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Factors Affecting the Oxidative Stability of Foods-Interesterified Soybean Oil with High Intensity Ultrasound Treatment and Trona Mineral in Packaged Fresh Meats

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FACTORS AFFECTING THE OXIDATIVE STABILITY OF FOODS-INTERESTERIFIED SOYBEAN OIL WITH HIGH INTENSITY ULTRASOUND TREATMENT AND TRONA MINERAL IN PACKAGED FRESH MEATS

by

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ABSTRACT

Factors Affecting the Oxidative Stability of Foods-Interesterified Soybean Oil with High Intensity Ultrasound Treatment and Trona Mineral in Packaged Fresh Meats

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Oxidation in oils and muscle foods has been studied for many years to understand its mechanism and furthermore to control and manage it. A series of different processing steps or different packaging techniques can alter oxidative stability. The objective of the current study was to examine oxidative stability of processed oil and to evaluate the effect of carbon dioxide generating mineral on quality of beef and chicken under different storage conditions. In Study 1 (Chapter 3), the effect of ultrasound on oxidative stability of interesterified soybean oil and soybean oil was examined. Sonication did not affect oxidation rate until the oils were highly oxidized. Sonicated interesterified soybean oil exhibited a slightly but significantly lower oxidation rate than non-sonicated oil during long-term storage, while sonication of non-interesterified soybean oil led to a significantly higher oxidation rate than in non-sonicated soybean oil after induction period. In Study 2 (Chapter 4), the feasibility of trona as a CO_2 producing product in a model system and in modified atmosphere packaging of beef steaks was investigated.

Trona was able to generate more carbon dioxide than sodium bicarbonate with salicylic acid in model systems. Steaks stored with trona/acid mixture had similar color stability and delayed lipid oxidation compared to those stored in high oxygen packaging. In Study 3 (Chapter 5), the effect of packets containing trona and acid placed in a simulated self serve retail case and closed butcher case on the quality of ground beef was studied. Mineral packets did not affect color, lipid oxidation, or microbial growth of ground beef since there was not a sufficient amount of moisture to generate CO_2 effectively. In Study 4 (Chapter 6), the quality of chicken breast/thigh portions stored with mineral packets was compared to those without mineral packets during extended storage, and mineral packets had an antimicrobial effect of CO_2 only on day 15. In conclusion, high intensity ultrasound did not affect the rate of oxidation of oil until the oil had already become noticeably rancid, and mineral packets containing trona and an acid with low water solubility can be used as CO_2 generating sachet if sufficient moisture is given.

(165 pages)

PUBLIC ABSTRACT

Factors Affecting the Oxidative Stability of Foods-Interesterified Soybean Oil with High Intensity Ultrasound Treatment and Trona Mineral in Packaged Fresh Meats

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Oxidation leads to rancid flavors or odors in oils and meat and causes discoloration in muscle foods. There is a great concern about economic loss when oxidation is not effectively controlled. Novel processing and storage methods such as ultrasound, interesterification, or gas emitter sachet in meat packaging have been studied to provide desirable quality properties of oils and meat products. However, there are not enough studies to explain their effect on oxidative stability. Better understanding of new techniques is required to manage the oxidative stability and even other quality properties.

High intensity ultrasound is an effective method to improve physical properties of oils. However, its effect on oxidative stability of interesterified soybean oil had not been clear. The effect of ultrasound on interesterified soybean oil appeared on the crystallization behavior and lipid oxidation. Ultrasound-treated interesterified soybean oil had a thick creamy consistency while non ultrasound treated interesterified soybean oil had a separation of crystals and liquid. Ultrasound-treated interesterified oil had a significantly lower level of primary oxidation products than non-treated one. Interesterified soybean oil was less resistant to oxidation compared to soybean oil, but it had much lower oxidation rate than interesterified oil once oxidation products started to increase dramatically. Ultrasound treated soybean oil had a slight higher oxidation rate than non treated soybean oil. However, ultrasound did not cause accelerated or delayed oxidation in oils until the oils were highly oxidized.

Carbon dioxide is often used in meat packaging because of antimicrobial effect. Trona mineral, a source of baking soda, was tested for its potential use for carbon dioxide emitter in meat packaging. The reaction of trona and acid in water was used for carbon dioxide generation. Carbon dioxide generating capacity of trona plus acid was better than baking soda in water and dependent on the amount of water available. The mixture (4 g) of trona/acid with steaks in master pack was able to generate a high CO_2 atmosphere (>20% in head space) with meat drip as the sole moisture source, prolonging color shelf life while minimizing lipid oxidation of the steaks. When a trona packet was used for extended chicken storage (after 15 days of storage), the growth of aerobic microorganisms was suppressed on day 15 using trona, but after 15 days there was no effect of using trona on microorganism growth. Chicken was spoiled and had strong odors after 15 days. There was no significant effect of carbon dioxide generated from trona on lipid oxidation or discoloration of chicken. Trona is a potential carbon dioxide emitter when it is used with acid and water. However, there are some important factors that should be considered such as the amount of available moisture and size of bags to give antimicrobial effect.

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LIST OF SYMBOLS, NOTATION, DEFINITIONS

	Abbreviation Key
a*	Redness
ANOVA	Analysis of variance
b*	Yellowness
CO_2	Carbon dioxide
dMb	Deoxymyoglobin
FA	Fatty acid
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
$FeCl_2$	Ferrous chloride
HIU	High intensity ultrasound
IESBO	Interesterified soybean oil
L*	Lightness
MAP	Modified atmosphere packaging
Mb	Myoglobin
MbO_2	Oxymyoglobin
MDA	Malondialdehyde
MES	2-(4-morpholine)ethanesulfone acid
MetMb	Metmyoglobin
N_2	Nitrogen
NMAP	80% nitrogen/20% carbon dioxide packaging

NS-	Non sonicated
O_2	Oxygen
OMAP	80% oxygen/20% carbon dioxide packaging
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
PVC	Polyvinylchloride
S-	Sonicated
SBO	Soybean oil
TAG	Triacylglycerol
TAC	Total aerobic plate count
TMAP	Trona containing modified atmosphere packaging
TBARS	Thiobarbituric acid reactive substances
Trt	Treatment
US	Ultrasound

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1. Introduction

Oxidation in foods is an important concern in the food industry. Oxidation can affect quality factors of food such as color, odor, taste, inherent nutrients and production of potentially toxic compounds (Frankel, 1998; Halliwell et al., 1995; Kubow, 1992, 1993; Liu & Huang, 1995; Nawar, 1996). In general, deterioration that results from exposure to oxygen is avoided by producers and consumers, even though it is desirable to some food products depending on the types of oxidation. The overall effect of oxidation is unnecessary economic losses and many health problems. Oxidative deterioration results in rancidity in meat products which causes a great economic loss concern since the quality and acceptability in muscle foods is limited by lipid oxidation (Morrissey, Sheehy, Galvin, & Kerry 1998; Renerre, 2000; Zamora & Hidalgo, 2001).

Lipid oxidation is one of the most important types of oxidation occurring in foods. It is a complex series of chemical reactions between unsaturated fatty acids and reactive oxygen species (Frankel, 1998; McClements & Decker, 2000; Min & Boff, 2002; Nawar, 1996). Proper food processing, packaging and storage are required to control oxidation of foods since managing lipid oxidation is necessary to control quality of foods. Oxidation in oils and meat products is often measured since their oxidative stability is important for the taste and odor.

For oils and fats, processing techniques such as high intensity ultrasound (HIU), hydrogenation, and interesterification can be used to improve their chemical and/or

physical properties. However, when oils go through multiple processing steps, the change in oxidative stability is not easy to predict because of the complication of their effects. Hydrogenation and interesterification are known to improve the oxidative stability of oils (Daniels, Kim, & Min, 2006). On the other hand, HIU has been found to accelerate deterioration (Chemat, Grondin, Sing, & Smadja, 2004). However, when full hydrogenation and interesterification are used with HIU, the oxidative stability is hard to predict. The effect of HIU on oxidative stability of interesterified blends of soybean oil and fully hydrogenated soybean oil stock is not available.

Oxidation in meat products is also important for color, taste and odor. In muscle food, lipid oxidation is important for rancid flavor development. Meat products with rancid flavors are often rejected by consumers. Myoglobin oxidation is an important oxidation that has to be considered in addition to lipid oxidation since it is closely related to color.

Modified atmosphere packaging (MAP) is a common meat packaging method used to control quality factors including color, taste, odor and texture. Oxidation can be controlled by changing the level of oxygen in the packaging environment by replacing O_2 with an inert gas. Carbon dioxide is often used in MAP for its antimicrobial effect in both low oxygen and high oxygen MAP. Flushing or removing a gas (or gases) is an essential technique in MAP. It is possible that a desirable atmosphere can be created by reaction of chemical substances within the packaging without the need for gas flushing. This would require identifying an alternative method capable of releasing the required level of gas to work as effectively as actual MAP on oxidation in meat, and identifying other factors that could be influenced by the alternative method.

2. Hypothesis

Processing methods and storage conditions can alter oxidative stability and other properties of lipids and lipid-containing food systems. Specifically: high intensity ultrasound will affect the oxidative stability of interesterified soybean oil (IESBO) and soybean oil (SBO); and mineral combinations capable of releasing CO_2 will create a modified atmosphere packaging environment, thus affecting quality and extending shelf life of raw beef and poultry.

3. Objectives

1. To compare oxidative stability of sonicated IESBO and SBO and non-sonicated IESBO and SBO and to evaluate their oxidation progress.
2. To measure carbon dioxide concentration generated by trona mineral or sodium bicarbonate in a model system, to evaluate atmosphere generated by trona mineral or sodium bicarbonate packets designed for steak storage and to compare the effect of trona mineral on steak under retail storage (1 ℃) on pH, color, microbial growth, and lipid oxidation in PVC packages with different MAP.
3. To evaluate the effect of packets containing trona mineral and a phenolic acid on fresh beef shelf life;
    a) PVC packaged ground beef held under retail (1 ℃) and home storage (4 ℃) conditions on pH, color, microbial growth, and lipid oxidation.
    b) Ground beef chuck held under retail display (1 ℃) conditions of drip loss.
4. To examine the effect of different amount of trona mineral concentration on pH, color, microbial growth, and lipid oxidation of chicken in PVC packages with air.

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CHAPTER 2

LITERATURE REVIEW

1. Oxidative stability

Oxidation in food is an important chemical process that deteriorates nutrition and quality of the food. Oxidative stability in oils and fats is important in that it determines quality and shelf life (Hamilton, 1994). Oxidative stability of oils can be defined as the resistance to oxidation during processing and storage (Guillen & Cabo, 2002). Oxidation of edible oils can be influenced by many different factors: energy input such as light or heat, composition of fatty acids, types of oxygen compounds, and minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglcerols, thermally oxidized compounds, and antioxidants (Choe & Min, 2006). In addition, numerous agents such as enzymes and transition metals can directly or indirectly catalyze these oxidative processes through enzymatic and nonenzymatic mechanisms.

2. Autoxidation

Autoxidation is an important chemical reaction between unsaturated lipids and active oxygen species (Frankel, 1998; McClements & Decker, 2000; Min & Boff, 2002; Nawar, 1996). The overall mechanism of autoxidation consists of three steps; initiation, propagation and termination (Allen & Hamilton, 1983; Gray, 1978; Raharjo & Sofos, 1993).

Initiation:

$RH + O_2 \text{-->} R\bullet + \bullet OOH$ (1)

Propagation:

$R\bullet + O_2 \text{ --> } ROO\bullet$ (2)

$ROO\bullet + RH \text{ --> } R\bullet + ROOH$ (3)

$ROOH \text{ --> } RO\bullet + \bullet OH$ (4)

Termination:

$R\bullet + R\bullet \text{ --> } RR$ (5)

$R\bullet + ROO\bullet \text{--> } ROOR$

$ROO\bullet + ROO\bullet \text{ --> } ROOR + O_2$ (6)

where RH is a fatty acid (free or bound to glycerol backbone), O_2 is diatomic oxygen, R• is a lipid free radical, H• is a hydrogen free radical, ROO• is a peroxy free radical, ROOH is a lipid hydroperoxide, RR is a lipid dimer and ROOR is a lipid peroxide.

Initiation is the step involving free radical formation. In the initial step, fatty acid radical (alkyl radical) is formed by abstraction of hydrogen from a fatty acid in the presence of an initiator such as a metal catalyst. Theoretically, non-radical state atmospheric O_2 and polyunsaturated fatty acid (PUFA) cannot interact with each other due to thermodynamic constraints caused by electronic spin conservation (Min & Bradley, 1992). Thus, ground state oxygen has to be altered to reactive oxygen species to initiate lipid peroxidation, and such reactive oxygen species (ROS) are hydroxyl radical (•OH), superoxide anion ($O_2\bullet^-$), hydrogen peroxide ($H_2O_2\bullet$), hydroperoxyl radical ($HO_2\bullet$), lipid peroxyl radical (LOO•), alkoxyl radical (LO•), iron-oxygen complexes (ferryl- and perferryl radical) and singlet oxygen ($^1O_2$). Propagation is the free chain oxidation reaction. Propagation sequence involves two steps; formation of a peroxyl radical ROO by the reaction of allyic radical R with triplet oxygen and hydrogen abstraction of peroxyl

radical to produce lipid hydroperoxide and a new alkyl radical (Girotti, 1985, 1990; Halliwell & Gutteridge, 1990; Kappus, 1985). Oxygen availability and temperature determine the formation rate of lipid peroxyl radicals and hydroperoxides (Velasco, Andersen, & Skibsted, 2003). The concentration of peroxyl radicals is higher than that of the lipid alkyl radicals since the reaction between alkyl radicals and triplet oxygen occurs instantly at normal oxygen pressure (Aidos, Lourenço, Van der Padt, Luten, & Boom, 2002). Finally, the radicals interact to form non radical (non-reactive) products in the termination step. In many food products, termination is not as important as initiation and propagation because rancidity is developed before termination step (Chaiyasit, Elias, McClements, & Decker, 2007)

2.1. Primary oxidation products

Primary oxidation products are hydroperoxides which are stable and do not provide any flavor change or off-odor. White (1994) reported that peroxides and hydroperoxides are intermediates that form volatile carbonyl compounds which are responsible for flavor developed by oxidation. Formation of hydroperoxides can be accelerated by heat, metal catalysts, and ultraviolet and visible light which accelerate free radical formation (Choe & Min, 2006). Peroxide value is an indicator of the degree of lipid oxidation and expressed in milliequivalents of peroxide per kg of sample (mEq/kg). Iodometric titration assay is one of the standard methods used to measure peroxide value, and this reaction is based on the oxidation of the iodide ion (I^-) by hydroperoxides (Antolovich, Prenzler, Patsalides, Mcdonald, & Robards, 2002). Chemical reactions involved are given below:

$2\ I^- + H_2O + ROOH \rightarrow ROH + 2OH^- + I_2$

$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2\ I^-$

A saturated solution of potassium iodide is added to oil samples to react with hydroperoxides. The liberated iodine is titrated with a standardized solution of thiosulfate using a potato starch suspension as an endpoint indicator (Antolovich et al., 2002; Kiritsakis, Kanavouras, & Kiritsakis, 2002; Shahidi & Wanasundara, 2002). Peroxide value does not directly measure rancidity or freshness of the oils and fats, but certain levels of peroxide values are associated with off flavors. The level of peroxide values that gives a perceptible rancid taste in products varies depending on the type of oils. Fish oils were suggested to have a lower standard peroxide values (2 mEq/kg) that show a rancid taste (Turner, McLean, & Silvers, 2006). Soybean oils that have peroxide values of 1-5 mEq/kg, 5-10 mEq/kg and >10 mEq/kg are considered as slightly, moderately and highly oxidized, respectively (AOCS, 1997; Nielson, 2003). According to Gunstone (1996), refined vegetable oils should have peroxide value below 1 mEq/kg, and a rancid taste becomes perceptible at approximately10 mEq/kg. It has been reported that a rancid taste is noticeable in many oils that have peroxide values between 20 and 40 mEq/kg (Kirk & Sawyer, 1991; O'Brien, 1998).

