives of this study were to: (1) evaluate lipid oxidation levels resulting from the cooking process of comminuted pork muscle, (2) establish STPP concentration-related effects on oxidative stability conferred during cooking, (3) compare TBARS products at 532nm, 495nm, 455nm and the wavelength ratios (WARS) of both water-based and acetic acid-based TBA solutions, (6) evaluate a rapid total carbonyl analysis for lipid oxidation assessment, and correlate it with the distillation TBA test.

MATERIALS AND METHODS

Treatment Preparation

Raw material selection and processing

Fresh, boneless pork picnic shoulders obtained from the Iowa State University (ISU) Meats Laboratory were closely trimmed of external fat, cut into strips for grinding, vacuum-packaged to limit oxygen exposure and stored in a covered box kept in a chill cooler at 1°C for 1-2 days prior to grinding.

On the day of experimentation, the meat strips were removed from storage and subjected to two grindings, first through a 9.52-mm (3/8-in) plate followed by a 3.18-mm (1/8-in) plate. Hand-mixing was performed after the first grinding, and again after the second grinding to promote homogeneity.

Phosphate addition

Sodium tripolyphosphate (STPP) preweighed for addition at the rate of 0.00%, 0.05%, 0.10%, 0.15%, 0.00%, 0.20%, 0.25%, 0.30% and 0.35% in 400-g meat samples was completely dissolved in 10.0 ml deionized distilled water. Meat samples and STPP were added together in a 1.5-L capacity stainless steel mixing bowl, and mixed with a KitchenAid® multifunction mixer, model KSM 90 WH (St. Joseph, MO) equipped with a dough attachment, at the "stir" speed setting for 1 min. After mixing, samples

of approximately 50 g each were removed for the raw meat lipid analysis; the remainder was transferred to 1-L beakers for the cooked treatment.

Cooking

The 1-L beakers of ground meat were transferred to a PRECISION shaking water bath (GCA Corp., Chicago, IL) set at 85°C with a shaker speed of 15 oscillations/min. Meat temperature was monitored via copper/constantin probes connected to an OMEGA Trendicator °C (OMEGA Engineering, Inc., Stamford, CT) digital temperature readout. The ground meat was stirred every 5 min; heat was applied to an endpoint of 70°C, upon which the beakers were removed and placed into an icewater bath until analyzed.

Analyses

Product Composition

Representative meat samples were analyzed for fat and moisture utilizing a CEM Moisture/Solids Analyzer, Model AVC 80 equipped with 100-g balance capacity and automatic extraction system (Matthews, NC). A representative raw meat sample from each replication was first thoroughly comminuted to a paste-like texture utilizing a Cuisinart Basic Food Processor (Greenwich, CT). A sample size of 4 g was used for the combination moisture/fat analyses. The CEM analyzer was

programmed selecting "Mode M=3" for alternating meat moisture/fat analysis. CEM analyzer moisture parameters were power=90% and time=4:00 min; fat parameters were power=100% and time=3:30 min.

2-Thiobarbituric acid (TBA) analysis

Sample distillation After mixing with STPP, the samples were prepared as described by Tarladgis et al. (1960) for distillation, with the exception that the mixed 10-g samples were transferred into 250-ml round-bottomed flasks, 96.5 ml deionized distilled water were added, followed by 2.5 ml 4N HCl. The 250-ml round-bottomed flasks were then transferred for homogenization to a Kinematica Polytron PT 10/35 equipped with a PTA 20 shaft assembly (Brinkman Instruments Co., Westbury, NY) at a speed setting of "6" for 30 sec. After homogenization, a few drops of antifoam C and glass boiling beads were added prior to randomized placement of the flasks onto distillation units. Heat was applied stepwise uniformly to all treatment samples. Approximately 25-30 min was required for 50 ml of distillate which was collected into 50-ml Erlenmeyer flasks, capped with parafilm, and stored in the dark at 1°C until analyzed within 24 hr.

Reaction with TBA For comparison, solutions of 0.02M 2-Thiobarbituric acid were prepared utilizing one of two different solvents: 90% glacial acetic acid ("acid-based") as

presented by Tarladgis et al. (1960) or water ("water-based") as recommended by other researchers (Tarladgis et al., 1962, 1964; Crackel et al., 1988). After removal from ≤24 hr storage and prior to uncapping, the 50-ml Erlenmeyer flasks were shaken/swirled to ensure homogenization of distillate prior to sample distillate extraction. Two aliquots of 5.0 ml of distillate were extracted; 1 aliquot was added to 5.0 ml of water-based and the other to 5.0 ml of acid-based 0.02M TBA solutions in test tubes and tightly capped. The test tubes then were incubated in a 95°C water bath for 35 minutes to promote color formation. Immediately after water bath removal, the test tubes were subjected to a cold water rinse in preparation for spectrophotometric analysis.

Spectrophotometric analysis Sample were then analyzed for 2-thiobarbituric acid reacting substances (TBARS) on a GILFORD Response® UV-VIS Spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH) equipped with a rapid sampler accessory set for a sample time of 0.5 sec and an equilibration time of 2 sec. Wavelength scans with bandwidth set at 1.0 nm and scan increment of 0.5 nm were utilized to verify wavelength peaks. Absorbance readings were then performed at the selected wavelengths of 455nm, 495nm and 532nm. Similar solvent-based (water- vs. acid-based) STPP treatments were analyzed together; order of solvent-based analyses was varied with each replication. Blanks based upon

the appropriate solvent were utilized at the beginning of each solvent family analysis.

Total Carbonyls

The method of Keller and Kinsella (1973) utilizing 2,4-dinitrophenylhydrazine (DNP) was used with modifications. Meat samples of 5.0 g were added to 125-ml capacity, opaque polyethylene bottles equipped with screw-on caps, to which 20.0 ml of deionized distilled water were subsequently added. Homogenization was then performed on a Kinematica Polytron PT 10/35 equipped with PTA 20 shaft assembly (Brinkman Instruments Co., Westbury, NY) at a speed setting of "6" for 30 sec.

After homogenization, 25.0 ml of DNP reagent (2 g of 2,4-dinitrophenylhydrazine/1 L 2N HCl) were added, oxygen was purged with a nitrogen stream, the bottles were capped and subsequently hand-shaken for 15 sec. The bottles were then placed in the dark for incubation; a time period of 30 min at ambient temperature was experimentally determined (data not presented) to be adequate reaction time.

After incubation, 50 ml hexane were added, the bottles capped, and shaken vigorously for 15 sec. The mixture from each sample was then transferred to two 50-ml round-bottomed polypropylene centrifuge tubes equipped with screw-on caps, and centrifuged for 10 min at 6500 rpm (at 5°C) on a Beckman

model J-21C centrifuge (Palo Alto, CA) with a JA-20 aluminum fixed-angle rotor.

After centrifuging, approximately 15-ml samples were decanted from the top layer and transferred to capped test tubes. Spectrophotometric analysis for 2,4-dinitrophenylhydrazones was performed as outlined above for the TBA test, except a wavelength of 340nm was used. Results were analyzed and reported as absorbance readings.

Statistical analysis

Experimental design was based on a split, split-plot format (Cochran and Cox, 1957; Steel and Torrie, 1980). Main plots were days of experimentation divided into heat treatments, subdivided into the different STPP levels which, in turn, were subdivided into type of TBA solvent (water vs. acid). Three replications were performed; duplicates were taken for every reading and averaged to yield one data figure for analysis. TBARS and total carbonyls absorbance readings were statistically analyzed directly without mathematical conversion. Data analysis was performed utilizing the Statistical Analysis System, version 6.06.01, (SAS Institute Inc., Cary, NC). Significance between treatment and subtreatment means was tested using the least significant difference (lsd) procedure, but recognized only if a treatment significant F test resulted [Fisher's (protected) lsd] (Steel

and Torrie, 1980). Standard errors and coefficients of variability were calculated from SAS results (Cochran and Cox, 1957; Steel and Torrie, 1980). All statistics for absorbance ratios (495nm/532nm, 455nm/532nm and 455nm/495nm) were calculated from the total model (versus data point wavelength averages); regression equations and coefficients derived for TBARS formation rates presented in the legend tables of plotted averages were calculated from the total model as well.

RESULTS AND DISCUSSION

Product Composition

Average fat and water content of the ground pork in this study was 13.74% and 65.76%, respectively.

Cooking Effects on Oxidation

2-Thiobarbituric acid reactive substances (TBARS)

Typical time for the ground pork to achieve an internal temperature of 70°C was 25 min in a 90°C water bath (Figure 1).

Cooking of the ground pork caused dramatic oxidative increases in both water and acid-based 532nm TBARS relative to the uncooked group (Tables 1 and 2, Figure 2). The increase of meat lipid oxidation due to cooking has been reported before (Younathan and Watts, 1960; Apte and Morrissey, 1987; Raharjo et al., 1989). The raw STPP curve served primarily as a baseline for comparison; the largest contrasts between raw and cooked are at STPP levels ≤0.10% (Figure 2). Comparison of the raw and cooked STPP concentration curves graphically demonstrates STPP antioxidative effect with increasing concentration; this effect will be discussed in a later section.

Average differences between the heat categories are discussed below according to TBA solvent category.

Water-based TBARS Cooking dramatically increased average water-based TBARS at 532nm ($P<0.05$) and 495nm ($P<0.01$), and the wavelength absorbance ratios (WARS) of 495nm/532nm ($P<0.01$), 455nm/532nm ($P<0.01$) and 455nm/495nm ($P<0.05$) (Table 1). No effects were detected for 455nm ($P>0.05$).

Acid-based TBARS Acid-based TBARS at 532nm ($P<0.05$), 495nm ($P<0.05$) and the 455nm/532nm WARS ($P<0.05$) increased due to cooking (Table 2). As with the water-based equivalents, 455nm was not affected.

The acid-based 455nm/495nm WARS differences were not significant due to aberrant low 495nm values in replication #2. More variability existed in the acid-based values than in the water-based, as evidenced by coefficients of variability (Table 1 and 2).

General cooking effects on oxidation The cooking procedure utilized in this study applied extreme oxidative stress on the ground pork; stirring every 5 min incorporated oxygen into the system and promoted homogeneous distribution of endogenous catalysts. Ramsey and Watts (1963) cooked ground muscle in the absence of air and observed no cooking-originated lipid oxidation. Water bath temperature also was a factor; Arganosa et al. (1989) demonstrated that cooking to an internal temperature of 65°C yielded higher TBARS with increasing water bath temperatures of 70, 85 and 100°C.

Different mechanisms to explain cooking-induced meat lipid oxidation have been suggested and possibly are simultaneously exerting their influences. Igene et al. (1979) concluded that cooking of meat released significant amounts of non-heme iron from heme pigments. In foods, the main catalysts of homolytic hydroperoxide decomposition are transition metal ions (Gardner, 1975). Additionally, it has been reported that cooking causes a large increase in free fatty acids (Yamauchi, 1973; Willemot et al., 1985), which are recognized to oxidize at a faster rate than esterified fatty acids (Kim and LaBella, 1987; Saidia and Hammond, 1989).

A study was performed (Wanous, 1990) to assess the influence of endpoint cooking temperature on the resultant water-based 532nm TBARS of ground pork muscle. Cooking endpoint temperatures were limited to 40°C (≈100°F), 50°C (≈120°F) and 60°C (≈140°F) (Figure 3); TBA analysis was performed simultaneously during cooking (Figure 4). Results indicated that endpoint temperatures of ≥50°C exhibited a greater increase of lipid oxidation as measured by 532nm TBARS (Figure 4). Potential mechanisms behind this observation were not investigated and further research in this area is needed. Several conceivable mechanisms may have been involved. (1) Denaturation of the myoglobin heme, with concomitant release of endogenous iron, could catalyze hydroperoxide decomposition. (2) Reaching the melting temperature of animal

fats would enable a change in transition state from solid to liquid, enhancing their mobility and increasing contact with oxidation catalysts. (3) Free fatty acid formation, with their characteristic increased susceptibility to oxidation, would cause increased oxidation levels. (4) Denaturation of amino acids/proteins which constitute endogenous reducing enzyme systems of the muscle would decrease its oxidative stability. (5) Reaching of a threshold temperature providing adequate heat energy would directly promote formation of secondary lipid oxidation products.

Total carbonyls

Though the total carbonyl values for the cooked pork appeared greater than those for the raw pork (Table 3, Figure 5), these differences were not statistically significant ($P<0.05$) due to variability among the readings for the two heat treatments. This inconsistency with the TBA trends questions the sensitivity of the total carbonyl analysis, or at least of this particular modification.

STPP Effect on Cooking-Originated Lipid Oxidation

The primary goal of this study was determination of STPP effects in controlling lipid oxidation during cooking. Heat*STPP level interactions for 532nm TBARS (water- and acid-based) were significant ($P<0.0001$); oxidation was significantly lowered in the cooked treatment with increasing

STPP concentration but no effects were demonstrated in the raw pork (Figure 2). STPP antioxidative abilities in cooked ground meat have been confirmed by other researchers by utilizing gas chromatography (Love and Pearson, 1976; Dupuy, 1987).

Because STPP level had no impact (P>0.05) on 532nm TBARS of the raw product (Figure 2) nor on any of the other oxidative measurements performed on the raw ground pork (data not presented), the raw treatment will not be analyzed further. The raw pork analyses confirmed the lack of STPP interference with the distillation TBA analysis as previously reported (Part II of this dissertation); these values served as a baseline to compare STPP effect (Figure 2), and provided useful WARS values for fresh pork to gauge the utility of these additional tools in meat lipid oxidation assessment (Tables 1 and 2).

2-Thiobarbituric Acid Reactive Substances (TBARS)

No interaction existed between STPP level and TBA solvent (P>0.05). However, differences in variability, and consequently statistical significance, of various TBARS measurements between the two solvent groups directly affected calculated STPP effects.

Wavelengths Because of its ability to detect multiwavelength absorbances of prominent lipid oxidation

aldehyde classes, the TBA test is a potentially useful tool for estimating a broad range of lipid oxidation products (Kosugi et al., 1989). Other research studies (Parts I, II and III of this dissertation) have analyzed meat lipid TBARS at 532nm, 495nm and 455nm (to include TBA solvent influence) and calculated changes in these wavelengths with oxidation as well as correlations between these measurements. This practice was continued in the current study.

Water-based TBARS As referred to earlier, Figure 2 graphically describes STPP concentration-related effectiveness in repressing oxidation arising during the cooking process; as STPP level increased, the 532nm TBARS cooked curve converged with the raw baseline curve (Figure 2).

STPP significantly lowered cooking-originated TBARS absorbances at 532nm ($P<0.0001$) and 495nm ($P<0.0001$) at increasing concentrations (Table 4, Figure 6). Though TBARS at 455nm appeared to decrease at STPP levels ≥0.30% (Figure 7), these differences were not significant ($P>0.05$).

Acid-based TBARS Acid-based TBARS were significantly lowered by increasing STPP levels at 532nm ($P<0.0001$), 495nm ($P<0.0001$) and at 455nm (Table 5, Figures 6 and 7). Acid TBA has been shown to favor expression of 455nm TBARS (Parts I and II of this dissertation), which has been associated with alkanal (e.g., hexanal) content (Part I of this dissertation). Shahidi et al. (1987b) evaluated STPP-

controlled lipid oxidation in cooked ground pork via the TBA distillation method and gas chromatographic analysis of hexanal; the two methods demonstrated high correlation in showing STPP was effective at limiting lipid oxidation.

Calculated STPP concentration effects The STPP concentration effects on 532nm and 495nm TBARS during cooking of ground pork appeared to occur above a threshold STPP level of 0.10% (Tables 4 and 5, Figure 6). Regression analysis of data from the total model for the boxed-in area of Figure 6, (i.e., inclusive of data from STPP levels in the range of 0.10%-0.35%), is presented in Figure 8.

A diminishing marginal ability of STPP to yield identical absorbance decreases existed for STPP levels in the range 0.10%-0.35%. Regression analysis suggested a very highly significant relationship ($P<0.0001$; $R^2 \geq 0.96$) between linear 532nm and 495nm TBARS (water- and acid-based) decreases, and logarithmic STPP level increases (Figure 8).

Results encompassed by these findings have been suggested in the literature. Ramsey and Watts (1963) observed some oxidative protection in cooked muscle at a STPP level of 0.10%. A threshold STPP level of approximately 0.15% to limit lipid oxidation in cooked muscle has been reported (Shahidi et al. 1986; Yun et al., 1987). Dupuy et al. (1987) reported 0.20% STPP in cooked ground meat to control lipid oxidation. Ande (1985) and Huffman et al. (1987) concluded that 0.25%

polyphosphate controlled lipid oxidation (TBARS formation) to the same extent as 0.50% polyphosphate.

Advantages exist in limiting STPP addition for antioxidative properties in cooked products to the most effective range, and using it in combination with other antioxidants. Chambers et al. (1990) recommended addition at ≤0.30% to minimize STPP flavor detection in meat products. Shahidi et al. (1987a) reported that 0.30% STPP yielded lower TBARS values, but was not as effective as phenolic antioxidants such as BHA and TBHQ, or sodium nitrite. Some benefits may exist in combination with phenolic antioxidants, however; STPP and rosemary oleoresin (a primary antioxidant) have demonstrated synergistic effects in a meat model system (Liu et al., 1991).

Wavelength absorbance ratios (WARS) Utilizing the alternate wavelengths, WARS have been calculated in other studies to assess their utility as additional tools in lipid oxidation analysis (Parts II and III of this dissertation). They are based on the differential rates at which the wavelength absorbances increase with advancing lipid oxidation (i.e., 532nm>495nm>455nm).

495nm/532nm Both water- and acid-based 495nm/532nm ratios increased nonsignificantly ($P>0.05$) with STPP level (Tables 4 and 5). As the level of lipid oxidation decreased, the 455nm/495nm absorbance ratio increased faster

than the 495nm/532nm ratio, and the two curves increasingly diverged with increased oxidative stability (Figure 9); this was seen earlier with refrigerated raw and cooked pork (Parts II and III of this dissertation). In a system of malondialdehyde as the TBARS reactant, a 495nm/532nm ratio of approximately 0.2500 was maintained when water was the TBA solvent (0.2200 for acid-based) (Part I of this dissertation). In this study, STPP at 0.00%-0.10% expressed the highest levels of water-based TBARS and had the lowest 495nm/532nm values (≈0.2800, Table 4); though STPP at 0.00%-0.10% expressed the highest acid-based TBARS, acid-based 495nm/532nm values ranged from ≈0.2400-0.2500 throughout the STPP concentration range of 0.00%-0.30% (Table 5).

455nm/532nm Neither the water-based or the acid-based 455nm/532nm WARS demonstrated significant reaction to STPP levels ($P>0.05$); standard errors and coefficients of variation were the highest for this measurement for both solvent type categories (Tables 4 and 5). The 455nm/532nm absorbance ratio curves approximately paralleled those for the 495nm/532nm ratios, though values for the 455nm/532nm figures were lower (Figure 10).

455nm/495nm STPP level significantly increased ($P<0.05$) the water-based 455nm/495nm WARS (Table 4, Figure 9). STPP influence on the acid-based equivalent approached significance ($P=0.08$); aberrant values for the 0.30% STPP

level contributed to variability for the acid-based category (Figure 9). The STPP-level associated values, and range of values, were greater for the acid-based than for the water-based 455nm/495nm WARS (Tables 4 and 5, Figure 9 and 10).

WARS summary It was previously observed (Parts II and III of this dissertation) that with increased pork lipid oxidation, the 455nm/495nm curve decreased and converged upon the 495nm/532nm ratio as their values approximated each other. Cooked pork with extensive lipid oxidation during refrigerated storage yielded curves for these two ratios which overlapped (Part II of this dissertation). In this study, values for these two ratios approached each other at 0.00%-0.10% STPP, and began their divergence with increasing STPP concentration-related antioxidative effectiveness (Tables 4 and 5, Figure 9).

The acid-based 495nm/532nm range of values was shorter than the water-based, demonstrating less responsiveness to STPP level (Tables 4 and 5, Figure 9); the converse was true for the acid-based 455nm/495nm ratio. As a result, the 455nm/495nm and the 495nm/532nm ratios demonstrated more divergence than the water-related equivalents (Figure 9); this was also observed for refrigerated raw pork (Part II of this dissertation). TBA solvent-related effects will be discussed later.

STPP antioxidant mechanism Traditionally, phosphoric acid derivatives have been recognized to extend the shelf life of lipid-containing foods because of their ability to chelate transition metals (Gordon, 1990) which are the main catalysts of homolytic hydroperoxide decomposition (Gardner, 1975). The work of Igene et al. (1985), however, would lead to the conclusion that STPP does not act as an antioxidant through sequestration of metals in the cooked pigment extract. Other plausible reactions/mechanisms/contributions of STPP have been proposed, such as hydroperoxide interaction through direct reduction (Frankel, 1980) or polymerization (Privett and Quackenbush, 1954), reaction with free fatty acids (Sherman, 1961; Craig et al., 1990; Schantz and Bowers, 1990), ionic strength contribution (McDonald and Hultin, 1987; Ang and Young 1989) and pH increase (Ande, 1985).

A study was performed (Wanous, 1990) to further assess the capability of STPP to inhibit cooking-originated lipid oxidation. Three treatments were placed in a heated waterbath, cooked to a 70°C internal temperature and cooled; analysis for water-based 532nm TBARS formation was performed during cooking. In addition to a control (0.00% STPP), one treatment was formulated with 0.15% STPP (precook addition) and the other treatment had 0.15% STPP added midway through cooking (Figure 11). The 532nm TBARS of the control rapidly increased throughout the cooking process and the 0.15% STPP

added before cooking inhibited cooking-originated TBARS formation. An interesting observation was that STPP added midway arrested further cooking-originated TBARS formation after the time of addition (Figure 11). No other lipid oxidation analysis was performed, but these results suggest further hydroperoxide decomposition into secondary oxidation products was inhibited, since other types of reactions (e.g., reaction with free fatty acids) would have allowed hydroperoxides formed prior to this point to degrade and contribute further to lipid oxidation levels. Because oxidation at this point would be in the autocatalytic propagation phase, halting of further oxidation initiation (with subsequent hydroperoxide formation) would not be expected. Further research is this area measuring other types of lipid decomposition products during cooked is warranted.

Total carbonyls

Effects of STPP on measurements of total carbonyls are in Table 6 and in Figure 5. No STPP effects were observed ($P>0.05$), in spite of a relatively small standard error and coefficient of variability.

An interpretation of these results is that STPP did not affect the level of total lipid oxidation, but affected the final secondary oxidation product mix. Melton (1983) concluded that the method reported by Lawrence (1965) or its

modification (Keller and Kinsella, 1973) was the most accurate method for assessment of total carbonyl compounds in muscle foods (Melton, 1985). McDonald and Hultin (1987) used a total carbonyl method based on spectrophotometric analysis of 2,4-dinitrophenylhydrazine (DNP) products and witnessed changes in values paralleling those for a TBA method.

Another interpretation is that the total carbonyl analysis, or this specific modification, lacks specificity in analyzing for off-flavor compounds. The TBA test analyzes the more volatile compounds, and therefore would be more sensitive to detection of off-flavor components than the total carbonyl test, which theoretically analyzes all of the volatile and nonvolatile carbonyl compounds. The volatile aldehydes responsible for off-flavor development associated with lipid oxidation comprise a small fraction of the secondary oxidation products; the vast majority are nonvolatile aldehydes and ketones. Fernandez et al. (1991) utilized a similar spectrophotometric DNP procedure but observed large fluctuations in their results. Keller and Kinsella (1973) stated that carbonyls of cooked meat include both flavor and off-flavor compounds; this nonspecificity contributes to its imprecision. Melton (1983) reported that some investigators analyzing total carbonyls have seen erratic changes or decreases during refrigerated and frozen storage. This method offers advantages to lipid oxidation analysis but more work

needs to be done regarding interpretation of its results relative to off-flavor development.