2.2. Secondary oxidation products

Secondary oxidation products produced from the decomposition of hydroperoxides are responsible for the off-flavor in oxidized edible oils (Frankel, 1998). Low molecular weight aldehydes, ketones, alcohols, and short chain hydrocarbons are produced through a beta-scission reaction (eq. 2). The secondary oxidation products can

be detected by various methods, such as gas chromatography and analysis for thiobarbituric acid reactive substances (TBARS). The TBARS assay is an economic and fast method of measuring secondary oxidation products. TBARS analysis is particularly useful to detect oxidative deterioration of fat-containing foods such as meat products and oils. The TBARS assay measures red pigment formation developed by a condensation reaction of one molecule of malonaldehyde (a secondary oxidation product common in meats) with two molecules of 2-thiobarbituric acid (Sinnhuber & Yu, 1958, 1977). The absorbance maxima of the red pigments are 450 nm and 530-532 nm, and traditional TBARS measurement of unsaturated fatty acids uses 532 nm (Stapelfeldt, Nielsen, & Skibsted, 1997). Thiobarbituric acid can react with other oxidation byproducts as well as common food components such as alkanals, 2-alkenals, 2,4-alkdienals, ketones, ketosteroids, acids, esters, proteins, sucrose, urea, pyridines, and pyrimidines, causing interference with the TBARS assay (Guillen-Sans & Guzman-Chozas, 1998; Jardine, Antolovich, Prenzler, & Robards, 2002). For instance, the reaction of TBA with various aldehydes such as 2-alkenals and 2,4-alkadienals form a yellow chromagen with an absorption maximum at 450 nm, which overlaps with the pink peak at 532 nm, causing artificially high TBARS values (Hoyland & Taylor, 1991; Jardine et al., 2002; Sun, Faustman, Senecal, Wilkinson, & Furr, 2001).

3. Processing soybean oil and Partial hydrogenation

Soybean oil (SBO) is one of the most popular oils in the world due to its high quality and low cost (Hui, 1996). SBO oil represents 85% of edible oils in the United States (Wang, 2002). Soybean oil contains approximately 55% linoleic acid and 8%

linolenic acid (Daniels, Kim, & Min, 2006). Its high content of polyunsaturated fatty acids, which are necessary in the human diet, makes it susceptible to oxidation. In addition, SBO has not been a good source of shortening, margarines, and salad dressings without modifications because of its low melting point (Daniels et al., 2006). To overcome this drawback, soybean oil is hydrogenated to improve oxidation stability and plasticity (Jang, Jung, & Min, 2005). Nawar (1996) found that hydrogenation improved the oxidative stability of oil and modifies the physical characteristics by reducing the double bonds. Hydrogenation significantly improved the oxidative stability of soybean oil based on the headspace volatile analysis and induction time determination (Daniels et al., 2006). Partially hydrogenated oil was used widely as shortening, salad dressing and cooking oil in the past because of its distinctive mouth feel and desirable flavor. However, partially hydrogenated soybean oil results in production of trans-fatty acids, and the use of partially hydrogenated oils has waned in the past few years due to the health concern of trans-fatty acids (Weggemans, Rudrum, & Trautwein, 2004). Trans-fatty acids increase the risk of cardiovascular diseases by increasing low density lipoprotein and decreasing high intensity lipoprotein (Enig, 1996; Gurr, 1990; Lichtenstein, 1993; Mensink & Katan, 1990). In 2006, labeling of trans-fatty acids on food labels became mandatory by the Food and Drug Administration. In addition, in July 2007 New York City banned the use of oils, margarines and shortenings containing more than 0.5 g of trans fat per serving in both chain and non-chain restaurants. There have been some efforts to find a substitute for partially hydrogenated oils which can also provide desirable functional properties without containing trans-fatty acids.

4. Alternative methods of partial hydrogenation

4.1. Fully hydogrenated oils

Fully hydrogenated oils are used for the manufacture of low to zero-trans commercial fats (De Greyt, Radanyi, Kellens, & Huyghebaert, 1996). In many studies, desirable hardness in oils was obtained when fully hydrogenated oil was blended with liquid oil without trans-fatty acids (Hurtova, Schmidt, Zemanovic, Simom, & Sekretar, 1996; List, Emken, Kwolek, Simpson, & Dutton, 1977; List, Mounts, Orthoefer, & Neff, 1995; Zeitoun, Neff, List, & Mounts, 1993).

4.2. Interesterification

Interesterification redistributes fatty acids within and among triglycerides to change the physical properties and behaviors of oil (Nawar, 1996). Interesterification of a blend of hard fats and liquid oils has been an effective method to produce zero-trans fats, yielding fat bases with excellent characteristics for preparation of margarines, shortenings and spreads (Karabulut, Turan, & Ergin, 2004; Khatoon & Reddy, 2005; List et al., 1995; Lo & Handel, 1983). Interesterified blends of soybean oil and fully hydrogenated soybean oil at different ratios provided the desirable texture of partially hydrogenated soybean oil, while increasing the melting point of the oils (Ribeiro, Grimaldi, Gioielli, & Goncalves, 2009).

However, interesterification alone can decrease oxidative stability (Daniels et al., 2006). According to Coenen (1976), oxidative stability of oil can be decreased during the interesterification process by initiation of lipid oxidation by extended exposure to high temperatures. Park, Terao, and Matsuhita (1983) suggested that interesterification led to

the loss of natural tocopherols and accelerated the autoxidation of randomized oils. After interesterification, α-tocopherol was not detectable, while γ-tocopherol and δ-tocoperol were reduced 12% and 39%, respectively.

Daniels et al. (2006) reported improved oxidative stability and extended induction time when hydrogenation was used together with random interesterification. In his study, combined hydrogenation and interesterification was a more effective method to increase melting point of oils than either hydrogenation or interesterification alone.

Neff et al. (1992) found that SBO stability improved through changes in triacylglycerol (TAG) composition and TAG fatty acid location. They found a positive correlation between the rate of peroxidation and average number of double bonds (r=0.81) and linolenic acid (r=0.63) at the sn-2 position. In addition, linoleic acid at the sn-2 position showed a positive correlation (r=0.72) with the rate of peroxide formation. Volatile formation was increased as the concentration of linolenic acid increased at the sn-1,3 positions and by linoleic acid at the sn-2 position. Neff, Mounts, Rinch, Konishi, and El-Agaimy (1994) reported linoleic acid concentration at the sn-2 position of TAG was decreased by blending and interesterifying soybean oil and palm olein.

Lampi, Piironen, Hopia, and Koivistoinen (1997) reported that the amount of unsaturated FAs and their degree of unsaturation is the most important factor affecting oxidation. Other factors, such as the position of unsaturated fatty acids in the TAG and the presence of anti- and prooxidants, also contribute to controlling susceptibility to oxidation.

5. High intensity ultrasound

Novel processing technologies are being sought to modify the physicochemical properties of lipids while maintaining their functional properties. Power ultrasound (US) is a non-thermal process method with high potential in the food industry and already has been used in various areas such as filtration, homogenization, extraction, cleaning, pasteurization, emulsification, oxidation, sterilization, freezing, thawing, meat tenderization, inactivation of microorganisms and enzymes, crystallization, drying and degassing (Mason, Riera, Vercet, & Buesa, 2005; McClements, 1995; Povey & Mason, 1998). The benefits from US include reduced processing times and increased efficiency. US travels like a sound wave through media. Cavitation bubbles are formed by the excessive rarefaction over attractive forces between molecules in a liquid phase at sufficiently high power (Rastogi, 2011). Cavitation bubbles produced by ultrasound collapse and create shear forces which can produce a mechanical effect (Rastogi, 2011). High intensity ultrasound (HIU) at lower frequencies (20 to 100 kHz) can cause cavitations with sound intensities of 10 to 1000 W/cm^2 (Feng & Yang, 2005; McClements, 1995).

Crystallization behavior (such as the number of crystals, crystal sizes and their network, crystallization rate, crystal morphology and crystallinity) is an important factor in fat containing products. Altering crystallization behavior by ultrasound can change the physicochemical properties of fats and oils. Accelerating nucleation in lipids is one of the important results of ultrasound application that will change the crystallization behavior (Higakia, Uenoa, Koyanob, & Satoa, 2001). Application of high intensity ultrasound to lipids modifies crystallization behavior in oils (Higakia et al., 2001; Ye, Wagh, & Martini,

2011). According to Ye et al. (2011), crystallization temperature, agitation time, and intensity of ultrasound power affect characteristics of fats. For interesterified soybean oil (IESBO) at 32 ℃, ultrasound generated smaller crystals without an effect on the total quantity of crystals generated. Additionally, when 10 seconds of HIU was applied to IESBO 10 min after stopping agitation (at 10 min), harder materials were obtained. However, deterioration of lipid molecules might occur due to sonication.

6. Meat color and Myoglobin

Myoglobin (Mb; Figure 2-1) is a very compact globular heme protein in muscle tissues that is responsible for meat color (Faustman & Phillips, 2001). Concentration and redox state of Mb are important factors that determine meat color, and the concentration of Mb is known to be influenced by both animal genetics and environment (Faustman, Chan, Lynch, & Joo, 1996; Giddings, 1974; Livingston & Brown, 1981). Thus, meat color is affected by genetics and environment as well. According to the United States

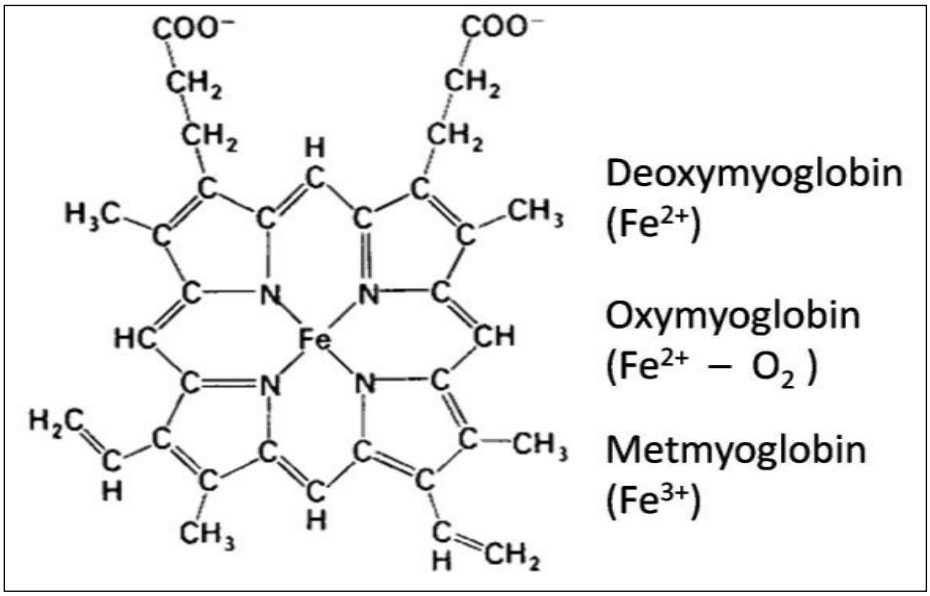

Figure 2-1. Chemical structure of myoglobin heme.

Department of Agriculture (2011), the species, sex, diet, age, and the amount of exercise of the animal affect the color. Darker meat can be obtained from older or more physically active animals because of higher myoglobin content. To understand the relationship betweencolor of meat and Mb, structure of Mb must be understood. Mb is made up of two main parts of two main parts, a globin and a prosthetic heme group (Hayashi, Takimura, Aoyama, & Hitomi 1998; Pegg & Shahidi, 1997). Globin is a single polypeptide chain of 153 amino acids (for bovine) in a globular tertiary structure, consisting of 8 right handed α-helices (A-H) connected by short nonhelical sections (Han, Dautrevaux, Chaila, & Biserte, 1970). The helices form a hydrophobic pocket of non-polar amino acids containing a heme group, with only polar residues in the pocket (His F8 and His E7). Polar residues on the surface of the Mb are hydrophilic, and this makes Mb water soluble protein. The prosthetic heme group contains an iron (II) within protoporphyrin-IX complex (Hayashi et al., 1998; Pegg & Shahidi, 1997), and the iron can form six coordinating bonds. Four of the six bonds are with nitrogen contained within the tetrapyrrole ring system, and the 5^{th} bond is with proximal histidine (His F8), anchoring the heme group within the hydrophobic pocket of the protein (Clydesdale & Francis, 1971). The 6th coordination position of the iron is available for a bond with oxygen or carbon monoxide, which is stabilized by the distal histidine (His E7; Pauling, 1964; Perutz & Mathews, 1966; Phillips & Schoenborn, 1981). Because of this oxygen binding property of Mb, Mb can store oxygen. Oxygen binds to the iron at 120 °angle, allowing oxygen to be removed easily from the iron (Makino et al., 2011). The state and structure of the iron affects the reactions and color changes of Mb (Livingston & Brown, 1981).

7. The chemistry of meat color

There are three major myoglobin forms that determine meat color: deoxymyoglobin, oxymyoglobin, and metmyoglobin. The chemistry of iron in the heme group is important for color (Livingston & Brown, 1981; Figure 2-2). Deoxymyoglobin occurs when the 6th coordination site is empty and heme iron is in its reduced ferrous deoxymyglobin can be maintained in its deoxygenated form under very low oxygen tension (<1.4 mm Hg). When deoxymyoglobin is oxygenated, oxygen is covalently bonded to heme iron center forming oxymyoglobin. The heme iron remains in its reduced ferrous state, and oxymyoglobin gives a bright cherry red color equated with freshness of meats (Young, Reviere, & Cole, 1988). Oxygenation occurs when oxygen concentrations are relatively high (Taylor, 1985). As exposure to oxygen increases, the oxymyoglobin

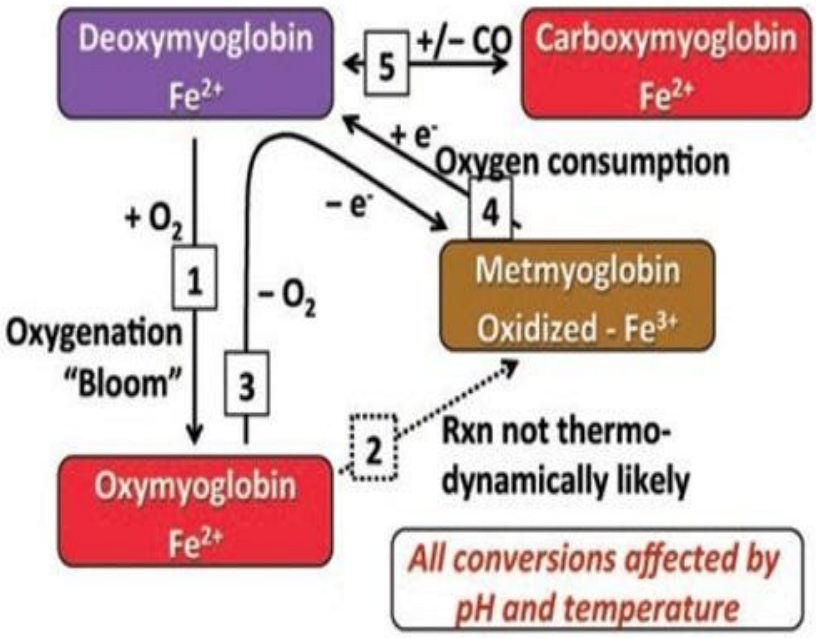

Figure 2-2. Schematic of the interconversions of myoglobin redox forms in fresh meat. Courtesy of Hunt and Cornforth in American Meat Science Association (2012).

layer moves deeper from the meat's surface. The meat's temperature, oxygen partial pressure, pH, and competition for oxygen by other respiratory processes are the important factors that affect depth of oxygen penetration and thickness of the oxymyoglobin layer (Mancini & Hunt, 2005).