TBA Solvent Effects

As has been observed previously, (Parts I, II and III of this dissertation), choice of TBA solvent affected wavelength absorbances and derived wavelength absorbance ratios (WARS). No significant STPP level*TBA solvent interactions were detected ($P>0.05$).

Wavelengths (532nm, 495nm, 455nm)

Water as the TBA solvent in cooked pork lipid analysis resulted in significantly higher absorbance values for 532nm ($P<0.05$) and 495nm ($P<0.0001$) TBARS (Table 7; Figures 6 and 7). Acid-based TBA favored higher TBARS expression at 455nm. Similar findings have been reported previously (Crackel et al., 1988; Parts I and II of this dissertation).

Wavelength absorbance ratios (WARS)

Water-based TBA yielded significantly higher 495nm/532nm ($P<0.001$) and lower 455nm/495nm ($P<0.05$) WARS (Table 7; Figure 9). Because 495nm appears in both ratios, as the numerator of one and the denominator of the other, it appears that water TBA solvent promotes the 495nm TBARS development (relative to acid-based TBA), thereby increasing 495nm/532nm values while decreasing those for 455nm/495nm. Water-based TBA expression

of 455nm TBARS is lower than acid-based, which would also decrease the 455nm/495nm ratio values.

Correlations

As explained earlier, TBA solvent exerted an influence on TBARS at the measured wavelenths, as well as the absorbance ratios calculated from these wavelengths. Correlations were therefore determined for the wavelengths and associated ratios based on TBA solvent.

Water-based TBARS As shown in other work (Parts I & II), 495nm was highly correlated with 532nm ($P<0.0001$) (Table 8). Unexplainable is the observation that absorbances at 455nm were not correlated either with 532nm or 495nm ($P>0.05$). The 455nm TBARS varied erratically with STPP level (Table 4, Figure 7); this variability among 24 observations probably contributed to the lack of correlation for this measurement (Table 8).

The highest wavelength correlation for each of the WARS was with 532nm (Table 8) ($R^2 \approx |0.71|$; $P<0.0001$); next highest correlation for each WARS was with 455nm ($R^2 \geq |0.53|$; $P<0.01$).

The highest correlations for total carbonyls existed with the three ratios ($P<0.01$, $R^2 \geq |0.56|$), which were negatively correlated. Total carbonyls were significantly correlated with 532nm and 455nm, though the correlations were not strong ($P<0.05$, $R^2 \leq |0.40|$). An interesting observation is that the

correlation with 532nm was positive, whereas that with 455nm was negative.

Acid-based TBARS The highest acid-based wavelength correlations were between 495nm and 532nm (R^2=0.98, P<0.0001); the second-highest were between 455nm and 495nm (R^2=0.60; P<0.0001), as has been seen in other research (Parts II and III of this dissertation). Acid-based 455nm was not correlated with any of the wavelength ratios (water-based 455nm was not correlated with either of the other two wavelengths). The highest WARS correlation was between 532nm and 495nm/532nm (R^2=|0.70|; P<0.0001).

The total carbonyls' significant (P<0.01) correlation with acid-based 532nm was greater than with the water-based equivalent (R^2=0.53 vs. 0.41). Correlations of total carbonyls with acid-based WARS were similar to those of the water-based. No correlation existed between total carbonyls and acid-based 455nm.

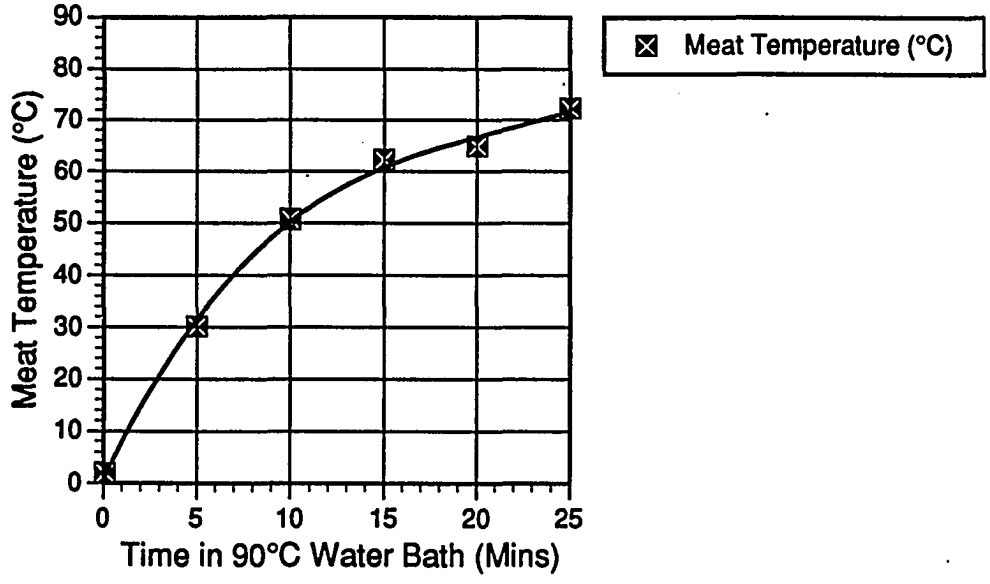

Figure 1. Heating rate for cooked comminuted pork

Table 1. Effect of heat treatment on *water-based* TBARS absorbances of comminuted pork[1,2,3]

Heat Trtmt	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
Cook	0.1263**a**	0.0384**a**	0.0167**a**	0.3331**b**	0.1759**b**	0.4716**b**
Raw	0.0263**b**	0.0147**b**	0.0147**a**	0.5572**a**	0.5599**a**	1.0130**a**
Avg.	0.0763	0.0266	0.0157	0.4452	0.3679	0.7423
S.E.	0.0127	0.0015	0.0015	0.0215	0.0254	0.0995
C.V	20.32%	7.05%	11.48%	5.91%	8.45%	16.41%
Sig.	(P<0.05)	(P<0.01)		(P<0.01)	(P<0.01)	(P<0.05)

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

[3]Abbreviations: Avg.= average, S.E.= standard error, C.V.= coefficient of variability, Sig.= level of statistical significance for treatment effect.

Table 2. Effect of heat treatment on *acid-based* TBARS absorbances of comminuted pork[1,2]

Heat Trtmt	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
Cook	0.1214**a**	0.0296**a**	0.0152**a**	0.2505**a**	0.1576**b**	0.7008**a**
Raw	0.0230**b**	0.0071**b**	0.0128**a**	0.2948**a**	0.5853**a**	2.2314**a**
Avg.	0.0722	0.0184	0.0140	0.2727	0.3715	1.4661
S.E.	0.0120	0.0035	0.0026	0.0245	0.0742	0.8146
C.V.	20.30%	23.04%	23.15%	10.99%	24.45%	68.01%
Sig.	(P<0.05)	(P<0.05)			(P<0.05)	

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

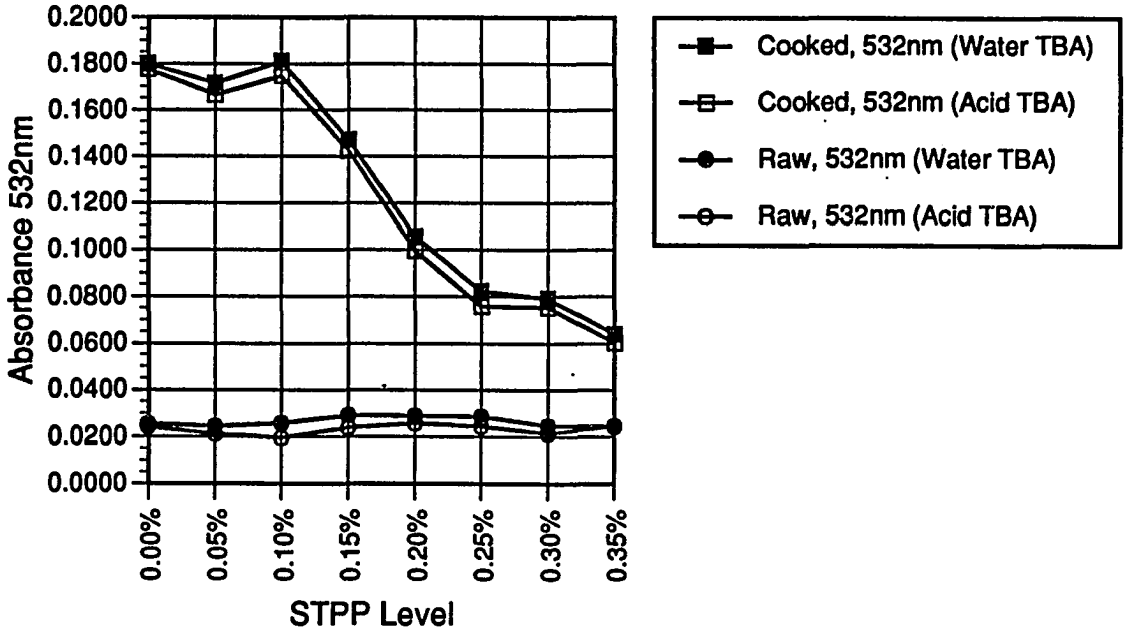

Figure 2. Effect of heat treatment, STPP level and TBA solvent on 532nm TBARS

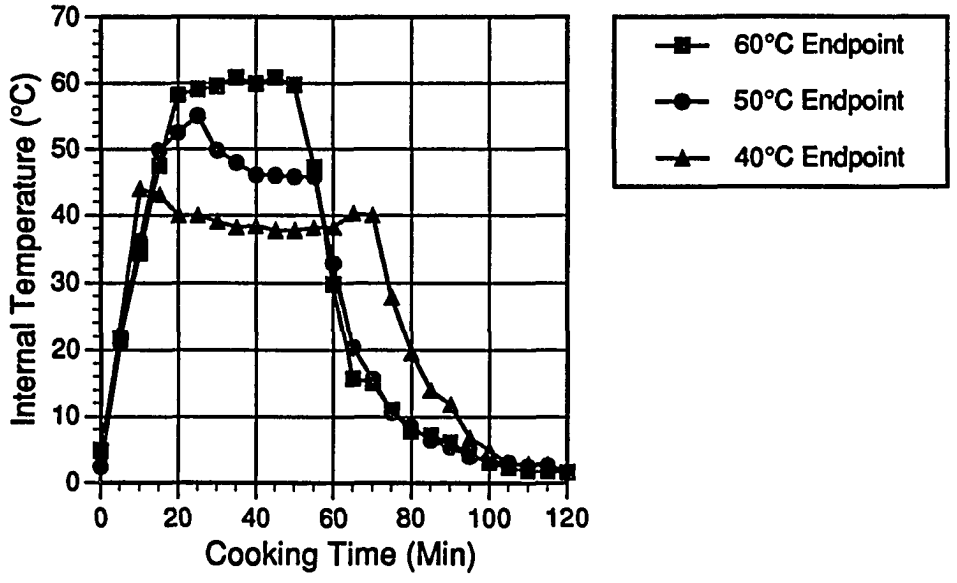

Figure 3. Heating curves for evaluation of endpoint temperature influence on water-based 532nm TBARS of cooked comminuted pork (Figure 4).

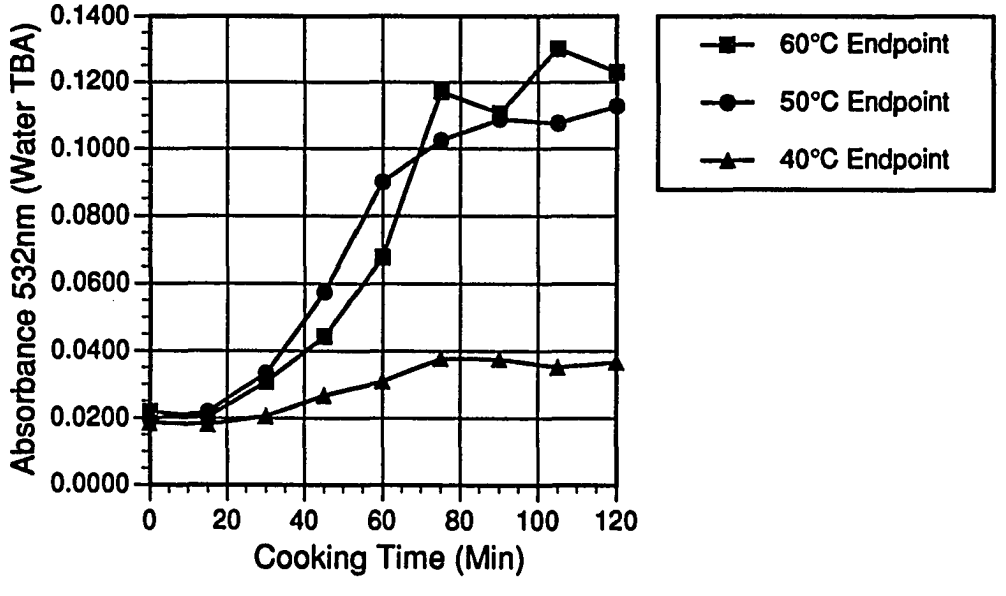

Figure 4. Effect of endpoint temperature on water-based 532nm TBARS of cooked comminuted pork

Table 3. Effect of heat treatment on total carbonyls of comminuted pork[1,2]

Heat Treatment	Absorbance 340nm
Cook	0.3345**a**
Raw	0.2601**a**
Avg.	0.2973
S.E.	0.0504
C.V.	20.77%
Sig.	

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

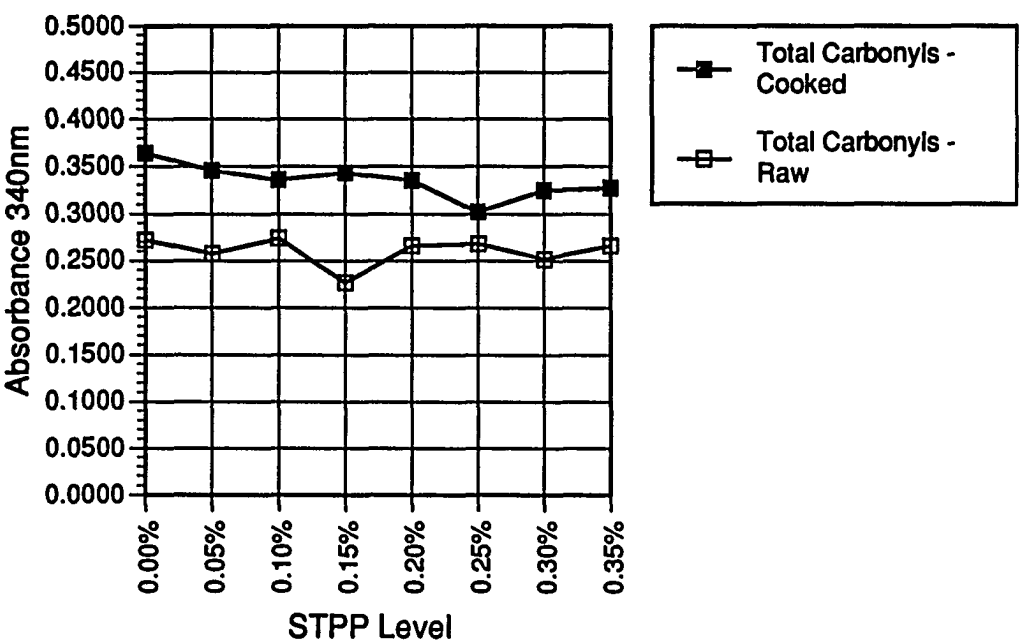

Figure 5. Effect of STPP on total carbonyls of raw and cooked comminuted pork

Table 4. Effect of STPP concentration on *water-based* TBARS absorbances of cooked comminuted pork[1,2]

Heat Trtmt	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	0.1799**a**	0.0503**a**	0.0181**a**	0.2811**a**	0.1003**a**	0.3536**c**
0.05%	0.1714**ab**	0.0487**a**	0.0171**a**	0.2846**a**	0.0997**a**	0.3442**c**
0.10%	0.1810**a**	0.0509**a**	0.0175**a**	0.2831**a**	0.0996**a**	0.3441**c**
0.15%	0.1471**b**	0.0441**a**	0.0164**a**	0.3033**a**	0.1132**a**	0.3710**bc**
0.20%	0.1056**c**	0.0337**b**	0.0174**a**	0.3381**a**	0.1954**a**	0.5410**abc**
0.25%	0.0821**cd**	0.0301**bc**	0.0181**a**	0.3874**a**	0.2563**a**	0.6278**a**
0.30%	0.0791**d**	0.0261**c**	0.0142**a**	0.3758**a**	0.2561**a**	0.5711**ab**
0.35%	0.0639**d**	0.0229**c**	0.0149**a**	0.4112**a**	0.2862**a**	0.6199**a**
Avg.	0.1263	0.0384	0.0167	0.3331	0.1759	0.4716
S.E.	0.0121	0.0034	0.0019	0.0617	0.0863	0.1005
C.V.	11.75%	10.75%	14.07%	22.69%	60.10%	26.05%
Sig.	(P<0.0001)	(P<0.0001)				(P<0.05)

[1]Treatment means represent results from 3 replications.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 5. Effect of STPP concentration on *acid-based* TBARS absorbances of cooked comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
0.00%	0.1775**a**	0.0449**a**	0.0207**a**	0.2525**a**	0.1157**a**	0.4555**a**
0.05%	0.1663**ab**	0.0420**a**	0.0172**ab**	0.2527**a**	0.1030**a**	0.4021**a**
0.10%	0.1745**a**	0.0411**ab**	0.0175**ab**	0.2358**a**	0.1016**a**	0.4290**a**
0.15%	0.1424**b**	0.0334**b**	0.0150**bc**	0.2254**a**	0.1065**a**	0.4491**a**
0.20%	0.0994**c**	0.0232**c**	0.0136**bc**	0.2373**a**	0.1565**a**	0.6439**a**
0.25%	0.0757**cd**	0.0182**c**	0.0132**bc**	0.2485**a**	0.2062**a**	0.7936**a**
0.30%	0.0752**cd**	0.0182**c**	0.0108**c**	0.2583**a**	0.1788**a**	0.6474**a**
0.35%	0.0601**d**	0.0158**c**	0.0133**bc**	0.2834**a**	0.2923**a**	0.9192**a**
Avg.	0.1214	0.0296	0.0152	0.2492	0.1576	0.5925
S.E.	0.0144	0.0037	0.0024	0.0241	0.0695	0.1748
C.V.	14.57%	15.48%	19.52%	11.82%	54.01%	36.05%
Sig.	(P<0.0001)	(P<0.0001)	(P<0.05)			*(P=0.08)*

[1]Treatment means represent results from 3 replications.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

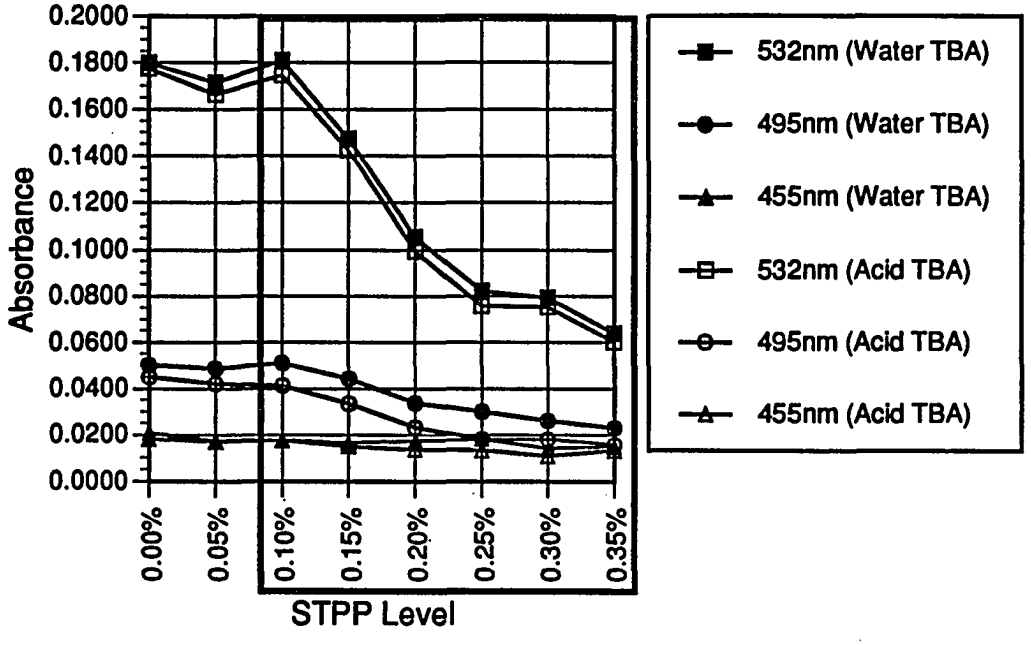

Figure 6. Effect of STPP and TBA solvent on 532nm, 495nm and 455nm TBARS of cooked comminuted pork

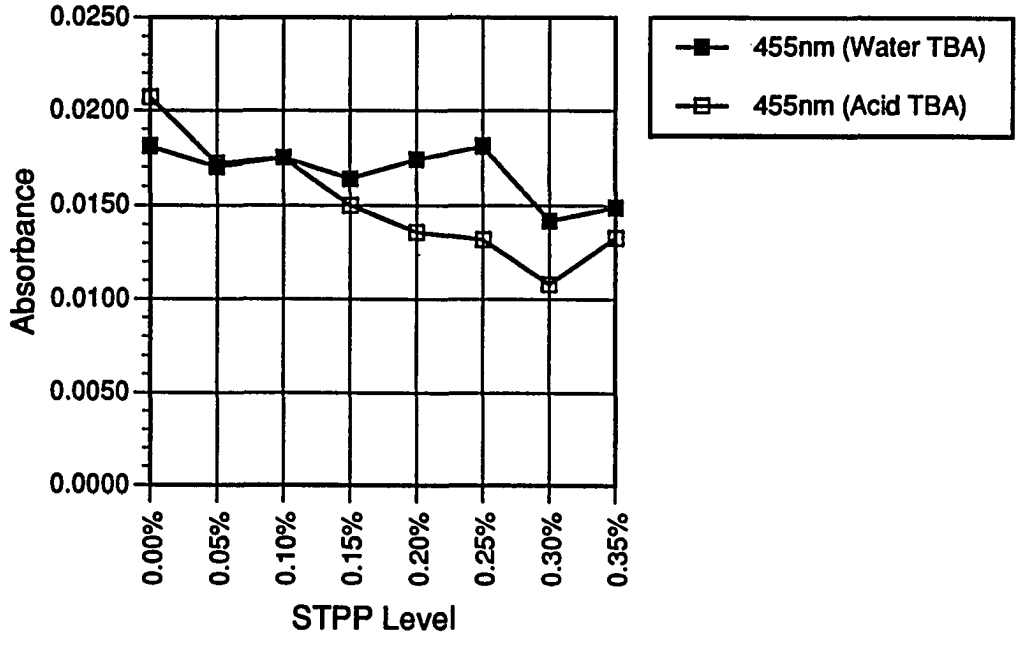

Figure 7. Detailed view comparing STPP and TBA solvent effects on 455nm TBARS of cooked comminuted pork