Metmyoglobin is formed when the unoccupied ferrous heme center in deoxymyoglobin is oxidized to its ferric state (Fe^{3+}; Livingstone & Brown, 1981; Wallace, Houtchens, Maxwell, & Caughey, 1982). Metmyoglobin cannot bind oxygen since iron in the ferric state pulls electrons more tightly and prevents the important back-donation of electrons (Livingstone & Brown, 1981). Brown metmyoglobin on the surface of red meats is considered as discoloration, and the amount of discoloration is often defined as the amount metmyoglobin covering the surface. Various factors can affect metmyoglobin formation such as oxygen partial pressure, temperature, pH, endogenous reducing activity, and in some cases micro-bacterial growth (Mancini & Hunt, 2005). Brown color caused by metmyoglobin formation is undesirable for the producer since perception of freshness is involved with discoloration. Smith, Belk, Sofos, Tatum, and Williams (2000) estimate that surface discoloration causes price discounts of approximately 15 % for retail beef, which corresponds to annual revenue losses of $1 billion within the industry.
Reducing ability of muscle products is also important for meat color life. It is dependent upon the activity of oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool (Mancini & Hunt, 2005).

To evaluate meat color, color of meats can be measured instrumentally using Hunter a* (+: redness/ -: greenness), b* (+: yellowness/ -: blueness), and L* (+: white/ -: black). Chroma (color saturation), or hue angle (true redness) are often calculated from

these measurements to evaluate color stability of meat.

8. Oxidation in meat

Similar to oils, lipid oxidation is important in meat products. Oxidation is a main reason for quality deterioration of meat products (Ladikos & Lougovois, 1990; Min & Ahn, 2005). From harvest to consumption, handling, processing and storage conditions can cause lipid oxidation which leads to discoloration, increased drip losses, off-odor and off flavor development, texture defects, and the production of potentially toxic compounds (Morrissey, Sheehy, Galvin, & Kerry, 1998; Richards, Modra, & Li, 2002). Lipid oxidation is an important oxidation reaction in muscle foods, and it has been shown to directly participate in myoglobin oxidation.

Lipid oxidation can be catalyzed by damaged heme pigments, including H_2O_2 denatured myoglobin (Han, Mcmillin, & Godber, 1994; Love, 1983). Active species such as perferrylmyoglobin ($^{\bullet}$MbFe(IV)=O) and ferrylmyoglobin (MbFe(IV)=O) produced from the interaction of H_2O_2 and metmyoglobin have also been shown to initiate lipid peroxidation in model system studies (Chan, Faustman,Yin, & Decker, 1997; Davies, 1990, 1991; Kanner, German, & Kinsella, 1987). Morey, Hansen, and Brown (1973) reported that altered oxidation state of the iron in myoglobin by H_2O_2 (oxidizing agent) led to red-brown color formation. Lipid oxidation products can alter myoglobin redox stability resulting in the promoted oxidation of oxymyoglobin (Lynch & Faustman, 2000) and formation of adducts with myoglobin through covalent modification (Michael Adducts; Faustman, Liebler, McClure, & Sun, 1999; Lee, Joo, Alderton, Hill, & Faustman, 2003; Lee, Phillips, Liebler, & Faustman, 2003; Phillips et al., 2001; Phillips,

Lee, Silbart, & Faustman, 2001). It has also been suggested that deoxymyoglobin can initiate lipid oxidation at low lipid hydroperoxide concentrations (Richards & Hultin, 2000). However, the extent of protein denaturation and damage was not measured, so the results of this study may also be explained by the presence of free heme in the system, which is known to contribute to oxidative damage.

9. Meat packaging

According to the Institute of Food Science and Technology guidelines (1993), shelf life is determined by safety, nutrition loss and desirable sensory, chemical, physical and microbiological characteristics. In addition, shelf life is determined by consumers' expectations in qualities such as appearance, taste, texture, and smell (Labuza & Schmidl, 1988). But display life, the possible period of time that a product can be displayed under refrigerated conditions without color change, is equally important.

In the traditional meat distribution scheme, retail stores receive primal and sub-primal cuts in vacuum packages; the primal cuts are trimmed and cut to consumer-size cuts and packaged in disposable retail polystyrene trays using O_2-permeable films such as PVC film (Cole, 1986). However, at the retail store, labor and time are still required for preparing final retail packs (Nortje & Shaw, 1989). Centralized packaging systems have slowly taken over the traditional system, combining the meat packer and retail levels (Jeyamkondan, Jayas, & Holley, 2000). Master packaging systems have been designed that are suitable for centralized packaging. Master packaging is a method that uses a large bag, called mother bag or master pack, which contains four to six individually packaged PVC wrapped meat cuts in foam or plastic trays. The mother bag is then flushed with the

desired gas after air is removed from the bag. Maximum storage life can be reached by creating a 100% CO_2 atmosphere (Spahl, Reineccius, & Tatini, 1981). Shay and Egan (1987) reported master packaged retail-ready meat containing O_2 had considerably shorter storage life. After delivery to stores, individual meat packs are removed from the mother bag and exposed to oxygen to bloom (turn red), a process that takes about 30 min (Penny & Bell 1993; Seideman, Cross, Smith, & Durland, 1984).

10. Effect of CO_2 on red meat

The effect of CO_2 can be explained by two different mechanisms: (1) the concentration of CO_2 in the package headspace; or (2) the concentration in food as absorbed CO_2. Löwenadler and Rönner (1994) reported that the concentration of CO_2 in the package headspace and the ratios of headspace to product volumes are closely related to the concentration of the dissolved gas in foods. Devlieghere, Debevere, and Van Impe (1998) reported that the initial CO_2 concentration in the gas-phase and the gas/product ratio were the major factors determining the amount of dissolved CO_2. Packaging collapse is a risk in MAP using a high percentage of CO_2 in the headspace because CO_2 dissolves in both muscle and fat tissue (Gill, 1988). Absorption of CO_2 by meat is dependent on temperature, pH, partial CO_2 pressure, headspace to meat volume ratio, meat surface area, total meat volume, and water to fat content (Jakobsen & Bertelsen, 2002). Jakobsen and Bertelsen (2002) reviewed the effect of CO_2 on chemical quality changes of packaged fresh red meats (such as beef, lamb and pork). They concluded that packaging and storage conditions were responsible for the amount of absorbed CO_2, with observed concentrations ranging from 0 to 1.79 L CO_2/kg in packaged red meats. The

maximum absorption (1.79 L CO_2/kg) was found in ground beef stored at 13 ℃ where the partial pressure of CO_2 was 230 kPa (Zhao, Wells, & McMillin, 1995)

Meat is high in protein, has high water holding capacity, and is a good buffer system. The pH of meat is closely correlated with meat texture and color. However, studies on the effect of CO_2 have been inconsistent. Some studies showed CO_2 lowered the pH of meat (Jakobsen & Bertelsen, 2002; Ledward, 1970). On the other hand, no change in pH was observed in a more recent study (Esturk & Ayhan, 2009). Carbonic acid, which rapidly dissociates to bicarbonate and hydrogen ions, is formed by exposing meat to CO_2, and this might be responsible for the decrease in pH (Dixon & Kell, 1989; Jakobsen & Bertelsen, 2002). Postmortem pH control is very important for managing final meat quality. A rapid decline in pH or low ultimate pH induces pale, soft and exudative (PSE) meat while dark meat with a dull and dry surface results from too high pH (Gill, 1988). Cornforth (1994) reported that meat with a high pH has a higher water-binding capacity, hence making it appear darker.

Storing beef in 20 to 25% CO_2 balanced with ambient air did not affect meat color (Brooks, 1933; Clark & Lentz, 1972; Ledward, 1971). Since CO_2 used in high O_2 MAP restricts bacterial spoilage, shelf life is dictated by color, not microbial growth (Gill & Jones, 1994). Extended lag phase and increased generation time were observed under CO_2 storage (Daniels, Krishnamurthi, & Rizvi, 1985; Stiles, 1991a, 1991b). However, the level of O_2 used in MAP (typically 80%) can lead to the development of off flavors and odors due to lipid oxidation (Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002). Bone discoloration may also occur, as was observed in beef lumbar vertebrae in meat cuts stored in high O_2 MAP (Grobbel et al., 2006).

11. Trona mineral

Few studies have been conducted on the possibility of generating CO_2 within the package itself, for example through acid-base reactions. One possible source of CO_2 is Trona (sodium sesquicarbonate), an evaporite mineral with the chemical formula $Na_3CO_3(HCO_3)_2(H_2O)$, is the primary source of sodium carbonate in the US (Virginia Department of Health, 2007). Six companies in the United States produce over 14 million tons of sodium carbonate annually, which is used in glass and fiberglass manufacture, powdered detergents, food leaveners, photography, and pH control of water.

Trona is composed of approximately 46% sodium carbonate (Na_2CO_3) and 36% sodium bicarbonate ($NaHCO_3$). Raw trona dissolved in water produces about 16% sodium carbonate and 10% sodium bicarbonate in solution without heat treatment (calcining) (Solvay Minerals Inc, 1997). Sodium bicarbonate can generate approximately 52% CO_2 by weight. Degradation of sodium bicarbonate ($NaHCO_3$) to carbon dioxide and sodium carbonate can occur after absorption of moisture at lower temperatures (Shefter, Lo, & Ramalingam, 1974). When sodium bicarbonate is stored as a powder, it can degrade over time as a result of moisture absorption, or can decompose directly to carbon dioxide and sodium carbonate at elevated temperatures (Shefter et al., 1974). Decomposition of sodium bicarbonate with loss of carbon dioxide may begin above 85% relative humidity (Rowe, Sheskey, & Owen, 1986). Kuu, Chilamkurti, and Chen (1998) reported sodium bicarbonate powder was stable below 76% relative humidity at 25 ℃ and the decomposition of sodium bicarbonate to sodium carbonate is very slow at low temperature (room temperature). They also reported that the weight of sodium bicarbonate powder increased rapidly at 93% relative humidity because of the fast rate of

moisture sorption and slow rate of losing carbon dioxide and water molecules. They suggested the generated CO_2 remained in the aqueous phase at low temperature due to its increased solubility, so the concentration of CO_2 in the aqueous phase has to increase to sufficient amount for CO_2 to be transferred from the aqueous phase to the gas phase. However, at elevated temperature (55 ℃) sodium bicarbonate lost its weight continuously even at 30% relative humidity due to rapid formation and release of CO_2 with relatively low rate of moisture sorption (Kuu et al., 1998). Direct thermal decomposition of sodium bicarbonate to CO_2, water, sodium carbonate was shown at 124 ℃ (Krupkowski, 1983). Sodium carbonate reacts with acid (HA) to form CO_2 gas in a three step process.

$$Na_2CO_3 + HA \rightarrow NaA + NaHCO_3 \quad (1)$$

$$NaHCO_3 + HA \rightarrow NaA + H_2CO_3 \quad (2)$$

$$H_2CO_3 \rightarrow H_2O + CO_2\,(g) \quad (3)$$

When sodium bicarbonate is used as a leavening agent in bread making, the CO_2 generation capacity of sodium bicarbonate is dependent on the type of acids (fast- or slow- acting acids; Conn, 1965, 1981; LaBaw, 1982). Sodium bicarbonate generates CO_2 with acid in the presence of water at room temperature. Fordtran, Morawski, Santa Ana, and Rector (1984) reported that sodium bicarbonate reacted with hydrochloric acid instantaneously generating CO_2, but CO_2 was slowly released into the gas phase in vitro method for measuring gas release after addition of sodium bicarbonate to a solution containing hydrochloric acid. They assumed that was because the generated CO_2 remained in the aqueous phase due to its higher solubility at relatively low temperature as Kuu et al. (1998) suggested.

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CHAPTER 3

EFFECT OF HIGH INTENSITY ULTRASOUND ON OXIDATIVE STABILITY OF SOYBEAN OIL AND INTERESTERIFIED SOYBEAN OIL

Abstract

The objective of this study was to evaluate the effect of high intensity ultrasound (HIU) on oxidative stability of soybean oil (SBO) and interesterified soybean oil (IESBO) by measuring peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). HIU was applied for 10 seconds using an acoustic power of 110W. After sonication, the sample was kept at a crystallization temperature (32 ℃) for 90 min to allow complete crystallization in the IESBO. Four different oil samples (Sonicated (S)-IESBO, Non Sonicated (NS)-IESBO, Sonicated (S)-SBO and Non Sonicated (NS)-SBO) were stored in amber bottles and exposed to oxygen by leaving the bottle caps unscrewed at 25 ℃ for 189 days or 40 ℃ after 105 days of storage at 25 ℃. SBO had a significantly lower initial peroxide value than IESBO regardless of HIU application ($P<0.0001$). IESBO had a shorter induction period than SBO (119 and 133 days, respectively) at 25 ℃ regardless of HIU application. However, the hydroperoxide formation rate of SBO (0.626 mEq/kg/day) was higher than that of IESBO (ca. 0.222 mEq/kg/day) after induction time. The samples transferred and stored at 40 ℃ after 105 days of storage at 25 ℃ oxidized faster than those at 25 ℃. The PVs of S-SBO and NS-SBO increased faster than S-IESBO and NS-IESBO. HIU application reduced the oxidation rate for semi solid oils (IESBO), while it increased the oxidation rate in liquid oils (SBO) in oils which had high PV. On days 35 and 42 the PVs of S-SBO were significantly higher than those of NS-SBO at 40 ℃ ($P<0.0001$ for all

values; 90.73 ± 0.46 and 77.09 ± 0.60 on day 35; 134.96 ± 0.37 and 116.94 ± 0.95 on day 42, respectively). NS-IESBO had a considerably higher PV than S-IESBO on day 42 ($P<0.0001$). TBARS values were maintained under 0.1 mg Malondialdehyde (MDA)/kg oil throughout the entire time of this study. NS-IESBO had a liquid-like texture and a visible separation of solid crystals and liquid. On the other hand, S-IESBO had a gel-like consistency and no separation was observed.

In conclusion, HIU improved consistency but did not affect oxidative stability of IESBO from a practical standpoint. Further study such as volatile headspace analysis will help to measure secondary oxidation products accurately and to evaluate rancid odor and flavor change over time.