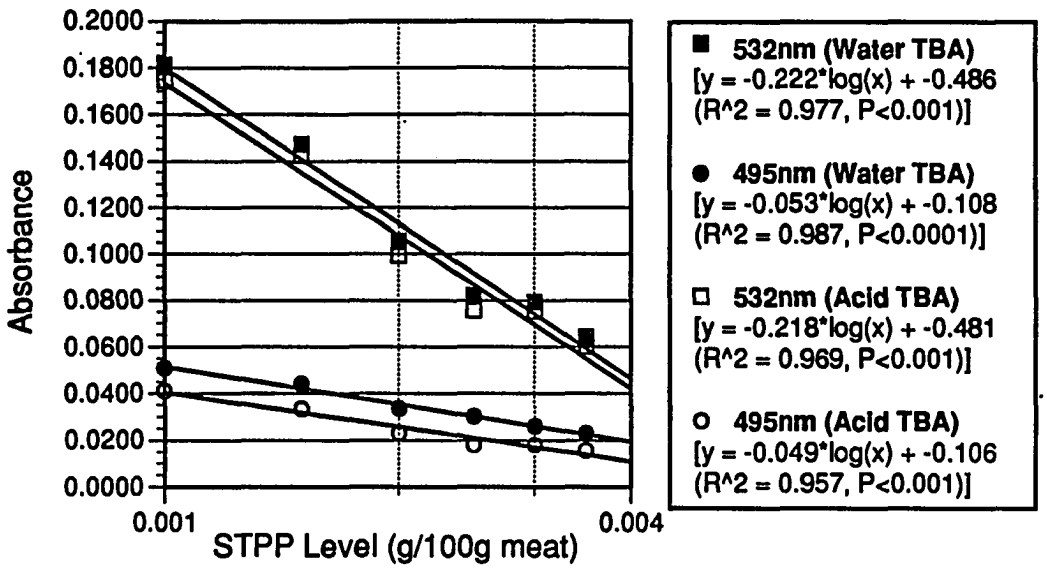

Figure 8. Semilogarithmic effects of STPP level on 532nm and 495nm TBARS *(water- and acid-based)* of cooked comminuted pork

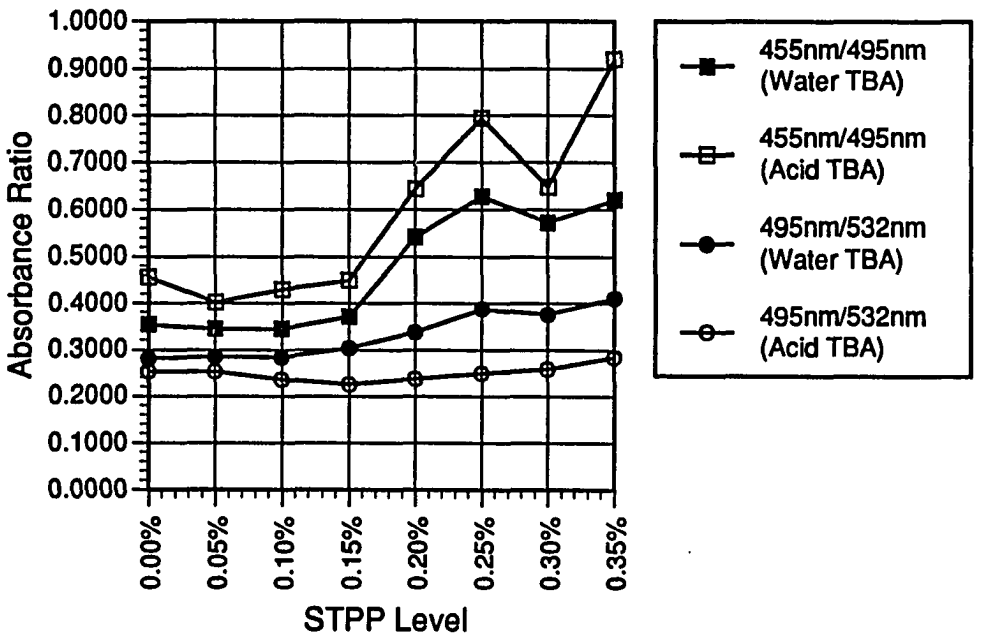

Figure 9. Effect of STPP and TBA solvent on 455nm/495nm and 495nm/532nm TBARS ratios of cooked comminuted pork

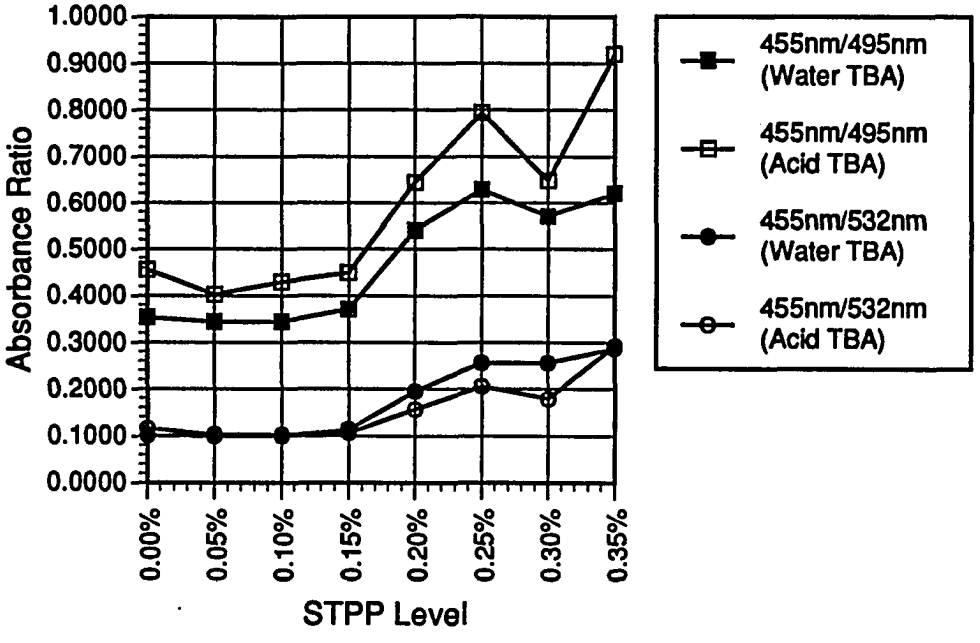

Figure 10. Effects of STPP and TBA solvent on 455nm/532nm TBARS ratios (relative to 455nm/495nm) of cooked comminuted pork

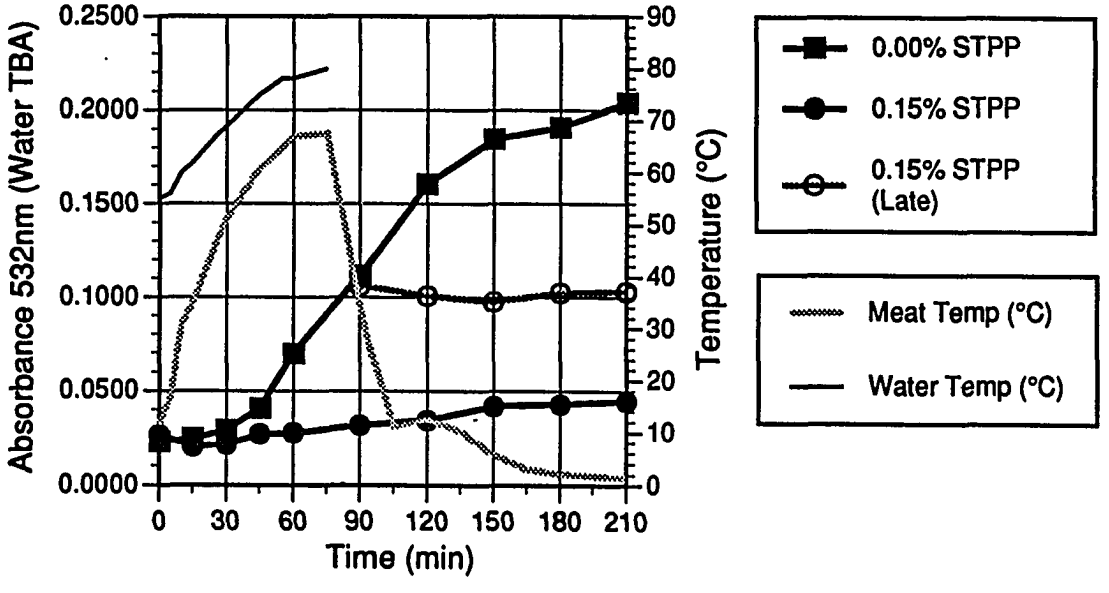

Figure 11. Effect of STPP on 532nm TBARS formation when introduced before, and during cooking of comminuted pork

Table 6. Effect of STPP concentration on total carbonyls of *cooked* comminuted pork[1,2]

STPP Level	Absorbance 340nm
0.00%	0.3640**a**
0.05%	0.3456**a**
0.10%	0.3358**a**
0.15%	0.3428**a**
0.20%	0.3347**a**
0.25%	0.3017**a**
0.30%	0.3241**a**
0.35%	0.3269**a**
Avg.	0.3344
S.E.	0.0332
C.V.	12.15%
Sig.	

[1]Treatment means represent results from 3 replications.

[2]Means within each column with different bold letters are significantly different ($P \leq 0.05$).

Table 7. Effect of TBA solvent *(water-based versus acid-based)* on absorbances of cooked comminuted pork[1,2]

STPP Level	Wavelength			Wavelength Ratio		
	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
Water	0.1263**a**	0.0383**a**	0.0167**a**	0.3331**a**	0.1759**a**	0.4716**b**
Acid	0.1214**b**	0.0296**b**	0.0152**a**	0.2505**b**	0.1576**a**	0.5925**a**
Avg.	0.1239	0.0340	0.0160	0.2918	0.1668	0.5320
S.E.	0.0021	0.0010	0.0012	0.0178	0.0151	0.0326
C.V.	5.82%	10.20%	25.85%	21.11%	31.30%	21.22%
Sig.	(P<0.05)	(P<0.0001)		(P<0.001)		(P<0.05)

[1]N=24 observations per mean.

[2]Means within each column with different bold letters are significantly different (P≤0.05).

Table 8. Correlations of *water-based* TBARS absorbances and ratios, and Total Carbonyls of cooked comminuted pork[1,2]

	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
532nm	1.0000	0.9242 (0.0001)		-0.7071 (0.0001)	-0.7194 (0.0001)	-0.7097 (0.0001)
495nm	0.9242 (0.0001)	1.0000		-0.4424 (0.05)	-0.5045 (0.05)	-0.5444 (0.01)
455nm			1.0000	0.5310 (0.01)	0.6171 (0.01)	0.6838 (0.001)
T.C.[3]	0.4079 (0.05)	*0.3690 (0.08)*	-0.4036 (0.05)	-0.5591 (0.01)	-0.6606 (0.001)	-0.6581 (0.001)

[1]N=24 observations.

[2]Numbers in parentheses under correlation coefficients represent significance level of correlation.

[3]T.C.=Total Carbonyls.

Table 9. Correlations of *acid-based* TBARS absorbances and ratios, and Total Carbonyls of *cooked* comminuted pork[1,2]

	532nm	495nm	455nm	495nm/ 532nm	455nm/ 532nm	455nm/ 495nm
532nm	1.0000	0.9788 (0.0001)	0.4553 (0.05)	-0.4378 (0.05)	-0.6968 (0.001)	-0.7388 (0.0001)
495nm	0.9788 (0.0001)	1.0000	0.5989 (0.01)		-0.5838 (0.01)	-0.6305 (0.001)
455nm	0.4553 (0.05)	0.5989 (0.01)	1.0000			
T.C.[3]	0.5330 (0.01)	0.4678 (0.05)		-0.5431 (0.01)	-0.6645 (0.001)	-0.6973 (0.001)

[1]N=24 observations.