1. Introduction

Shelf life of oils and fats is closely related to oxidation. Lipid oxidation affects chemical, sensory, and nutritional properties of oils and fats (Frankel, 1982). One of the major problems caused by oxidation is rancidity development, which gives an off flavor and odor to products. Rancidity in foods is not usually acceptable to consumers (Labuza, 1971; Paquette & Kanaan, 1985; Robards, Kerr, & Patsalides, 1988). Storage conditions and processing methods are very important in rancidity development. Processing factors such as temperature, chemicals, and the length of time a product is exposed to either, can accelerate or retard oxidative stability. In addition, storage conditions such as temperature, exposure to light or oxygen, and time period need to be considered to predict shelf life during storage.

Processing can improve functionality of lipids, but it can give an adverse effect

on storage stability such as oxidative deterioration. Much current research focuses on finding products that are stable from oxidation after processing with desirable functionality. Physical and chemical properties and the stility of soybean oil (SBO) have been studied in the past for practical purposes (DeMan, 1992; Formo, 1979; Pryde, 1980). Hydrogenation used in oil and fat processing improves texture and oxidative stability of SBO by lowering unsaturated fatty acid content (Dutton, 1979). After partial hydrogenation became undesirable due to health concerns related to trans-fatty acids, interesterification of blends of fully hydrogenated vegetable oil with liquid oils was studied intensively (Hurtova, Schmidt, Zemanovic, Simon, & Sekretar, 1996; List, Emken, Kwolek, Simpson, & Dutton., 1977; List, Mounts, Orthoefer, & Neff, 1995; Zeitoun, Neff, List, & Mounts, 1993). Interesterification can improve the physical properties of a product, but oxidative stability is not guaranteed. Hydrogenation and interesterification together can provide desirable functionality along with improved oxidative stability of SBO (Daniels, Kim, & Min, 2006).

Harder materials can be obtained by high intensity ultrasound (HIU) since it can modify texture and hardness by changing crystallization behavior such as crystal size, the number of crystals and induction time (Martini, Suzuki, & Hartel, 2008; Suzki, Lee, Padilla, & Martini, 2010; Ye, Wagh, & Martini, 2011). However, HIU application can accelerate deterioration. Deterioration in sensory and chemical stability by high power ultrasound was observed by Chemat et al. (2004a), who found peroxide values to dramatically increase in sonicated (for 1 min) refined sunflower oil after 30 days of storage. In addition, Chemat, Grondin, Sing, & Smadja (2004b) found compounds responsible for off flavors in sonicated (for 1 hr) sunflower oil such as hexanal, hept-2-

enal, decadi-2,4-enal and limonene by solid phase extraction and GC-MS. However, the length of ultrasound application time is an important factor that should be considered to evaluate the deteriorative effect of HIU. Few studies have examined the stability of sonicated interesterified soybean oil shortening under long term storage at room temperature. Also, there is insufficient information available that evaluates the effect of short periods of HIU application on the stability of interesterified blends of fully hydrogenated hard stock and soybean oil for long term storage at room temperature.

The aim of this study was to evaluate oxidative stability of IESBO with HIU application. The oxidative stability of IESBO was compared to SBO since the effect of HIU and interesterification on oxidative stability can be evaluated together.

2. Materials and Methods

2.1. Interesterified soybean oil and Soybean oil

A low saturated fat shortening (IESBO; product no. 76-240-0), produced by interesterification of liquid soybean oil and a fully hydrogenated hard stock, was provided by Archer-Daniels-Midland (Decatur, IL). Liquid soybean oil (SBO) from Archer-Daniels-Midland (Decarut, IL) was used for this experiment.

2.2. Crystallization and high intensity ultrasound application

SBO and IESBO were sonicated and prepared as described by Ye et al. (2011). Briefly, IESBO or SBO (100 g) was heated to 80 ℃ and kept at this temperature for 30 min to allow complete melting of the triacylglycerol (TAG). The melted lipid sample was placed in a thermostatized crystallization cell at 32 ℃. IESBO was crystallized with

agitation (200 rpm). HIU was applied after 20 min into the crystallization experiments. Before HIU was applied, agitation was stopped. HIU was applied for 10 seconds (Misonix S-3000 sonicator, Misonix Inc., Farmingdale, NY) using acoustic power (110W). After sonication, the sample was kept at the crystallization temperature (32 ℃) for 90 min to allow complete crystallization. After 90 min, the sample was placed in an incubator at 25 ℃ until all required sample was prepared. Crystallization process was repeated five times to obtain total of 500 g of each sample, then the collected sample (500 g) was mixed with a glass stir bar in a beaker (1000 ml). Aliquots of mixed sample (17 g) were transferred into amber bottles (60 ml volume) to avoid light exposure. The cap was loosely placed on the top of the bottle to allow for air exposure then stored in an incubator at 25 ℃ for 6 months. After 109 days of storage at 25 ℃, six bottles from each condition (sonicated or non sonicated oils) were transferred to an incubator 40 ℃ and stored for 6 weeks. For the comparison to sonicated oils, non-sonicated SBO and IESBO was prepared in the same manner as described above, except HIU was not applied.

2.3. Peroxide Value (PV)

PV was measured as described in AOCS official method Cd 8-53, on samples (5.00 ± 0.05 g) in a 250 ml stoppered glass Erlenmeyer flask. Thirty milliliters of an acetic acid - chloroform (3:2, v/v) solution was added to the flask. The flask was swirled until the sample was completely dissolved. Saturated potassium iodide (KI) solution was prepared by dissolving an excess of KI crystals in recently boiled distilled water, with the presence of undissolved crystals taken as the indication of saturation. The saturated KI solution (0.5 ml) was added to the flask, which was then stoppered and the contents of the

flask were swirled for exactly 1 min. Distilled water (30 ml) was immediately added by graduated cylinder. The mixture was titrated with 0.01 N sodium thiosulfate. If the starting color of the solution was deep red orange, it was titrated slowly with mixing until the color lightened. Starch solution was prepared by dissolving a paste (1 g of potato starch in a small amount of cold distilled water) to 100 ml of boiling water. The starch solution (2 ml) was added into the flask as an indicator, and the mixture was titrated until the blue gray color disappeared in the aqueous phase. Three replicates were performed per each oil sample. Values were calculated as follows:

$$\text{Peroxide value (milliequivalents peroxide/1 kg sample)} = \frac{(\text{S-B}) \times \text{N} \times 1000}{\text{mass of sample, g}}$$

where B=volume of titrant (mL) for blank; S=volume of titrant (mL) for sample; N= normality of sodium thiosulfate solution.

2.4. Thiobarbituric acid reactive substances (TBARS)

TBARS was performed according to AOCS official method Cd 19-90. Oil samples (50 to 200 mg) were accurately weighed into a 25-ml volumetric flask. Three replicates were performed per oil sample. The sample was dissolved in a small amount of 1-butanol, then brought to volume with 1-butanol and mixed thoroughly. The sample solution (5 ml) was transferred to a stoppered glass test tube. A 0.2% TBA solution in 1-butanol (5 ml) was added. The glass stopper was placed on the tube. A reagent blank (5 ml each of 1-butanol and 0.2% TBA) was prepared at the same time. The test tubes were placed in a 95 ℃ water bath for 2 hr. The tubes were then removed from the bath and cooled under running tap water for 10 min (until they reached room temperature). A Shimadzu UV 2100 U spectrophotometer (Columbia, MD) was turned on and allowed to

warm up ≥30 min before taking any readings. Two absorbance readings were taken per test solution at 532 nm then averaged. The spectrophotometer was zeroed with the reagent blank and then the absorbance of the test sample was measured using a glass cuvette (10 mm pathlength). TBA values were calculated as follows:

$$\text{TBA}=\frac{50\times(\text{A-B})}{\text{m}} = \text{mg Malondialdehyde (MDA)/kg}$$

where: A is the absorbance of the test solution; B is the absorbance of the reagent blank; m is the mass (mg) of the sample; and 50 is the dilution factor when using a 25 ml volumetric flask and a pathlength of 10 mm.

2.5. Statistical Analysis

Analysis of variance using the proc mixed function was performed in SAS version 9.1 (SAS Institute, Inc., Cary, NC). PV and TBARS were measured three times per sample. The main effect of time (day), type of oils, treatment (HIU application) and interactions of the factors was analyzed. The effect of type of oils and treatment on peroxide value was analyzed for means comparisons for each storage time separately. Statistical significance was identified at the 95% confidence level, and post-hoc means comparisons were made based on *p* values obtained using the Tukey-Kramer adjustment.

3. Results and Discussion

3.1. Apprearance after sonication

Differences in appearance were observed between non sonicated (NS-IESBO) and sonicated interesterified soybean oils (S-IESBO) stored at 25 ℃. Crystals (solid portion) in NS-IESBO started to settle in the bottom of the bottle, with liquid remaining

on the upper part (see Appendix A). This separation of crystals and liquid became even more obvious over time. A thin layer of crystals formed at the top. Liquid and crystals in NS-IESBO stayed separated through the entire storage period, and the sample appeared runny. On the other hand, there was no separation of crystals and liquid in S-IESBO. S-IESBO had a thick, creamy consistency. S-IESBO was more consistent in terms of crystal distribution than NS-IESBO. Both sonicated soybean oil (S-SBO) and non sonicated soybean oil (NS-SBO) stayed clear liquid through the entire storage period.

3.2. Peroxide value (PV)

As shown in Figure 3-1, SBO had significantly lower initial PV than IESBO regardless of HIU application ($P<0.0001$). The initial PVs of S-SBO and NS-SBO were 0.39 ± 0.06 mEq/kg and 0.59 ± 0.06 mEq/kg, respectively, while those of S-IESBO and NS-IESBO were 1.19 ± 0.12 and 1.26 ± 0.00 mEq/kg, respectively. There was a significant interaction between oil type and HIU treatment ($P<0.0001$; see Appendix B for detailed statistics). Oxidative stability of oils is defined as the resistance to oxidation during processing and storage and is often based on the induction period (Guillen & Cabo, 2002). Induction period is the time required to reach a sudden increase in oxidation rate or give a sensorial change (Frankel, 1993; Silva, Borges, & Ferreira, 2001). The induction period of IESBO stored at 25℃ was 119 days regardless of HIU application, based on the sudden increase in PV from 0.22 to 0.56 mEq/kg/day (Figure 3-1). SBO had a longer induction period (133 days) than NS-IESBO and S-IESBO. The increased induction period of SBO as compared to IESBO indicates that SBO had a better oxidative stability during the initial storage period. However, both SBO treatments had higher PVs

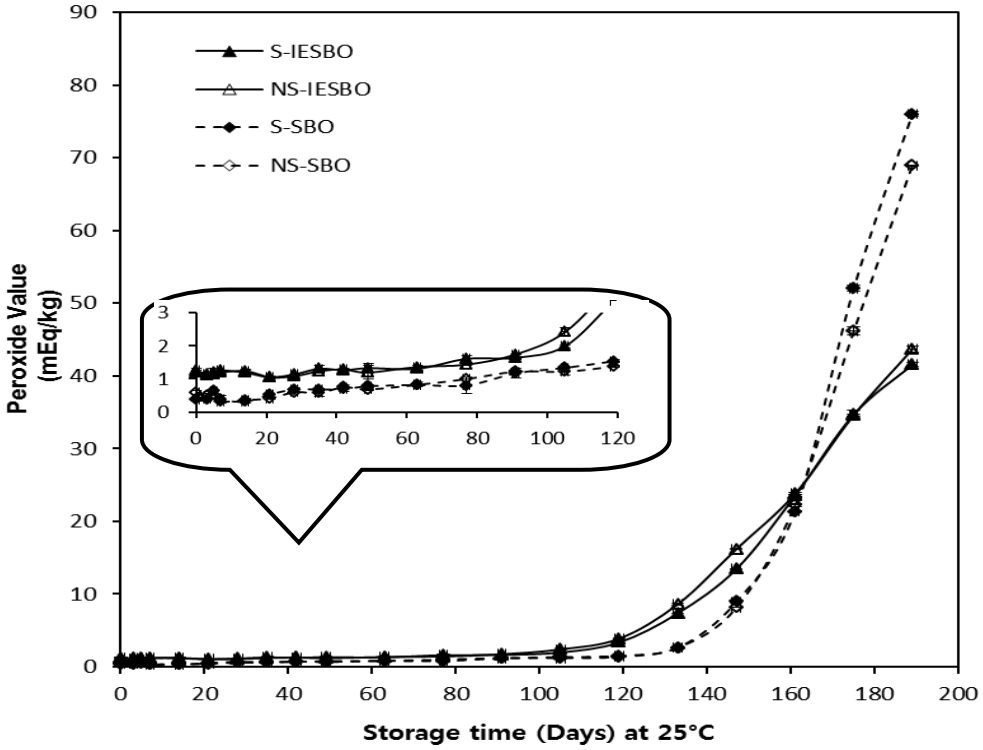

Figure 3-1. Peroxide value change of interesterified soybean oil and soybean oil with or without high intensity ultrasound stored at 25 ℃ for 189 days.
S-IESBO: IESBO with HIU application; NS-IESBO: IESBO without HIU application; S-SBO: SBO with HIU application; and NS-SBO: SBO without HIU application.
Error bars: ± standard deviation.

($P<0.0001$; S-SBO: 76.01 ± 0.31 mEq/kg; NS-SBO: 68.01 ± 0.06 mEq/kg) than those of the IESBO samples (S-IESBO:41.57 ± 0.26 mEq/kg; NS-IESBO; 43.67 ± 0.40 mEq/kg) on day 189, and this trend was first noticeable on day 175. HIU application accelerated peroxide formation of SBO in long term storage. S-SBO had significantly higher PVs than NS-SBO on days 147, 175, and 189 ($P<0.0001$). On the other hand, NS-IESBO had slightly but significantly higher PVs than S-IESBO on days 105, 119, 133, 147, and 189 ($P<0.05$).

A similar trend was observed in samples stored at 40 ℃ for the final portion of

the storage time (Figure 3-2). There was significant effect of storage time (day), oil type, HIU treatment and the interactions of oil type*HIU treatment, oil type*day, HIU treatment*day and oil type*HIU treatment*day (P<0.0001, see Appendix B). The PVs of NS-SBO and S-SBO indicate longer induction times, but ultimately exhibited higher oxidation rate than NS-IESBO and S-IESBO. S-IESBO had significant lower PVs than NS-IESBO on days 14, 35, and 42 (P<0.0001). There were significant differences in PVs between S-SBO and NS-SBO throughout 42 days at 40 ℃. S-SBO had significant higher PVs than NS-SBO on days 35 and 42. Rancidity becomes noticeable at different PV

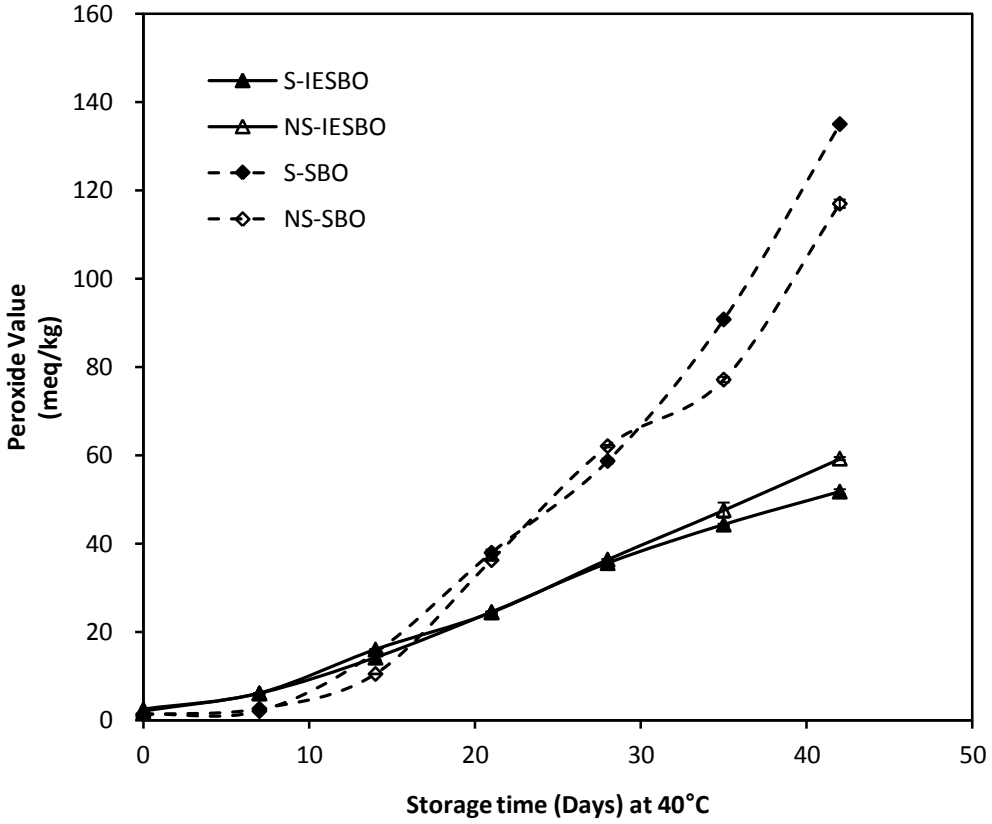

Figure 3-2. Peroxide value change of interesterified soybean oil and soybean oil with or without high intensity ultrasound stored at 25 ℃ for 105 days then 40 ℃ for 42 days. S-IESBO: IESBO with HIU application; NS-IESBO: IESBO without HIU application; S-SBO: SBO with HIU application; and NS-SBO: SBO without HIU application. Error bars: ± standard deviation.

levels depending on the type of oil. However, a rancid taste is often noticeable when PV is above 10 mEq/kg, while rancidity in refined oils may become noticeable at PV levels below 1 mEq/kg (Gunstone, 1996). SBO Soybean oils that have peroxide values of 1-5 mEq/kg, 5-10 mEq/kg and >10 mEq/kg are considered as slightly, moderately and highly oxidized, respectively (AOCS, 1997; Nielson, 2003). Both IESBO samples exceeded 10 mEq/kg after 133 days of storage at 25 ℃ and between days 7 and 14 of storage at 40 ℃ (112 to 119 days total). The PVs of NS-IESBO and S-IESBO exceeded 10 mEq/kg more quickly than S-SBO and NS-SBO. S-SBO had significantly higher PVs than NS-SBO on the last day of storage period, while S-IESBO had significantly lower PVs than NS-IESBO at both temperatures ($P<0.05$).

A significant difference between S-IESBO and NS-IESBO was found on day 14, but this difference was not significant from a practical standpoint. This difference between NS-IESBO and S-IESBO in taste and odor might not be obvious to consumers since the differences in PV were only 2-3 mEq/kg, and all samples were already rancid based on PVs (>10 mEq/kg). The final PVs of S-SBO and NS-SBO stored at a higher temperature (day 42 at 40 ℃; 147 days total) were approximately double those for oils stored only at 25 ℃ (189 days total).

The higher initial PV and shorter induction period of NS-IESBO and S-IESBO as compared to NS-SBO and S-SBO (Figures 3-1 and 3-2) is due to interesterification. Oxidative stability of oil can be decreased during interesterification process by initiating lipid oxidation due to extended exposure to high temperature (Coenen, 1976). In addition, the alkaline catalyst reagent used during interesterification can decrease oxidative stability by destroying endogenous antioxidants such as tocopherols. Tocopherol content

of edible oils is dependent on the cultivar, processing and storage of the oil (Deiana et al., 2002). According to Park, Terao, and Matsushita (1983), loss of antioxidants such as tocopherols led to accelerated autoxidation of random oils. They found that after interesterification, α-tocopherol was not detectable, while γ-tocopherol and δ-tocoperol reduced 12% and 39%, respectively. Antioxidants retard lipid oxidation by donating hydrogen to free radicals and converting them to more stable forms (Decker, 2002). Antioxidant radicals formed after hydrogen donation must be present in large quantities to initiate a chain or free radical propagating oxidation reaction since they are relatively stable at low levels (Wanasundara, & Shahid, 2005). In addition, antioxidant radicals are capable of reacting with free radicals (e.g. peroxy and alkoxy radicals) to stop propagation.

Despite the longer induction period of NS-SBO and S-SBO compared to NS-IESBO and S-IESBO (Figures 3-1), oxidation rate of SBO (ca. 1.25 mEq/kg/day) was higher than IESBO (ca. 0.56 mEq/kg/day) after induction period. There are two possible reasons for the higher oxidation rate. First, more rapid oxidation can be seen in oils that contain more unsaturated fatty acids (FA) compared to more saturated oils (Parker, Adams, Zhou, Harris, & Yu, 2003). SBO contains more unsaturated FAs than the IESBO used in this study (Table 3-1). The percent of unsaturated FAs is 84.8% in SBO, compared to 65.8% in IESBO. Second, triacylglycerol (TAG) composition and structure affect hydroperoxide formation (Neff et al., 1992). A decrease in linolenic (C18:3) and linoleic (C18:2) acid content and an increase in oleic (C18:1) acid content can result in improved oxidative stability in vegetable oils with modified FA composition (Liu & White, 1992; Mounts et al., 1988; O'Keefe, Wiley, & Knauft, 1993; Przybylski et al.,

Table 3-1. Fatty acid composition of IESBO and SBO.

FA	IESBO	SBO
C16:0	10.62	10.60
C18:0	21.66	4.00
C18:1	17.38	23.30
C18:2	41.69	53.70
C18:3	6.57	7.60
C20:0	0.39	0.30
C20:1	0.17	-
C22:0	1.52	0.30
C16:1	-	0.10
C17:1	-	0.10
Total	100.00	100.00

Source: Adapted from Ye et al. (2011) for IESBO fatty acid composition and O'Brien (1998) for SBO fatty acid composition.
Note: Percent of fatty acids were recalculated by omitting values under 0.1%.

1993). However, SBO contains more oleic acid (which improves oxidative stability), more linoleic acid (which decreases oxidative stability), and a similar percent of linolenic acid compared to IESBO. Hence, the structure and composition of TAGs for SBO and IESBO are more important to explain their different oxidation rates. TAGs containing unsaturated FAs in the sn-2 position have been reported to be more oxidatively stable than those with those acids in the sn-1 or -3 positions (Hoffmann, Stroink, Polman, & Van Ooster, 1973; Raghuveer & Hammond, 1967; Wada & Koizumi, 1983). In agreement with these previous studies, rate of peroxide formation showed a strong negative correlation with oleic acid at the sn-2 position of TAGs in variety of crude SBOs (Neff et al., 1992). However, a high ratio of linoleic at the sn-2 position of TAGs increased the rate of peroxide formation (Neff et al., 1992; Neff, Mounts, Rinsch, & Konishi, 1993). The same results were seen in modified canola oils (Neff, El-Agaimy, & Mounts, 1994a; Neff, Mounts, Rinsch, Konishi, & El-Agaimy, 1994b). The position of linolenic acid in

TAGs did not influence the rate of peroxide formation (Neff et al., 1992, 1994b). An interesterified blend of 20% fully hydrogenated SBO and 80% SBO (which has similar solid fat content to that of the IESBO used in this study) was found to contain ca.30% less linoleic acid on the sn-2 position of TAGs as compared to the refined SBO (Ribeiro, Grimaldi, Gioielli, & Goncalves, 2009). In addition, the refined SBO had a 5% higher rate of oleic acid incorporation at sn-2 position than the interesterified oil blend.

3.3. Thiobarbituric acid reactive substances (TBARS)

There were significant effects of time, HIU, type and HIU, type*HIU*time at 25 ℃ and 40 ℃ ($P<0.05$). TBARS values stayed very low (below 0.1 mg MDA/kg) during the entire 6 month storage period, but fluctuated between 0.01 and 0.08 mg MDA/kg at 25 ℃ and 40 ℃ (Figures 3-3 and 3-4). Lower TBARS values of soybean oil have been reported in other studies, and according to Pokorny and Dieffenbacher (1989), the mean value for the TBARS of soybean oil stored at 25 ℃ for 2 yr from 20 different laboratories was 0.165 mg MDA/kg. Lee, Kim, Kim, Kim, and Lee (2003) reported that TBARS values of SBO stored at 60 ℃ were ranged from 0.10 to 0.15 mg MDA/kg where PVs were ranged from 50 to100 mEq/kg oils. The TBARS values of the study are higher than the values in the current study, and the higher storage temperature might be a reason for those higher values. Primary oxidation products begin to decompose to secondary oxidative products around 20 mEq peroxide/kg of oil (Cocks & Van Rede, 1966). However, no decrease in PV (indicative of decomposition primary oxidation products) was observed through the entire storage period, regardless of temperature (25 ℃ and 40 ℃). A larger increase in TBARs might have been seen if samples had been stored long

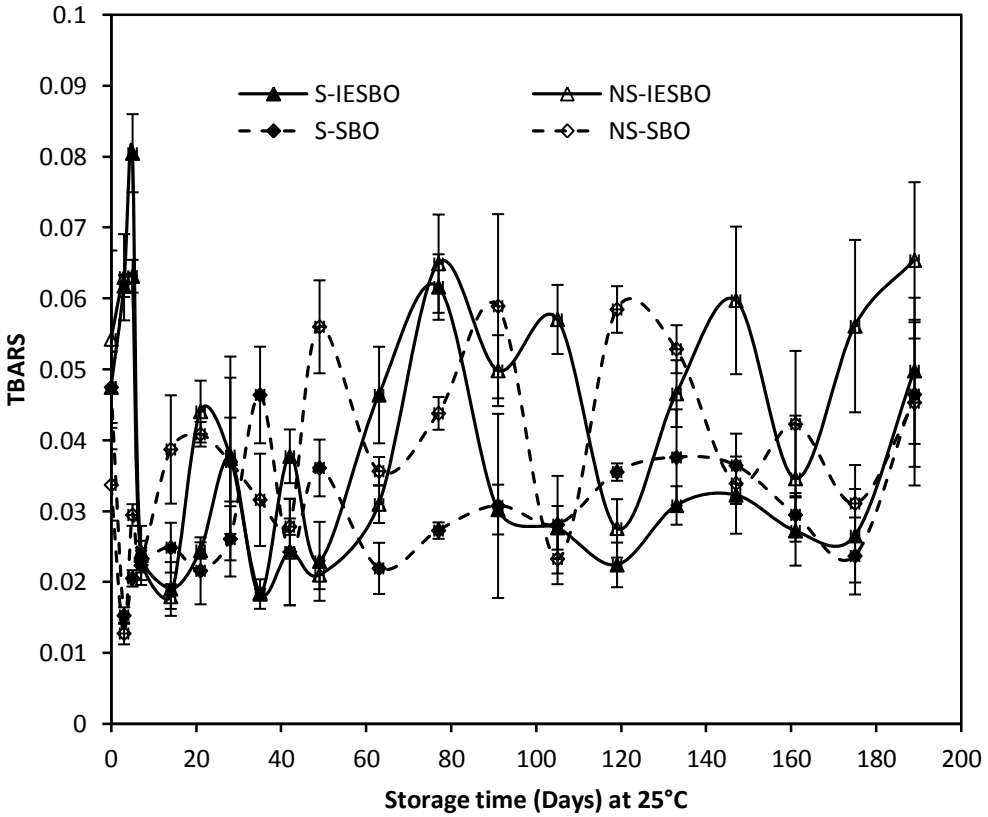

Figure 3-3. TBARS value change of interesterified soybean oil (IESBO) and soybean oil (SBO) stored at 25 ℃ for 189 days with/without high intensity ultrasound.
S-IESBO: IESBO with HIU application; NS-IESBO: IESBO without HIU application; S-SBO: SBO with HIU application; and NS-SBO: SBO without HIU application.
Error bars: ± standard deviation.

enough to see a drop in PV, especially for samples held at 40 ℃.

Secondary oxidation products such as aldehydes and ketones are responsible for rancid flavor and odor, and were measured as TBARS. TBARS assay measures red chromagen (532 nm) developed by the reaction between lipid oxidation products and thiobabituric acid (Buege & Aust, 1978), and it has been generally accepted that MDA is the major TBARS (Sinnhuber & Yu, 1958; Tarladgis, Pearson, & Dugan, 1962; Tarladgis, Watts, Younathan, & Dugan, 1960; Yu & Sinnhuber, 1957). However, aldehydes other than MDA such as alkanals, 2-alkenals and 2,4- alkadienals can also react with

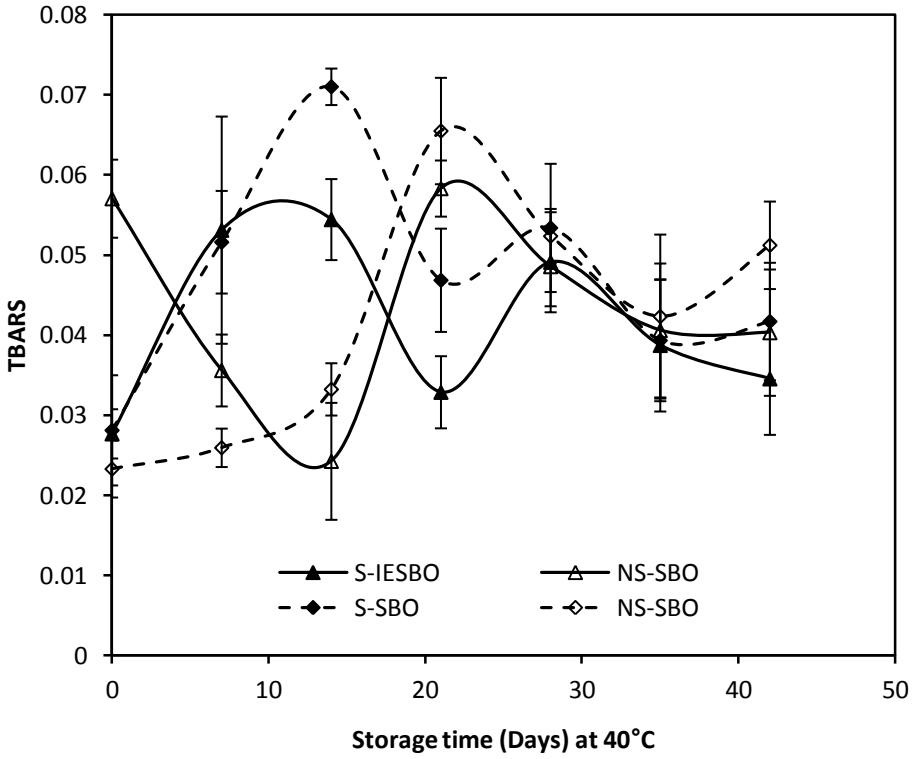

Figure 3-4. TBARS value change of interesterified soybean oil (IESBO) and soybean oil (SBO) with/without high intensity ultrasound stored at 40 ℃ after being stored at 25 ℃ for 105 days.
S-IESBO: IESBO with HIU application; NS-IESBO: IESBO without HIU application; S-SBO: SBO with HIU application; and NS-SBO: SBO without HIU application.
Error bars: ± standard deviation.

thiobarbituric acid, developing yellow (with an absorption maximum at 450 nm) or orange (at 495 nm) pigments (Asakawa, Nomura & Matsushita, 1975; Esterbauer Cheeseman Dianzani, Poli, & Slater, 1982; Jacobson, Kirkpatrick, & Goff, 1964; Kosugi & Kikugawa, 1985; Kosugi & Kikugawa, 1986; Marcuse & Johansson, 1973; Patton & Kuttz, 1955; Pryor, 1980; Witz, Zaccaria, Lawrie, Ferran, & Goldstein, 1985; Yu, Latriano, Duncan, Hartwick, & Witz, 1986; Yu & Sinnhuber, 1962). This can result in artificially high TBARS values (Hoyland & Taylor, 1991; Jardine, Antolovich, Prenzler, & Robards, 2002; Sun, Faustman, Senecal, Wilkinson, & Furr, 2001). The major

headspace volatiles that were reported in SBOs were hexanal, 1-penten-3-ol, (E)-2-heptenal, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptandienal, and (E,E)-2,4-decadienal (Yang, Chu, & Liu, 2005). Hexanal was the dominant headspace volatile in the SBOs stored for 10 days at 60 ℃, followed by (E)-2-heptenal, 1-penten-3-ol, (E,E)-2,4-heptandienal, (E,Z)-2,4-heptadienal, and (E,E)-2,4-decadienal (Yang et al., 2005). Ullrich and Grosch (1988) reported 2-hexanal, 3-hexenal, octanal, nonanal, and 2-nonenal in soybean bean oil stored at room temperature in the dark.