[2]Numbers in parentheses under correlation coefficients represent significance level of correlation ("P" values).

[3]T.C.=Total Carbonyls.

CONCLUSIONS

Cooking in itself resulted in significant meat lipid oxidation relative to the raw state. Significant TBARS formation occurred during the cooking process of freshly comminuted pork, under conditions promoting air incorporation. This oxidation was detectable immediately after cooking, prior to any storage.

STPP was effective in limiting cooking-originated lipid oxidation at STPP levels greater than 0.10%. STPP demonstrated a diminishing marginal antioxidative ability with concentration; linear decreases in absorbance of TBARS required logarithmic increases in STPP levels. The greatest STPP antioxidant efficiency was at levels $0.10\% < [STPP] \leq 0.25\%$.

Results from the total carbonyl test, as modified in this study, were inconclusive. Nonspecificity and/or imprecision of the test may have contributed to these observations.

The 455nm/495nm and 495nm/532nm wavelength absorbance ratios (WARS) converged at higher levels of oxidation and diverged with increased oxidative stability. The water-based version of the 455nm/495nm and 495nm/532nm WARS appeared to be have better utility as indicators, demonstrating a closer relationship at higher oxidation levels.

REFERENCES

Ande, C. F. 1985. The influence of polyphosphate on meat pH and its relationship to lipid and myoglobin oxidation. Ph.D. dissertation, Auburn University.

Ang, C. Y. W. and Hamm, D. 1986. Effect of salt and sodium tripolyphosphate on shear, thiobarbituric acid, sodium, and phosphorous values of hot-stripped broiler breast meat. Poult. Sci. 65: 1532.

Ang, C. Y. W. and Young, L. L. 1989. Factors relating to oxidative stability of cooked broiler breast patties treated with sodium tripolyphosphate. J. Food Sci. 54(5): 1151.

Apte, S. and Morrissey, P. A. 1987. Effect of haemoglobin and ferritin on lipid oxidation in raw and cooked muscle systems. Food Chem. 25: 127.

Arganosa, G. C., Godber, J. S., Tanchotikul, U., McMillin, K. W. and Shoa, K. P. 1989. Oxidative stability of restructured beef roasts as affected by cooking temperature. J. Food Sci. 54(4): 1072.

Chambers, B., Chambers, E., IV, Bowers, J. A. and Craig, J. A. 1990. Detection and population thresholds for sodium tripolyphosphate in cooked ground turky muscle. Paper No. 178, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Cochran, W. G. and Cox, G. M. 1957. *Experimental Designs*, 2nd ed. John Wiley & Sons, New York, NY.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M. and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28: 187.

Craig, J., Bowers, J. and Seib, P. 1990. Sodium tripolyphosphate and sodium ascorbate monophosphate as inhibitors of lipid oxidation and off-flavor development in cooked, vacuum-packages, frozen ground turkey. Paper No. 177, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Diosady, L. L. 1988. Private communication. Dept. of Chem. Engineering and Appl. Chem., Univ. of Toronto.

Dupuy, H. P., Bailey, M. E., St. Angelo, A. J., Vercellotti, J. R. and Legendre, M. G. 1987. Instrumental analyses of volatiles related to warmed-over flavor of cooked meats. In *Warmed-over Flavor of Meat*, A. J. St. Angelo and M. E. Bailey (Ed.), p. 165. Academic Press, Inc., Orlando, FL.

Fernandez, M. C. D. and Rodriguez, J. M. Z. 1991. Lipolytic and oxidative changes in 'chorizo' during ripening. Meat Sci. 29: 99.

Frankel, E. N. 1980. Lipid oxidation. Prog. Lipid Res. 19: 1.

Gardner, H. W. 1975. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J Agric. Food Chem. 23(2): 129.

Gordon, M. H. 1990. The mechnism of antioxidant action *in vitro*. Ch. 1. In *Food Antioxidants*, B. J. F Hudson (Ed.), p. 1. Elsevier Science, Publishers Ltd, Essex, England.

Gray, J. I. 1978. Measurement of lipid oxidation: a review. J. Am. Oil Chem. Soc. 55(6): 539.

Huffman, D. L., Ande, C. F., Cordray, J. C., Stanley, M. H. and Egbert, W. R. 1987. Influence of polyphosphate on storage stability of restructured beef and pork nuggets. J. Food Sci. 52(2): 275.

Igene, J. O., King, J. A., Pearson, A. M. and Gray, I. J. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem., 27(4): 838.

Igene, J. O., Yamauchi, K., Pearson, A. M. and Gray, J. I. 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavour (WOF) in cured meat. Food Chem. 18: 1.

Keller, J. D. and Kinsella, J. E. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. J. Food Sci. 38: 1200.

Kim, R. S. and LaBella, F. S. 1987. Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. J. Lipid Res. 28: 1110.

King, A. J. and Earl, L. A. 1988. Effect of selected sodium and potassium salts on TBA values of dark meat turkey patties. J. Food Sci. 53(3): 723.

Kolodziejska, I., Skonieczny, St. and Rubin, L. J. 1990. Malondialdehyde-nitrite interactions in meat and model systems. J. Food Sci. 55(4): 925.

Koniecko, E. S. 1979. *Handbook for Meat Chemists*. Avery Publishing Group, Inc., Wayne, NJ.

Kosugi, H., Kojima, T. and Kikugawa, K. 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24(10): 873.

Lawrence, R. C. 1965. Use of 2,4-dinitrophenylhydrazine for the estimation of micro amounts of carbonyls. Nature 203: 1313.

Liu, H. F., Booren, A., M. and Gray, J. I. 1991. Antioxidant efficacy of oleoresin rosemary and sodium tripolyphosphate in restructured pork steaks and in a meat model system. Paper No. 443, presented at 51st Annual Meeting of Inst. of Food Technologists, Dallas, TX, June 1-5.

Love, J. D. and Pearson, A. M. 1976. Metmyoglobin and nonheme iron as prooxidants in egg-yolk phospholipid dispersions and cooked meat. J. Agric. Food Chem. 24(3): 494.

Mann, T. F., Reagan, J. O., Lillard, D. A., Campion, D. R., Lyon, C, E. and Miller, M. F. 1989. Effects of phosphate in combination with nitrite or Maillard reaction products upon warmed-over flavor in precooked, restructured beef chuck roasts. J. Food Sci. 54(6): 1431.

Matlock, R. G., Terrell, R. N., Savell, J. W., Rhee, K. S. and Dutson, T. R. 1984. Factors affecting properties of precooked frozen sausage patties made with various NaCl/phosphate combinations. J. Food Sci. 49: 1372.

McDonald, R. E. and Hultin, H. O. 1987. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J Food Sci. 52(1): 15.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7): 105.

Melton, S. L. 1985. Methods of assessing oxidation. In *Proceedings of the Meat Industry Research Conference*, Chicago, IL, October 18-19. American Meat Institute, Washington, DC.

Privett, O.S. and Quackenbush, F. W. 1954. The relation of synergist to antioxidant in fats. J. Am. Oil Chem. Soc. 31(8): 321.

Raharjo, S., Sofos, J. N., Maga, J. A. and Schmidt, G. R. 1989. Influence of meat restructuring systems on lipid oxidation in beef. Lebensm. Wiss. Technol. 22: 199.

Ramsey, M. B. and Watts, B. M. 1963. The antioxidant effects of sodium tripolyphosphate and vegetable extracts on cooked meat and fish. Food Technol. 17: 102.

Roozen, J. P. 1987. Effects of Types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavour. Food Chem. 24: 167.

Saidia, B. and Hammond, E. G. 1989. Quantification of carbonyls produced by the decomposition of hydroperoxides. J. Am. Oil Chem. Soc. 66(8): 1097.

Sato, K. and Hegarty, G. R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36: 1098.

Schantz, R. M. and Bowers, J. A. 1990. Optimization of reduced sodium chloride and phosphate salts for sensory characteristics in turkey frankfurters. Paper No. 176, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Shahidi, F., Rubin, L. J., Diosady, L. L., Kassam, N., and Li Sui Fong, J. C. 1986. Effect of sequestering agents on lipid oxidation in cooked meats. Food Chem. 21: 145.

Shahidi, F., Rubin, L. J., Wood, D. F. 1987a. Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. Food Chem. 23: 151.

Shahidi, F., Yun, J. and Rubin, L. J. 1987b. The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. Can. Inst. Food Sci. Technol. J. 20(2): 104.

Sherman, P. 1961. The water binding capacity of fresh pork. II. The influence of phosphates on fat distribuion in meat products. Food Technol. 15: 87.

Smith, L. A., Simmons, S. L., McKeith, F. K., Bechtel, P. J. and Brady, P. L. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. J. Food Sci. 49(6): 1636.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C., Jr. and Dupuy, H. P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. J. Food Sci. 52(5): 1163.

Steel, R. G. D. and Torrie, J. H. 1980. *Principles and Procedures of Statistics*, 2nd ed. McGraw-Hill Book Company, New York, NY.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. R., Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39(1): 34.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Tims, M. J. and Watts, B. M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

Wanous. M. P. 1990. Unpublished data. Dept. of Food Science and Human Nutrition, Iowa State University, Ames.

Willemot, C., Poste, L. M., Salvador, J. and Wood, D. F. 1985. Lipid degradation in pork during warmed-over flavour development. Can. Inst. Food Sci. Technol. J. 18(4): 316.

Yamauchi, K. 1973. Effect of heat treatment on the amount of free lipids released from isolated mitochondrial fraction of skeletal muscle tissue. Jap. J. Zootech. Sci. 44(4): 201.

Younathan, M. T. and Watts, B. M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25: 538.

Yun, J., Shahidi, F., Rubin, L. J. and Diosady, L. L. 1987. Oxidative stability and flavour acceptability of nitrite-free meat-curing systems. Can. Inst. Food Sci. Technol. J. 20(4): 246.

GENERAL SUMMARY

Sodium tripolyphosphate (STPP) is a multifunctional ingredient utilized in meat processing that can control meat lipid oxidation. Recent observations by researchers, however, have questioned whether this inhibition is exaggerated due to interference with the distillation 2-thiobarbiturc acid (TBA) test, which is most commonly used to determine meat lipid oxidation.

The focus of this study was to investigate any potential interference under combinations of oxidative conditions and TBA variations. (1) Analysis of the distillation TBA test for its potential to detect major aldehydic classes of lipid oxidation products was performed, to include monitoring of alternate wavelengths and evaluating TBA solvent influence on wavelength absorbances. (2) Raw and cooked comminuted pork were individually monitored during a 2-week refrigerated lipid oxidation cycle to determine if changes in the oxidative product constituency during meat lipid degradation altered the susceptibility of the distillation TBA test to STPP interference. (3) Meat lipid oxidation during cooking was investigated, as well as STPP effectiveness in limiting these cooking-originated oxidative changes.

The distillation 2-thiobarbituric acid (TBA) test was studied to determine its ability in monitoring alkanals and

2,4-alkadienals, the major aldehydic products of meat lipid oxidation, at the wavelengths of 532nm, 495nm and 455nm. Both aldehydes expressed TBA reactive substances (TBARS) at all three wavelengths. Choice of TBA solvent (90% glacial acetic acid versus deionized water) influenced absorbance and order of wavelength predominance regarding relative absorbance.

Potential interference with the TBA test was analyzed via STPP incorporation immediately prior to the assay of stored raw and cooked comminuted pork muscle, and compared with a control. STPP did not significantly ($P>0.05$) influence TBA absorbance values at 532nm, 495nm or 455nm wavelengths, in either water- or acid-based TBA solution, during two weeks of refrigerated storage of raw comminuted pork. TBARS formation in raw refrigerated pork demonstrated a first order logarithmic rate with time. In cooked comminuted pork, increasing STPP levels significantly ($P<0.01$) decreased 2-thiobarbituric acid absorbance values at 532nm and 495nm, in both water- and acid-based TBA, during two weeks of refrigerated storage, though these differences were of minimal practical significance. Oxidation rate in refrigerated cooked pork TBARS exhibited a logarithmic decrease during storage.

Relative rates of increase for TBARS formation during refrigerated storage of both raw and cooked comminuted pork were 532nm>495nm>455nm. The greatest correlations existed between absorbances of adjacent wavelengths (i.e., 532nm and

495nm, 495nm and 455nm). The 455nm/495nm wavelength absorbance ratios (WARS) were the most highly correlated with the wavelength absorbances. Convergence of the 455nm/495nm and 495nm/532nm WARS was associated with higher rates of TBARS formation; as lipid oxidation advanced to a maximum, a similar absorbance relationship was achieved between adjacent wavelengths (i.e., 455nm/495nm = 495nm/532nm). Water-based TBA consistently demonstrated higher absorbances at 532nm and 495nm but acetic acid increased 455nm absorbances of stored raw pork; TBA solvent differences increased with oxidation levels.

Oxidative stress and lipid decomposition during cooking were analyzed. Cooking of comminuted pork resulted in significant meat lipid oxidation relative to the raw state, prior to any postcooking storage. STPP was effective in limiting these cooking-originated changes at levels >0.10%, but demonstrated a diminishing marginal antioxidative ability with increasing concentration; linear decreases in absorbance of TBARS at primary and secondary wavelengths required logarithmic increases in STPP levels. The greatest STPP antioxidant efficiency was at levels $0.10\% < [STPP] \leq 0.25\%$. The 455nm/495nm and 495nm/532nm wavelength absorbance ratios (WARS) converged at higher levels of oxidation and diverged with increased oxidative stability. The 455nm/495nm WARS demonstrated high correlation with 532nm in both TBA solvents;

the water-based versions of the 455nm/495nm and 495nm/532nm WARS demonstrated a more convergent relationship at higher oxidation levels than did the acid-based equivalents.

GENERAL CONCLUSIONS

1. The major aldehyde groups associated with meat lipid oxidation, the alkanals and 2,4-alkadienals, reacted with 2-thiobarbituric acid in the meat lipid oxidation analysis method described by Tarladgis et al. (1960).

2. STPP did not influence TBA absorbance values at 532nm, 495nm or 455nm wavelengths, in either water- or acid-based TBA solution, of raw or cooked comminuted pork. Relative rates of increase for TBARS formation during refrigerated storage of both raw and cooked comminuted pork were 532nm>495nm>455nm.

3. Water-based TBA consistently demonstrated higher absorbances at 532nm and 495nm in raw and cooked pork; acetic acid increased 455nm absorbances of stored raw pork. Water as the TBA solvent was preferred over 90% glacial acetic acid.

4. TBARS formation in raw refrigerated pork demonstrated a first order logarithmic rate with time. Oxidation rates in refrigerated cooked pork TBARS exhibited logarithmic decreases during refrigerated storage.

5. The greatest correlations existed between TBARS absorbances of adjacent wavelengths (i.e., 532nm and 495nm, 495nm and 455nm). Values of the 455nm/495nm and 495nm/532nm wavelength absorbance ratios (WARS) approached each other with higher TBARS levels, especially when utilizing water-based TBA solvent. High meat lipid oxidative instability could be verified by increased 532nm TBARS absorbances and convergence of values from these ratios.

6. STPP demonstrated a diminishing marginal antioxidative ability toward cooking-originated oxidation, when added prior to cooking of ground pork. Antioxidative effectiveness increases required logarithmic increases in STPP concentrations greater than 0.10%. The greatest STPP antioxidant efficiency was at levels $0.10\% < [STPP] \leq 0.25\%$.

7. Cooking of comminuted pork resulted in significant meat lipid oxidation. Cooking raw meat products prior to lipid oxidation analysis tests the product's oxidative stability under stress, and better represents the true oxidative state before consumption.

8. Variation inherent in the distillation method of the TBA test exercises a much lesser relative role in cooked than in raw meat products. This is due to higher TBARS values

associated with greater oxidation of the cooked products, and allows for enhanced precision in treatment differentiation to be realized.

LITERATURE CITED

Addis, P. B., Csallany, A. S., and Kindom, S. E. 1983. Some lipid oxidation products as xenobiotics. In *Xenobiotics in Foods and Feeds*, D. E. Schwass and J. W. Finley (Ed.), p. 85. ACS Symp. Series No. 234. American Chemical Society, Washington, D.C.

Addis, P. B. 1990. Coronary heart disease. An update with emphasis on dietary lipid oxidation products. *Food & Nutrition News*, 62(2): 1. National Live Stock and Meat Board, Chicago, IL

Allen, J. C., Farag, R. S. and Crook, E. M. 1979. The metal-catalyzed oxidation of aqueous emulsions of linoleic acid and trilinolein. J. Appl. Biochem. 1:1.

Ande, C. F. 1985. The influence of polyphosphate on meat pH and its relationship to lipid and myoglobin oxidation. Ph.D. dissertation, Auburn University.

Ang, C. Y. W. and Hamm, D. 1986. Effect of salt and sodium tripolyphosphate on shear, thiobarbituric acid, sodium, and phosphorous values of hot-stripped broiler breast meat. Poult. Sci. 65: 1532.

Ang, C. Y. W. and Young, L. L. 1989. Factors relating to oxidative stability of cooked broiler breast patties treated with sodium tripolyphosphate. J. Food Sci. 54(5): 1151.

Anonymous, 1987. Phosphates in meat, poultry, and seafood. Bull. No. 2. FMC Corporation, Philadelphia, PA.

Asakawa, T., and Matsushita, S. 1979. Thiobarbituric acid test for detecting lipid peroxides Lipids 14(4): 401.

Asakawa, T., and Matsushita, S. 1980. Coloring conditions of thobarbituric acid test for detecting lipid hydroperoxides. Lipids 15(3): 137.

Asghar, A., Gray, J. I., Buckley, D. J., Pearson, A. M. and Booren, A. M. 1988. Perspectives on warmed-over flavor. Food Technol. 42(6): 102.

Betts, A. T. and Uri, N. 1966. The conversion of metal catalysts into inhibitors of autoxidation. Makromol. Chem. 95: 22.

Black, J. F. 1978. Metal-catalyzed autoxidation. The unrecognized consequences of metal-hydroperoxide complex formation. J. Am. Chem. Soc. 100(2): 527

Boling, C. S., Graham, P. P., Marriot, N. G. and Kelly, R. F. 1990. Effect of butylated hydroxyanisole and ascorbic acid on storage stability of restructured pork. J. Anim. Sci. 68(1): 108.

Chen, H. 1989. Paper No. Z8 presented at 80th Annual Meeting of The American Oil Chemists' Society, Cincinnati, OH, May 3-6.

Chen, H. 1990. The inhibitory effect of water on the decomposition of methyl linoleate hydroperoxides catalyzed by transition metals. Paper No. QQ5 presented at 81st Annual Meeting of The American Oil Chemists' Society, Baltmore, MD, April 22-25.

Choi, Y. I., Kastner, C. L. and Kropf, D. H. 1987. Effects of hot boning and phosphate on microbila, TBA, and pH values of preblended pork during cooler storage. J. Food Protect. 50(12): 1037.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M. and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28: 187.

Craig, J., Bowers, J. and Seib, P. 1990. Sodium tripolyphosphate and sodium ascorbate monophosphate as inhibitors of lipid oxidation and off-flavor development in cooked, vacuum-packaged, frozen ground turkey. Paper No. 177, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Das, N. P. and Pereira, T. A. 1990. Effects of flavonoids on thermal autoxidsation of palm oil: structure-activity relationships. J. Am. Oil Chem. Soc. 67(4): 255.

Decker, E. A. and Crum, A. D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56(5): 1179.

DeVore, V. R. 1988. TBA values and 7-ketocholesterol in refrigerated raw and cooked ground beef. J. Food Sci. 53(4): 1058.

Dziedzic, S. Z. and Hudson, B. J. F. 1984. Phosphatidyl ethanolamine as a synergist for primary antioxidants in edible oils. J. Am. Oil Chem. Soc. 61(6): 1042.

Empson, K. L., Labuza, T. P. and Graf, E. 1991. Phytic acid as a food antioxidant. J. Food Sci. 56(2): 560.

Frankel, E. N. 1980. Lipid oxidation. Prog. Lipid Res. 19: 1.

Frankel, E. N. 1982. Volatile lipid oxidation products. Prog. Lipid Res. 22: 1.

Frankel, E. N. 1984. Lipid oxidation: mechanisms, products and biological significance. J. Am. Oil Chem. Soc. 61(12): 1908.

Frankel, E. N., Neff, W. E., Selke, E., and Brooks, D. D. 1987. Thermal and metal-catalyzed decomposition of methyl linolenate hydroperoxides. Lipids 22(5): 322.

Gardner, H. W. 1975. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J Agric. Food Chem. 23(2): 129.

Gardner, H. W. 1983. Effects of lipid hydroperoxides on food components. In *Xenobiotics in Foods and Feeds*, D. E. Schwass and J. W. Finley (Ed.), p. 63. ACS Symp. Series No. 234, American Chemical Society, Washington, DC.

Gordon, M. H. 1990. The mechnism of antioxidant action *in vitro*. Ch. 1. In *Food Antioxidants*, B. J. F Hudson (Ed.), p. 1. Elsevier Science, Publishers Ltd, Essex, England.

Gray, J. I. 1978. Measurement of lipid oxidation: A review. J. Am. Oil Chem. Soc. 55: 539.

Grosch, W. 1987. Reactions of hydroperoxides - products of low molecular weight. Ch. 4. In *Autoxidation of Unsaturated Lipids*, H. W.-S. Chan (Ed.). Academic Press Inc., London.

Gutheil, R. A., Bailey, M. E. and Heymann, H. Interaction of warmed over flavor volatiles and muscle myofibrillar proteins. Paper No. 665, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Hammond, E. G. 1986. Private communication. Dept. of Food Science and Human Nutrition, Iowa State Univ., Ames.

Huffman, D. L., Ande, C. F., Cordray, J. C., Stanley, M. H. and Egbert, W. R. 1987. Influence of polyphosphate on storage stability of restructured beef and pork nuggets. J. Food Sci. 52(2): 275.

Husain, S. R., Terao, J. and Matsushita, S. 1986. Effect of browning reaction products of phospholipids on autoxidation of methyl linoleate. J. Am. Oil Chem. Soc. 63(11): 1457.

Hwang, S.-Y., Bowers, J. A. and Kropf, D. H. 1990. Flavor, texture, color and hexanal and TBA values of frozen cooked beef packaged in modified atmosphere. J. Food Sci. 55(1): 27.

Igene, J. O. and Pearson, A. M. 1979. Role of phospholipids and triglycerides in warmed-over flavor develpment in meat model systems. J. Food Sci. 44: 1285.

Igene, J. O., King, J. A., Pearson, A. M. and Gray, J. I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27: 838.

Igene, J. O., Pearson, A. M., Dugan, L. R. Jr. and Price, J. F. 1980. Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. Food Chem. 5: 263.

Igene, J. O., Yamauchi, K., Pearson, A. M., Gray, J. I. and Aust, S. D. 1985. Evaluation of 2-thiobarbituric acid reactive substances (TBRS) in relation to warmed-over flavor (WOF) development in cooked chicken. J. Agric. Food Chem. 33: 364.

Irani, R. R. and Morgenthaler, W. W. 1963. Iron sequestration by polyphosphates. J. Am. Oil. Chem. Soc. 40(7): 283.

Janero, D. R. and Burghardt, B. 1989. Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed lipid peroxidation: influence of peroxidtion conditions. Lipids 24(2): 125.