4. Conclusion

HIU altered crystallization behavior and created a thick, gel like fat product in IESBO. S-SBO and NS-SBO had longer induction times than S-IESBO and NS-IESBO when they were exposed to oxygen. It took about 133-147 days for S-IESBO and NS-IESBO and about 147-161 days for S-SBO and NS-SBO to reach the range that has poor odor and flavor (over 10 mEq/kg) at room temperature. The effect of HIU on oxidation rate was dependent on the physical status of oils and fats after HIU application. When HIU was applied to oil with a low melting point at room temperature, it decreased oxidation rate. On the other hand, when HIU was applied to semisolid fats with higher melting points, it created smaller, more evenly distributed crystals, slightly lowering the oxidation rate. The type of oil (SBO or IESBO) was important for hydroperoxide formation rate. SBO was more prone to oxidize than IESBO at 25 ℃ and 40 ℃ due to SBO's higher unsaturated fatty acids content, regardless of HIU application. Even though there was a significant effect of HIU on oxidation of SBO and IESBO, the effects appeared after they were already highly oxidized.

In conclusion, HIU did not affect the oxidative stability of SBO or IESBO until the oils had already become noticeably rancid, as evidenced by high PV. Further studies such as sensory study and headspace volatile analysis can be useful to study whether ultrasound treatment causes a change in sensory acceptability.

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CHAPTER 4

CARBON DIOXIDE GENERATION BY TRONA MINERAL IN A MODEL SYSTEM AND ITS USE IN MODIFIED ATMOSPHERE PACKAGING

Abstract

The objective of this experiment was to evaluate CO_2 generating capacity of trona mineral compared to sodium bicarbonate in a model system and to examine the feasibility of trona as a CO_2 producing product in modified atmosphere packaging of steaks. Trona/salicylic acid mixture was able to generate CO_2 significantly faster than the mixture of sodium bicarbonate and salicylic acid ($P<0.05$). Mixture (2 g) of trona/salicylic acid generated 75.25 ml of CO_2 in an excess of water (100 ml). For steak storage, the quality of PVC overwrapped short loins (133.4 g; 1.9 cm thickness) packaged with an absorbent pad containing 4 g of trona/salicylic acid then placed in a non-gas permeable master package were compared with those stored 80% N_2/20% CO_2 and 80% O_2/20% CO_2. Hunter color, pH, total aerobic plate count (TAC), thiobarbituric acid reactive substances (TBARS) and myofilbrillar fragmentation index (MFI) were measured at 0, 6, 12, and 18 days. The level of CO_2 generated from the trona/acid mixture in the bags was 20% in 3 days and 30% by day 6. On day 12 it dropped to 25.8%, and then to 16% on day 18. Accelerated surface browning observed in 80% N_2/20% CO_2 packaging was most likely due to metmyoglobin formation by residual O_2 higher than 0.05% despite its low TBARS value. Hunter a*(redness) in 80% N_2/20% CO_2 was significantly lower than that seen in trona or 80% O_2/20% CO_2 MAP on days 6 and 12 ($P<0.05$). Color stability of the meat in 80% O_2/20% CO_2 was similar to those in trona

packaging, though some discoloration was detected on the steaks in the bags with trona/acid mixture and with 80% O_2/20% CO_2 on day 6. The steaks in 80% N_2/20% CO_2 had significantly higher TACs than 80% O_2/20% CO_2 and trona packaging on days 6 and 12 ($P<0.05$). The steaks stored with trona/acid mixture had significantly higher TBARS values than those in 80% N_2/20% CO_2 but lower TBARS values than those in 80% O_2/20% CO_2 on day 12. There was no significant difference between different packaging methods in MFI, for entire 18 days of experiment period. In conclusion, the mixture of trona and salicylic acid is an effective source of CO_2, and has potential for use as CO_2 generating system in meat packaging delaying lipid oxidation and providing color stability of steaks in master pack. Measuring the amount of absorbed CO_2 in meat may help to explain the effect of CO_2 on meat quality.

1. Introduction

Master pack/mother bag storage systems are becoming popular for centralized meat packaging since it is an efficient method that allows retailers to provide consistently high quality meat cuts to consumers at a lower price (Jeyamkondan, Jayas, & Holley, 2000). Master packs, containing individual PVC wrapped meat cuts, are flushed with a desired gas or gas mixture to create modified atmosphere packaging (MAP). MAP has been used to increase shelf and display life, thus increasing customer satisfaction. Modified atmospheres used in master packaging allow retailers to provide safe and high quality meats without the need for in-house butchering or fabrication (Scholtz, Jordan, Kruger, & Nortje, 1992). Common atmospheric gases used for MAP are oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). Vacuum packaging, involving the removal of all

headspace gases, is also widely used. Each gas has unique properties, and different mixes of these gases are used for various applications to improve the efficiency of MAP.

Vacuum packaging can control the growth of aerobic spoilage bacteria, reducing counts by as much as 2 $\log_{10}$/g (Tsigarida, Skandamis, & Nychas, 2000). Low O_2 MAP that contains a mixture of O_2, CO, and N_2 was used in Norway from 1985 to 2004, as low concentrations of CO (0.5 - 5%) have been shown to stabilize red color in fresh meats (Brewer, Wu, Field, & Ray, 1994; El-Badawi, Cain, Samuels, & Anglemeier, 1964; Gee & Brown, 1978; Hunt et al., 2004; Jayasingh, Whittier, Carpenter, & Cornforth, 2001; Krause, Sebranek, Rust, & Honeyman, 2003; Kusmider, Sebranek, Lonergan, & Honeyman, 2002). High O_2 MAP containing 80% O_2 and 20% CO_2 is commonly used for fresh meats, since beef stored in high O_2 MAP has a bright red color desirable to consumers (Behrends, Mikel, Armstrong, & Newman., 2003; Seyfert et al., 2005). CO_2 gas is routinely used in MAP due to its antimicrobial effect; with a minimum of 20% to 30% needed to significantly inhibit the growth of spoilage bacteria (Stiles, 1991). CO_2 used in high O_2 MAP restricts bacterial spoilage, so shelf life is dictated by color, not microbial growth (Gill & Jones, 1994). However, the level of O_2 used can lead to the development of off flavors and odors due to lipid oxidation (Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002).

In the typical MAP scheme, gas flushing is a common method to produce modified atmosphere, but sachets are also widely used. The most frequently used sachet in food packaging is an oxygen scavenger, to remove residual oxygen from the package. A combination sachet, responsible for absorbing (O_2) and emitting (CO_2) gasses is used for dual action. CO_2 emitters can be used alone to introduce antimicrobial gas. Verifrais™

made by SARL codimer (Paris, France), is a CO_2 emitting sachet used in fresh meat and fish packaging, which relies on the reaction of sodium bicarbonate and ascorbate to generate CO_2. Sodium bicarbonate generates a salt and CO_2 in the presence of an acid and water (juice exudates from meat or fish drip). Continuous CO_2 generation from the sachet helps to avoid pack collapse by replacing the CO_2 absorbed by the meat (Rooney, 1995). Trona mineral has the potential to be used in the same manner. Trona, which is a primary source of sodium carbonate in the US, can release CO_2 in the presence of moisture and acid. However, it is unknown if trona mineral can generate enough CO_2, given the limited amount of juice exudates from meat, to work as an alternative method for high O_2 packaging without the need for gas flushing. To examine this possibility, trona and sodium bicarbonate were compared to evaluate their CO_2 producing capacity with an excessive amount of water or a limited amount of water. In addition, trona and salicylic acid were incorporated into a soaker pad, and then used in an attempt to create 20% CO_2 within the MAP headspace. Finally, the effect of trona was tested by comparing to gas flushed high and low O_2 MAP packaged steak.

2. Materials and Methods

2.1. Trona mixture and sodium bicarbonate

The mixture of trona and salicylic acid (Trona Fresh) was obtained from Trona Life (Salt lake City, Utah, USA). The content of salicylic acid was about 14% of the total mixture weight. Sodium bicarbonate (baking soda) was purchased from a local grocery store and was mixed with salicylic acid crystalline powder (Fisher Scientific, Hampton, NH) at the same ratio of the Trona Fresh mixture.

2.2. Bench experiment

This experiment was performed to evaluate CO_2 generating capacity of trona mineral with an excess amount of water within a fixed volume capacity environment. Six 250 ml Erlenmeyer flasks were prepared to create 6 different conditions at room temperature. The flasks were labeled 1 to 6. The trona/acid mixture (4 g; 14% salicylic acid) was placed in flasks 1, 2 and 3. The sodium bicarbonate/acid mixture (4 g) was placed in flasks 4, 5 and 6. Distilled water (100 ml) was added to flasks 1 and 4 (reagent blanks). Acetic acid (100 ml; pH 5.6) was added to flasks 2 and 5 to simulate the pH of meat drip. A mixture of 2-(N-morpholino) ethanesulfonic acid (MES buffer) and acetic acid (100 ml; pH 5.6) was added to flasks 3 and 6 to simulate the buffering capacity and pH of meat drip. All flasks were covered with parafilm and a sticky nickel adhesive rubber septum was attached to the top of the film to prevent air leakage. The level (%) of carbon dioxide in the headspace was measured every 10 min for 1 hr and after 24 hr using a PAC CHECK 550 gas analyzer (Mocon, Minneapolis, MN), which was warmed up for 30 min then registered with carbon dioxide concentration of room air between -0.5 and 0.5% three times to verify the accuracy of the reading. These procedures were replicated five times for each condition.

2.3. Packaging experiment

This experiment was conducted to evaluate CO_2 generating capacity of trona mineral with a limited amount of water in packaging bags. Beef steaks in vacuum package loses between 2.7 and 5.3% moisture after thawing at 1 ℃ for 24 hr in the form of drip loss (Raharjo et al., 1995). The estimated minimum drip loss from a 2 lb steak is

approximately 25 ml, so this amount of liquid was used for the packaging experiment. Six low-gas permeable master bags (15.24 cm x 30.48 cm) were placed in a walk-in cooler. The master bags (Vacmaster, Kansas City, MO) used for packaging were of 3-mil thickness (0.75-gauge nylon,2.25-gauge polyethylene) with an oxygen permeability of 0.6 cm^3/100 m^2/24 h at 0 ℃ and a water vapor transmission rate of 0.6 g/100 m^2/24 h at 38 ℃ and 100% relative humidity. Bags 1, 2 and 5 contained the trona/acid mixture (4 g), and bags 3 and 4 contained the sodium bicarbonate/acid mixture (4 g). An absorbent pad containing distilled water (25 ml) was placed into bag 5. Distilled water (25 ml) was added directly to bags 1 and 3. A buffered acetic acid solution (MES buffer; 25 ml) was added directly to bags 2 and 4. All bags were flushed with ambient air and sealed with a heat sealer. The internal size of the bags was 21 x 15 cm^2 after they were heat sealed. A sticky nickel adhesive rubber septum was attached to each bag for atmosphere sampling. The level of carbon dioxide in the headspace was measured after 0, 20, 40, 60, 80, and 1440 min by use of a puncture probe through the sticky nickel disc, using a PAC CHECK 550 gas monitor (Mocon, Minneapolis, MN), which was warmed up for 30 min then registered with carbon dioxide concentration of room air between -0.5 and 0.5% three times to verify the accuracy of the reading. Procedures were replicated five times for each condition.

2.4. Maximum carbon dioxide generating capacity of trona/acid mixture

This experiment was performed to determine the maximum CO_2 generating capacity of the trona/acid mixture. A glass graduated cylinder (250 ml) was fully filled with water and wrapped with parafilm to avoid water loss. The cylinder was placed

upside down in a plastic water tub completely filled with water and fixed to a stand with clamps. As parafilm was removed from the cylinder under the water, a long rubber tube was placed inside of the glass cylinder in the water tub. The other end of the tube was connected to the side arm of an Erlenmeyer flask (250 ml) containing the trona/acid mixture (2 g). 100 ml of distilled water was added to the flask, which was immediately stoppered. The flask was gently stirred every 5 min and the volume of CO_2 generated from the trona/acid mixture was measured in the glass cylinder every 5 min. After 35 min, the level of CO_2 was measured every 30 min for an additional 2 hr without stirring. This experiment was repeated four times.

2.5. Steak experiment

2.5.1. Preparation of steaks

Choice grade short loins (IMPS 1180; 133.4 g) were sliced into 1.9 cm thick steaks. Short loins from three different animals were used for replication. The sliced steaks were placed in foam trays (13 cm x 13 cm) with a soaker pad and wrapped with a single layer of PVC film. Gas permeability of PVC film (Kansas City, MO, USA) was as follows: O_2 permeability = 8400 $cm^3/(24\ h \times m^2 \times atm)$ at 23 ℃; water vapor transmission = 83 $g/(24\ h \times m^2)$ at 23 ℃ and 50% relative humidity. The packaged steaks were placed in non-gas permeable bags containing three different headspace compositions. Bag 1 (NMAP) was flushed with an 80% N_2/20 % CO_2 mixture, bag 2 (OMAP) was flushed with an 80% O_2/20% CO_2 gas; and bag 3 (TMAP) contained soaker pads including 4 g trona/acid mixture (no gas flush). Heat sealed bags were stored in a walk-in cooler at 1 ℃. Samples were pulled after 0, 12, 15, and 18 days for Hunter color, TBARS, pH

measurement, and microbial load. The CO_2 headspace level in mother bags was monitored during storage with a PAC CHECK 550 gas analyzer.

2.5.2. pH measurement

A portable pH meter (HANNA Instruments, Ann Arbor, MI) fitted with a semi-solid foods probe was used to measure pH. The pH probe was directly inserted to steaks and held until pH reading was constant.

2.5.3. Hunter color

Hunter L*, a* and b* were measured on the surface of PVC film by a Miniscan colorimeter(Hunter Labs, Reston, VA) with a 5 mm diameter aperture set to use illumninant D-65 and 2 °observer angle. The colorimeter was standardized with both black and white tiles covered with a single layer of PVC film before use. Three measurements were taken for each sample. Hue angle (true redness) was calculated as [arctangent (b*/a*)]. Chroma (color saturation) was calculated as $\sqrt{(a^{*2} + b^{*2})}$.

2.5.4. Thiobarbituric acid reactive substances (TBARS)

TBARS assay was performed according to Buege and Aust (1978) with modifications. Thiobarbituric acid (TBA) reagent was made with 15% (W/V) tricholoacetic acid, 0.375% (W/V) thiobarbituric acid, and 0.25 N hydrochloric acid. Minced sample (1 g) was added to a centrifuge tube. TBA reagent (3 ml) was added to the centrifuge tube, which was then capped loosely and heated for 10 min in a water bath (100 ℃) to develop pink color. Then, it was cooled with tap water and centrifuged for 25 min at 3200 x g. Two absorbance readings were taken per sample at 532 nm using a

Shimadzu UV 2100 U spectrophotometer (Columbia, MD), then averaged. Two replicates were performed per steak. TBA values were calculated according to the following equation:

$$\text{TBA}\left(\frac{\text{mg}}{\text{kg}}\right) =$$

$$A_{532} \times \frac{1\text{ M chromagen}}{156{,}000} \text{ x } \frac{1\text{ mol/L}}{\text{M}} \times \frac{0.004\text{ L}}{1.0\text{ g meat}} \times \frac{72.07\text{ g MDA}}{\text{mole}} \times \frac{1{,}000{,}000\text{ mg}}{\text{kg}}$$

2.5.5. Total aerobic plate count (TAC)

Swabbing method was used for microbial growth test. A 5 cm x 5 cm area on the surface of steaks was swabbed with a 3M™ Swab-Sampler 10mL Buffered Peptone Water Broth (3M Corporation, St. Paul, MN) in three different directions. Peptone water for dilutions was prepared by dissolving 15 g of dehydrated peptone water culture media (Neogen, Lansing MI) in 1 L of distilled water, and autoclaving for 15 min at 121 ℃. The swab was placed in the peptone water, diluted to subsequent concentrations and plated to Petrifilm^{TM} aerobic count plates (3M Corporation, St. Paul, MN) in duplicate. Plates were incubated at 34 ℃ for 48 hr. The number of colonies were counted according to manufacturer recommendations and expressed as CFU/cm^2.

2.5.6. Myofibrillar fragmentaion index (MFI)

This experiment was performed to examine tenderness of meat. The extraction was performed in duplicate per steak. Sample was collected from two different spots on steak using a metal core (d=1.5 cm), then minced, and connective tissue and fat were removed. Minced sample (2.5 g) was place in a 50 ml centrifuge tube with 25 ml of ice-cold buffer (100 mM KCl; 1 mM Ethylene gluycol-bis(b-aminoethyl ether)-N,N,N’,N’-

tetraacetic acid (EGTA); 1 mM $MgCl_2$; 20 mM potassium phosphate, pH 7.0; 1 mM NaN_3) and homogenized with a Ultra-Turrax T18 Basic (IKA, Wilmington, NC) at 1000 x g for 30 seconds. After homogenization, the tubes were stored in ice water and centrifuged at 1000 x g for 15 min. The myofibrils were re-suspended with a glass rod and centrifuged again. The myofibrils were re-suspended and passed through a 400 μm^2 nylon mesh to remove connective tissue and debris before protein determination. Bradford protein assay was conducted in duplicate for each suspension for protein determination. Albumin standard (BSA) was diluted to the final concentration of 0, 25, 125, 250, 500, 750, 100, 1500 and 2000 μg/ml. The standard curve prepared by reading absorbance of the mixture of 30 μl of the diluents and 1.5 ml Coomassie Protein Assay Reagent (Thermo Scientific, Rockford, IL) at 595 nm using a Shimadzu UV 2100 U spectrophotometer (Columbia, MD). The myofbrils were diluted to a protein concentration of 0.5 mg/ml with distilled water, and the absorbance reading at 540 nm was multiplied by 200 to give the index termed MFI.

2.6. Statistical analysis

Analysis of variance was performed using the proc mixed function in SAS version 9.1 (SAS Institute, Inc., Cary, NC). For both bench and packaging experiments, values within each time point were compared separately. Statistical significance was identified at the 95% confidence level, and post-hoc means comparisons were made based on p-values obtained using the Tukey-Kramer adjustment.

3. Results and Discussion

3.1. Bench experiment

The amounts of headspace CO_2 generated in model systems are shown in Table 4-1. There were significant effects of treatment on generation of CO_2 ($P<0.0001$; see Appendix C for detailed statistics). In all cases, CO_2 generation occurred very rapidly after water was added to flasks, and then leveled out. The most dramatic increase in percent headspace CO_2 was observed for during the first 10 min, with significantly higher ($P<0.05$) levels of headspace CO_2 flasks containing the trona/acid mixture, regardless of pH or buffer. The solubility of chemicals affects the rate of chemical reactions. According to Solvay North America, LLC (2010), the solubility of trona mineral is 15.6 g/100 ml at 20 ℃. The solubility of sodium bicarbonate is about 9.6 g/100 ml at 20 ℃ (OECD SID, 2002). Trona generated more CO_2 than sodium bicarbonate in this experiment, however, the solubility of compounds was not important since excess water was used, allowing both trona and sodium bicarbonate to dissolve completely.

Theoretically, one mole of trona and sodium bicarbonate can generate 38.9% and 52.4% headspace CO_2, respectively, based on calculations by weight. When the same amount of trona and sodium bicarbonate are used, sodium bicarbonate is able to generate more CO_2. However, the percent headspace CO_2 generated from trona was almost 30% higher than seen for sodium bicarbonate in this experiment. This could be due in part to the different crystalline sizes of the salicylic acid used. The salicylic acid/trona mixture obtained from the supplier was a more fine powder form, while the salicylic acid that was mixed with sodium bicarbonate in-house was present as larger crystals. This difference

Table 4-1. Percent headspace CO_2 generated from trona/salicylic acid or sodium bicarbonate/salicylic acid with 100 ml of water in 250 ml flask at room temperature.

Treatment	0 min	10 min	20 min	30 min	40 min	50 min	60 min	24 hr
1. TR (pH5.9)	9.54 ±1.89 a	58.58 ±1.31 a	56.72 ±1.46 a	53.76 ±1.55 a	51.66 ±1.69 a	47.62 ±1.84 a	44.98 ±1.73 a	34.86 ±1.51 ab
2. TR (pH5.6)	8.88 ±1.06 a	58.90 ±0.79 a	57.66 ±0.74 a	56.34 ±0.66 a	53.16 ±0.74 a	50.42 ±0.72 a	48.58 ±0.55 a	41.18 ±0.92 a
3. TR(pH5.6, MES)	11.94 ±1.85 a	63.16 ±0.98 a	62.02 ±1.00 a	61.00 ±0.80 a	59.26 ±0.84 a	55.64 ±0.79 a	52.68 ±0.91 a	41.02 ±1.73 a
4. SB (pH5.9)	3.12 ±0.48 a	27.12 ±0.56 b	29.08 ±0.48 b	28.94 ±0.27 b	26.34 ±0.48 b	25.86 ±0.29 b	24.30 ±0.24 b	20.84 ±0.74 c
5. SB (pH5.6)	3.94 ±1.20 a	21.04 ±1.39 b	27.42 ±0.61 b	27.86 ±0.46 b	24.72 ±0.67 b	25.50 ±0.27 b	23.24 ±0.54 b	19.34 ±1.13 c
6. SB(pH5.6, MES)	6.6 ±1.89 a	28.66 ±0.45 b	30.16 ±0.61 b	31.60 ±0.30 b	31.18 ±0.19 b	30.14 ±0.36 b	29.32 ±0.51 b	27.42 ±1.89 bc

TR: trona + salicylic acid; SB: sodium bicarbonate + salicylic acid; pH 5.6: pH adjustment with acetic acid; and MES: Buffer 2-(N-morpholino) ethanesulfonic acid.
Values sharing letters within (but not between) column groupings are not significantly different (P>0.05).
Values represent mean +/- standard error of the mean.

might have affected the solubility of salicylic acid and subsequent gas generation. Buffering capacity is an important characteristic of meats. The pH of distilled water used in this experiment was about 5.9, which was slightly higher than the ultimate pH of meat (5.5- 5.7). Thus, MES buffered water with pH adjustment was required to simulate the conditions in meat drip. Slightly higher percentages of headspace CO_2 were generated in buffered, pH adjusted water than in plain distilled water; this trend was consistent even though it was not statistically significant except at 30 and 50 min. ($P>0.05$).The percent headspace CO_2 in the flasks containing trona dropped continuously after 10 min. CO_2 can be dissolved in water since it is a water soluble gas (1.45 g/L at 25 ℃). Thus, dissolved CO_2 in water may be responsible for the decrease of the CO_2 percent. In addition, according to Henry's law, increased pressure caused by CO_2 produced within the closed flasks can increase the CO_2 solubility:

Henry's law: $p = k_H c$

where p is the partial pressure (atm) of the solute in the gas above the solution, c is the concentration of the gas in the liquid (mol/L) and k_H is a constant with the dimensions of pressure divided by concentration (Moore, 1972). The constant, known as the Henry's law constant, depends on the solute, the solvent and the temperature.

3.2. Packaging experiment

Table 4-2 shows the percentage of headspace CO_2 generated from trona and sodium bicarbonate in 25 ml of water at 4 ℃. Using an absorbent pad to supply moisture did not influence CO_2 generation ($P<0.05$; see Appendix C for detailed statistics).

Table 4-2. Percent headspace CO_2 generated from trona or sodium bicarbonate with 25 ml of water in low-gas permeable master bags[a] at 4 ℃.

Treatment	0 min	20 min	40 min	60 min	80 min	24hr
1. TR (pH5.9)	2.38 ±0.48 a	25.18 ±0.76 a	25.74 ±0.72 a	26.02 ±0.71 a	26.06 ±0.73 a	23.98 ±0.41 a
2. TR (pH5.6, MES)	1.24 ±0.25 a	27.98 ±0.36 a	28.74 ±0.28 a	28.78 ±0.33 a	29.08 ±0.29 a	25.62 ±0.69 a
3. SB	1.32 ±0.26 a	12.54 ±0.12 b	12.92 ±0.14 b	13.38 ±0.13 b	13.60 ±0.13 b	13.66 ±0.11 b
4. SB (pH 5.6, MES)	2.22 ±0.44 a	16.42 ±0.52 b	17.10 ±0.56 b	17.50 ±0.50 b	17.74 ±0.48 b	14.16 ±0.51 b
5. TR+ pad (pH 5.9)	1.70 ±0.34 a	26.32 ±0.80 a	26.68 ±0.82 a	26.62 ±0.80 a	26.98 ±0.79 a	23.80 ±0.38 a

[a] Oxygen permeability: 0.6 cm^3/100 m^2/24 h at 0 ℃; water vapor transmission rate: 0.6 g/100 m^2/24 h at 38 ℃ and 100% relative humidity.

TR: trona + salicylic acid; SB: sodium bicarbonate + salicylic acid; pH 5.6: pH adjustment with acetic acid; MES: Buffer 2-(N-morpholino) ethanesulfonic acid; and pad: absorbent pad + 25 ml distilled water.

Significantly higher CO_2 was produced with trona than with sodium bicarbonate through whole experiment time ($P<0.05$), consistent with the results of the bench experiment. In meat packaging, juice exudates from meat are usually the main source of water. Since the moisture amount is limited, solubility of chemicals used is very important. For trona and salicylic acid to react, both ingredients must be dissolved.

Since salicylic acid has a limited water solubility (2 g/L) compared to sodium bicarbonate and trona, the amount of the acid dissolved was the limiting factor for the rate of CO_2 generation. In addition, the amount of CO_2 generated from the mixtures was limited by the quantity of salicylic acid used (0.226 mol of salicylic acid per 1 mole of trona). Even with a limited amount of water (25 ml), more than 20% headspace CO_2 was produced in the bags containing trona. However, this is simply a percent of headspace CO_2, which is relative to the volume of the individual bags. Thus, it is not possible to calculate the absolute amounts of CO_2 generated in this experiment. The percents of headspace CO_2 generated from trona and salicylic acid in this experiment were lower than those in packaging experiment. This is because the volume of packaging bags changed with CO_2 production in the packaging experiment while the total volume of flasks was fixed during the bench experiment. In addition, the percent of headspace CO_2 in the master bags was balanced with existing air present in the packaging bags, and a smaller amount of water was used.

3.3. Maximum carbon dioxide generating capacity of trona/acid mixture

There was no change in the level of CO_2 between 35 and 120 min, thus the data from 0 to 35 min was used. The volume (ml) of CO_2 generated from the trona mixture is

shown in Figure 4-1. The majority of the CO_2 was generated during the first 5 min, and then the level of CO_2 increased relatively slowly. The amount of CO_2 generated from 2 g of the trona/acid mixture was 75.25 ml, or 0.0025 mol.