Karel, M. 1980. Lipid oxidation, secondary reactions, and water activity of foods. Ch. 12. In *Autoxidation in Food and Biological Systems*, M. G. Simic and M. Karel (Ed.), p. 191. Plenum Press, New York, NY.

Keller, J. D. and Kinsella, J. E. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. J. Food Sci. 38: 1200.

Kim, R. S. and LaBella, F. S. 1987. Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. J. Lipid Res. 28: 1110.

King, A. J. and Earl, L. A. 1988. Effect of selected sodium and potassium salts on TBA values of dark meat turkey patties. J. Food Sci. 53(3): 723.

King, M. F. and Boyd, L, C. 1991. A rapid method for determining the oxidation of N-3 fatty acids. Paper No. 254, presented at 52nd Annual Meeting of Inst. of Food Technologists, Dallas, TX, June 1-5.

Knable, S. J., Walker, H. W. and Hartman, P. A. 1991. Inhibition of *Aspergillus flavus* and selected Gram-positive bacteria by chelation of essential metal cations by polyphosphates. J. Food Protect. 54(5): 360.

Kolata, G. 1988. New theory explains how cholesterol threatens the heart. In *The New York Times*, October 25.

Kolodziejska, I., Skonieczny, St. and Rubin, L. J. 1990. Malondialdehyde-nitrie interactions in meat and model systems. J. Food Sci. 55(4): 925.

Kwon, T. W., Menzel, D. B. and Olcott, H. S. 1965. Reactivity of malonaldehyde with food constituents. J. Food Sci. 30: 808.

Labuza, T. P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Technol. 2: 355.

Lamkey, J. W., Mandigo, R. W. and Calkins, C. R. 1986. Effect of salt and phosphate on the texture and color stability of restructured beef steaks. J. Food Sci. 51(4): 873.

Lee, J.-H., Fujimoto, K. and Kaneda, T. 1983. Peroxide-decomposing activities of antarctic krill lipids and certain other oils. Agric. Biol. Chem. 47(9): 2001.

Lenga, R. L. (Ed.). 1985. *The Sigma-Aldrich Library of Chemical Safety Data, Edition 1.* Sigma-Aldrich Corp., Milwaukee, WI.

Lillard, D. A. 1985. Chemistry of lipid oxidation. Proc. Meat Ind. Res. Conf., American Meat Institute Foundation, Washington, D.C.

Liu, H. F., Booren, A., M. and Gray, J. I. 1991. Antioxidant efficacy of oleoresin rosemary and sodium tripolyphosphate in restructured pork steaks and in a meat model system. Paper No. 443, presented at 51st Annual Meeting of Inst. of Food Technologists, Dallas, TX, June 1-5.

Lundberg, W. O. 1962. Mechanisms. Ch. 2. In *Symposium on Foods: Lipids and Their Oxidation*, H. W. Schultz, E. A. Day and R. O. Sinnhuber, (Ed.), p. 31. AVI Publishing Co., Inc., Westport, CT.

Mann, T. F., Reagan, J. O., Lillard, D. A., Campion, D. R., Lyon, C, E. and Miller, M. F. 1989. Effects of phosphate in combination with nitrite or Maillard reaction products upon warmed-opver flavor in precooked, restructured beef chuck roasts. J. Food Sci. 54(6): 1431.

Marion, W. W. and Forsythe, R. H. 1962. Protection of lipids of turkey meat by butylated hydroxyanisole, egg white solids, gelatin and tripolyphosphate. Poult. Sci. 41: 1663.

Matlock, R. G., Terrell, R. N., Savell, J. W., Rhee, K. S. and Dutson, T. R. 1984. Factors affecting properties of precooked frozen sausage patties made with various NaCl/phosphate combinations. J. Food Sci. 49: 1372.

McDonald, R. E. and Hultin, H. O. 1987. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J. Food Sci. 52(1): 15.

McNeill, J., Kakuda, Y. and Findlay, C. 1988. Influence of carcass parts and food additives on the oxidative stability of frozen mechanically separated and hand-deboned chicken meat. Poult. Sci. 67: 270.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7): 105.

Melton, S. L. 1985. Methods of assessing oxidation. In *Proceedings of the Meat Industry Research Conference*, Chicago, IL, October 18-19. American Meat Institute, Washington, DC.

Miller, M. F., Davis, G. W., Seideman, S. C., Ramsey, C. B. and Rolan, T. L. 1986. Effects of various phosphates onthe palatability, appearance and storage traits of flaked and formed restructured beef steaks. J. Food Sci.51(6): 1435.

Miyashita, K. and Takagi, T. 1986. Study on the oxidative rate and prooxidant activity of free fatty acids. J. Am. Oil Chem. Soc. 63(10): 1380.

Moerck, K. E. and Ball, H. R., Jr. 1974. Lipid autoxidation in mechanically deboned chicken meat. J. Food Sci. 39: 876.

Molins, R. A., Kraft, A. A., Walker, H. W., Rust, R. E., Olson, D. G. and Merkenich, K. Effect of inorganic polyphosphates on ground beef characteristics: some chemical, physical and sensory effects on frozen beef patties. J. Food Sci. 52(1): 50.

Nair, V. and Turner, G. A. 1984. The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. Lipids 19(10): 804.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351.

Park, S. W. and Addis, P. B. 1987. Cholesterol oxidation products in some muscle foods. J. Food Sci. 52(6): 1500.

Patel, S. B., King, A. J. and Lee, Y. B. 1988. Effect of sodium salts and added water on physical, chemical and sensory properties of dark turkey meat subjected to long-term frozen storage. J. Food Sci. 53(6): 1610.

Paterson, B. C., Parrish, F., C., Jr., and Stromer, M. H. 1988. Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle. J. Food Sci. 53(5): 1258.

Pearson, A. M., Gray, J. I., Wolzak, A. M. and Horenstein, N. A. 1983. Safety implications of oxidized lipids in muscle foods. Food Technol. 37(7): 121.

Pikul, J., Leszczynski, D. E. and Kummerow, F. A. 1983. Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. J. Agric. Food Chem. 31: 1338.

Privett, O.S. and Quackenbush, F. W. 1954. The relation of synergist to antioxidant in fats. J. Am. Oil Chem. Soc. 31(8): 321.

Pryor, W. A., Stanley, J. P. and Blair, E. 1976. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids 11(5): 370.

Raharjo, S., Sofos, J. N., Maga, J. A. and Schmidt, G. R. 1989. Influence of meat restructuring systems on lipid oxidation in beef. Lebensm. Wiss. Technol. 22: 199.

Rauch P. and Kas, J. 1983. Phospholipid changes in the course of beef meat storing at 4°C. Nahrung 27(10): 939.

Rhee, K. S. 1978. Minimization of further lipid peroxidation in the distillation 2-thiobarbituric acid test of fish and meat. J. Food Sci. 43: 1776.

Roozen, J. P. 1987. Effects of Types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavour. Food Chem. 24: 167.

Saidia, B. and Hammond, E. G. 1989. Quantification of carbonyls produced by the decomposition of hydroperoxides. J. Am. Oil Chem. Soc. 66(8): 1097.

Salih, A. M., Price, J. F., Smith, D. M. and Dawson, L. E. Lipid degradation in turkey breast meat during cooking and storage. Poult. Sci. 68: 754.

Sato, K. and Hegarty, G. R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36: 1098.

Schantz, R. M. and Bowers, J. A. 1990. Optimization of reduced sodium chloride and phosphate salts for sensory characteristics in turkey frankfurters. Paper No. 176, presented at 51st Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 16-20.

Schmedes, A. and Holmer, G. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Am. Oil Chem. Soc. 66(6): 813.

Schricker, B. R., Miller, D. D. and Stouffer, J. R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47: 740.

Schwartz, W. C. and Mandigo, R. W. 1976. Effect of salt, sodium tripolyphosphate and storage on restructured pork. J. Food Sci. 41: 1266.

Shahidi, F., Rubin, L. J., Diosady, L. L., Kassam, N., and Li Sui Fong, J. C. 1986. Effect of sequestering agents on lipid oxidation in cooked meats. Food Chem. 21: 145.

Shahidi, F., Rubin, L. J., Wood, D. F. 1987a. Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. Food Chem. 23: 151.

Shahidi, F., Yun, J. and Rubin, L. J. 1987b. The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. Can. Inst. Food Sci. Technol. J. 20(2): 104.

Sherman, P. 1961. The water binding capacity of fresh pork. II. The influence of phosphates on fat distribution in meat products. Food Technol. 15: 87.

Shimp, L. A. 1981. The advantages of STPP for cured meat production. Meat Processing, August.

Sinnhuber, R. O., Yu, T. C. and Yu, T. C. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23(6): 626.

Siu, G. M. and Draper, H. H. 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43: 1147.

Smith, L. A., Simmons, S. L., McKeith, F. K., Bechtel, P. J. and Brady, P. L. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. J. Food Sci. 49(6): 1636.

Sofos, J. N. 1986. Use of phosphates in low-sodium meat products. Food Technol. 40(9): 52.

St. Angelo, A. J., Vercellotti, J. R., Dupuy, H. P. and Spanier, A. M. 1988. Assessment of beef flavor quality: a multidisciplinary approach. Food Technol. 42(6): 133.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C., Jr. and Dupuy, H. P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. J. Food Sci. 52(5): 1163.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. R., Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. J. Am. Oil Chem. Soc. 39(1): 34.

Tarladgis, B. G., Pearson, A. M., Dugan, L. R., Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Tay, H. C., Aberle, E. D. and Judge, M. D. 1983. Iron catalyzed oxidative rancidity in prerigor ground pork. J. Food Sci. 48: 1328.

Tims, M. J. and Watts, B. M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

Trout, G. R. and Schmidt, G. R. 1983. Utilization of phosphates in meat products. *Proceedings of the 36th Annual Reciprocal Meat Conference*. American Meat Science Association, Chicago, IL.

Wanous, M. P. 1986. Effects of meat grinder wear and freezing rate on the oxidatin of lipid and myoglobin in commercial fresh pork sausage. M.S. thesis, Iowa State University, Ames.

Wanous. M. P. 1990. Unpublished data. Dept. of Food Science and Human Nutrition, Iowa State University, Ames.

Willemot, C., Poste, L. M., Salvador, J. and Wood, D. F. 1985. Lipid degradation in pork during warmed-over flavour development. Can. Inst. Food Sci. Technol. J. 18(4): 316.

Wilson, B. R., Pearson, A. M.,and Shorland, F. B. 1976. Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem. 24(1): 7.

Wu, T. C. and Sheldon, B. W. 1988. Flavor components and factors associated with the development of off-flavors in cooked turkey rolls. J. Food Sci. 53(1): 49.

Yamauchi, K. 1973. Effect of heat treatment on the amount of free lipids released from isolated mitochondrial fraction of skeletal muscle tissue. Jap. J. Zootech. Sci. 44(4): 201.

Yamauchi, K., Nagai, Y. and Ohashi, T. 1982. Quantitative relationship between alpha-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in chicken skeletal muscle. Agric. Biol. Chem. 46: 2719.

Yasuhara, A. and Shibamoto, T. 1989. Analysis of aldehydes and ketones in the headspace of heated pork fat. J. Food Sci. 54(6): 1471.

Younathan, M. T., Marjan, Z. M. and Arshad, F. B. 1980. Oxidative rancidity in stored ground turkey and beef. J. Food Sci. 45: 274.

Yu, L. W., Latriano, L., Duncan, S., Hartwick, R. and Witz, G. 1986. High-performance liquid chromatography analysis of the thiobarbituric acid adducts of malonaldehyde and *trans, trans*-muconaldehyde. Anal. Biochem. 156: 326

Yun, J., Shahidi, F., Rubin, L. J. and Diosady, L. L. 1987. Oxidative stability and flavour acceptability of nitrite-free meat-curing systems. Can. Inst. Food Sci. Technol. J. 20(4): 246.