3.4. Steak experiment

3.4.1. Headspace CO_2 concentration

Headspace percentage of CO_2 in the bags containing trona/acid mixture (TMAP)

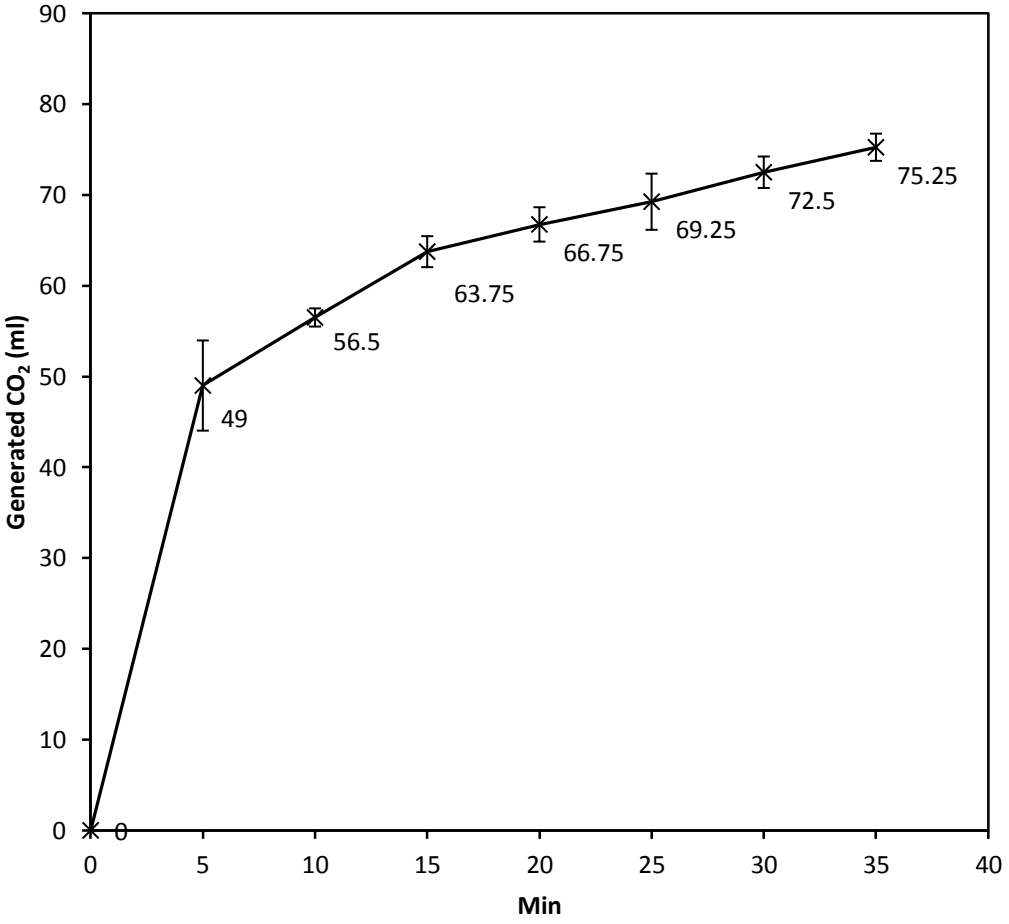

Figure 4-1. Volume of CO_2 generated from mixture (2 g) of trona and salicylic acid (14% by weight) in 250 ml graduated cylinder at room temperature.
Error bars: +/- standard error of the mean.

was measured every 3 days until day 9, and then measured on days 12 and 18 (see Appendix Table C3). Headspace CO_2 levels reached over 20% in 3 days, and increased to 30% by day 6. On day 12 it was 25.8% and then dropped to 16% on day 18. These bags were open for testing on days 6, 12 and 18. Headspace percentage of CO_2 in 80% N_2/20% CO_2 bags (NMAP) and 80%O_2/20% CO_2 bags (OMAP) were measured, then bags were opened for measurement of pH, Hunter color, TBARS, APC and MFI. The headspace percentage of CO_2 in both NMAP and OMAP was 15 % and 16% CO_2 in the bags on days 12 and 18, respectively.

The minimum concentration of CO_2 to have an inhibitory effect on microbial growth is 20% in atmosphere (Stile, 1991). The bags containing the trona/acid mixture maintained CO_2 level above 20% from day 3 to 12. However, the detected CO_2 level in OMAP and NMAP were under 20% on days 6, 12, and 18. Continuous CO_2 generating capacity of the trona/acid mixture could replace the CO_2 absorbed by steaks, thus maintaining a headspace CO_2 level above 20% within the package for 12 days of storage. On the other hand head space CO_2 level in OMAP and NMAP stayed below 20% during the storage period.

3.4.2. pH measurement

pH changes over 18 days of storage are shown in Table 4-3. A slight decrease in pH was observed on day 6 in all packaging systems. The pH in NMAP increased steadily through day 18 while the pH of steaks packaged in OMAP or TMAP through day 12 then on day 18. The pH in NMAP was significantly lower than the other two packaging methods on day 12 ($P<0.05$). However, a higher pH in NMAP was observed than in

Table 4-3. pH change of steaks stored in different packaging at 1 ℃ for 18 days.

Packaging Method	Day 0	Day 6	Day 12	Day 18
80%N_2/20%CO_2	5.52 ± 0.03 a	5.47 ± 0.02 a	5.59 ± 0.01 b	5.76 ± 0.00 a
80%O_2/20%CO_2	5.52 ± 0.03 a	5.50 ± 0.02 a	5.76 ± 0.01 a	5.57 ± 0.02 b
Trona	5.52 ± 0.03 a	5.51 ± 0.02 a	5.80 ± 0.02 a	5.58 ± 0.02 b

Values for individual time points represent mean +/- standard error of the mean at a given time point. Values sharing letters within, but not between, columns are not significantly different (P>0.05).

TMAP and OMAP on day 18 (P<0.05).

3.4.3. Hunter color

Redness (a*), which is the most important measurement of red meat color, decreased over time for all packaging types (Table 4-4; see Appendix C for detailed statistics). On days 6 and 12, steaks in NMAP had significantly lower a* values (P<0.05), consistent with their brown appearance. Even though the steaks in OMAP and TMAP maintained a red color, some small brown spots on the meat surface were observed on day 6. After the steak cuts in NMAP were exposed to oxygen, it was expected that some degree of red color would be regained within 30 min. However, the surface of the steak in NMAP was brown and did not bloom, even though their internal color was red. According to Ledward (1970), low oxygen concentration accelerates oxidation of oxymyoglobin to metmyoglobin. Oxygen levels below 0.05% are required to minimize the metmyoglobin formation (Faustman & Cassen, 1990). The detected oxygen level in NMAP ranged from 0.46 to 0.74%, high enough to accelerate metmyoglobin formation. An oxygen scavenger is commonly used to remove oxygen from the package for this reason. After 18 days of storage, no differences were seen in a* values. After 18 days of storage, the surface of the steaks in NMAP appeared totally brown when they were

Table 4-4. Hunter a*, b* and L* of steaks stored in different MAP at 1 ℃.

Packaging Method	a* (Redness)[a]			
	Day 0	Day 6	Day 12	Day18
N_2/CO_2	12.7 ±0.1 a	5.1 ±0.1 a	4.7 ±0.1 a	3.7 ±0.1 a
O_2/CO_2	12.7 ±0.1 a	14.1 ±0.1 b	10.8 ±0.4 b	6.4 ±0.4 a
Trona	12.7 ±0.1 a	12.2 ±0.2 b	10.4 ±0.3 b	4.1 ±0.1 a
Packaging Method	L* (Lightness)[a]			
	Day 0	Day 6	Day 12	Day 18
N_2/CO_2	36.0 ±0.3 a	33.3 ±0.2 a	35.7 ±0.2 a	33.7 ±0.3 a
O_2/CO_2	36.0 ±0.3 a	35.6 ±0.3 a	36.6 ±0.3 a	36.3 ±0.4 a
Trona	36.0 ±0.3 a	34.9 ±0.3 a	34.1 ±0.4 a	38.5 ±0.4 a
Packaging Method	b* (Yellowness)[a]			
	Day 0	Day 6	Day 12	Day 18
N_2/CO_2	13.7 ±0.2 a	10.6 ±0.1 a	10.0 ±0.1 a	11.7 ±0.1 a
O_2/CO_2	13.7 ±0.2 a	15.3 ±0.1 b	13.5 ±0.2 b	13.9 ±0.1 a
Trona	13.7 ±0.2 a	14.6 ±0.1 b	13.4 ±0.1 b	12.0 ±0.9 a

[a]Values for individual time points represent mean +/- standard error of the mean at a given time point. Values sharing letters within, but not between, columns are not significantly different (P>0.05).
N_2/CO_2: 80%N_2/20%CO2; O2/CO_2: 80% O_2/20%CO_2; and Trona: trona and salicylic acid (4 g) packaging.

visually inspected. There was no significant effect of different packaging method on L* (lightness) for 18 days of storage (P>0.05). The meat cuts in OMAP and trona packaging had higher b* values (more yellow) for 14 days than those NMAP. There was no significant difference in any color parameters between OMAP and TMAP over the course of the study (P>0.05). Hue angle increased thought 18 days for all treatments (Table 4-5). The steaks in NMAP had higher hue angle values (which means it is redder and less yellow) than those for other treatments by day 12. Chroma values for the steaks in NMAP did not change for entire 14 days of study. On the other hand, chroma values of the steaks in OMAP and TMAP decreased over time, but were considerably higher (more intensive color) than those in NMAP.

Table 4-5. Hue angle and chroma of steaks stored in different MAP at 1 ℃.

Packaging Method	Hue Angle[a]				Chroma[a]			
	Day 0	Day 6	Day 12	Day 18	Day 0	Day 6	Day 12	Day 18
N_2/CO_2	47.2 ± 0.4 a	64.3 ± 2.6 a	65.0 ± 3.0 a	72.5 ± 1.8 a	18.7 ± 0.2 a	11.8 ± 1.1 a	11.0 ± 1.2 a	12.3 ± 1.3 a
O_2/CO_2	47.2 ± 0.4 a	47.5 ± 2.4 b	52.4 ± 6.7 b	66.8 ± 12.1 a	18.7 ± 0.2 a	20.8 ± 1.2 b	17.5 ± 3.4 b	15.6 ± 2.4 a
Trona	47.2 ± 0.4 a	50.4 ± 3.2 b	52.9 ± 6.2 b	71.0 ± 3.5 a	18.7 ± 0.2 a	19.0 ± 2.4 b	17.0 ± 2.7 b	12.7 ± 1.1 a

[a]Values for individual time points represent mean +/- standard error of the mean at a given time point. Values sharing letters within, but not between, columns are not significantly different (P>0.05).
Hue angle (true redness) was calculated as [arctangent (b*/a*)]. Chroma (color saturation) was calculated as $\sqrt{(a^{*2} + b^{*2})}$.
N_2/CO_2: 80%N_2/20%CO_2; O_2/CO_2: 80% O_2/20%CO_2; and Trona: trona and salicylic acid (4 g) packaging.

3.4.4. Thiobarbituric acid reactive substances (TBARS)

TBARS values increased during 18 days of storage (Table 4-6). There was no significant difference in TBARS values among treatments on day 6. Significantly (P<0.05) lower TBARS values of TMAP compared to those of in OMAP were observed only on day 12, even though steaks in TMAP were less oxidized than those in OMAP for the entire storage period. TBARS values in OMAP increased more rapidly than other packaging during 18 days of storage. On day 18, TBARS values in OMAP were significantly higher than those of NMAP (P<0.05), and there is no significant difference in TBARS values between TMAP and OMAP or TMAP and NMAP on day 18. It is hard to compare TBARS values directly between studies, but rancid flavor is typically detectable by sensory evaluation for red meat products between TBARS values of 0.5 and 2.0 mg MDA/kg (Gray & Pearson, 1987). Lanari, Schaefer, and Scheller (1995) also suggested that 0.5 mg MDA/kg was the detection limit for rancid off-flavors in fresh pork.

Table 4-6. TBARS change of steaks stored in different packaging at 1 ℃ for 18 days.

Packaging Method	Day 0	Day 6	Day 12	Day 18
80%N_2/20%CO_2	0.19 ± 0.00 a	0.24 ± 0.01 a	0.18 ± 0.02 a	0.37 ± 0.02 a
80%O_2/20%CO_2	0.19 ± 0.00 a	0.51 ± 0.08 a	0.89 ± 0.11 b	1.61 ± 0.23 b
Trona	0.19 ± 0.00 a	0.38 ± 0.00 a	0.55 ± 0.07 a	0.87 ± 0.02 ab

Values for individual time points represent mean +/- standard error of the mean at a given time point (n=3). Values sharing letters within, but not between, columns are not significantly different (P>0.05).

TBARS values on the steaks in OMAP exceeded 0.5 mg/MDA kg on day 6 which is earlier than in TMAP. TBARS values of the steaks in NMAP stayed under 0.5 mg MDA/kg during the study.

3.4.5. Total aerobic plate count (TAC)

As shown in Figure 4-2, TAC was increased from the initial counts. Removing O_2 in headspace suppress aerobic bacteria growth. Farber (1991) reported that displacement of O_2 by N_2 showed an inhibitory effect on the growth of aerobic microorganisms. However, steaks in NMAP had significantly higher TACs than those stored in other packaging methods (which contained O_2) on day 6 and 12. The maximum TAC was 2.8 log cfu/cm^2 in NMAP on day 6.

Overall, OMAP had lowest TAC values among all the treatments. TAC in OMAP were significantly lower than other packaging on days 6 and 12 ($P<0.05$). Even though the headspace percentage of CO_2 in OMAP was not maintained over 20%, the aerobic bacterial growth was lowest among all the treatments. In addition, even though the headspace CO_2 level in TMAP was maintained over 20% through days 3 to 12, the aerobic counts of the steaks in TMAP was higher than those in OMAP. There was no significant difference between treatments by day 18. The overall TAC measured in this

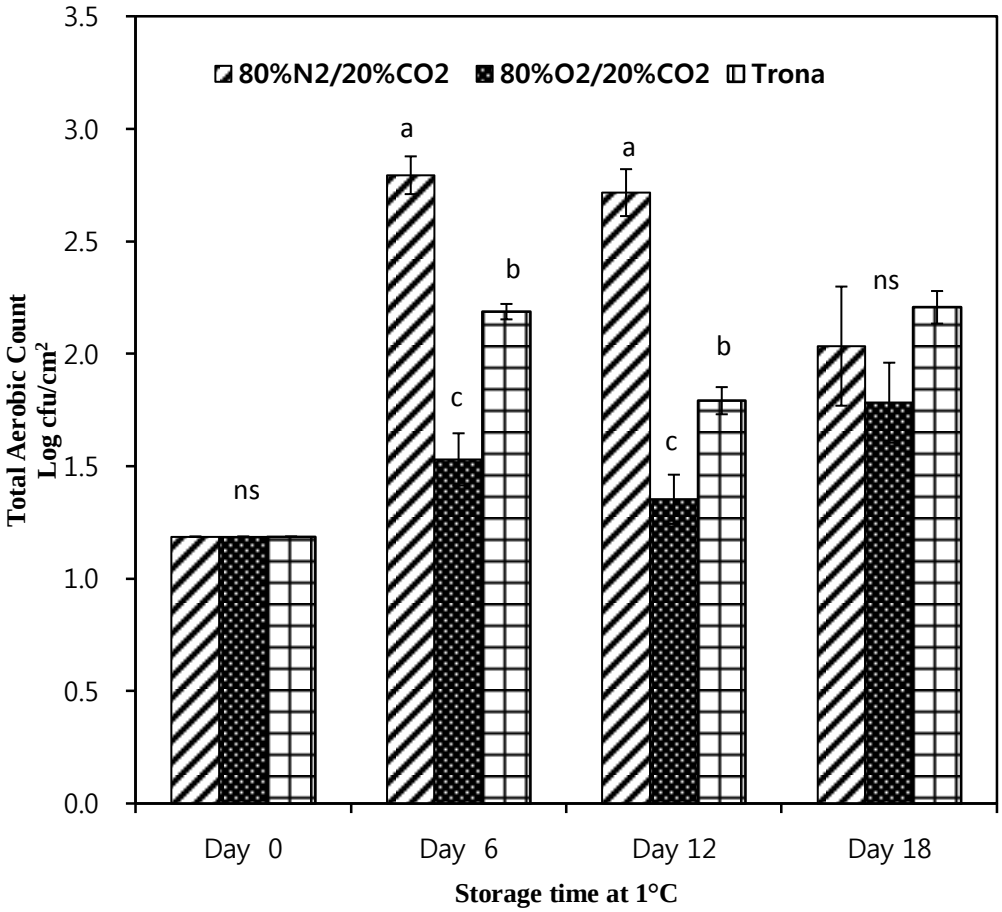

Figure 4-2. Total aerobic plate count in steaks in three different packaging bags at 1 ℃ for 18 days. Values sharing letters are not significantly different (P>0.05).
"ns" indicates no significant difference (P>0.05). Error bars: +/- standard error of the mean at a given time point.

study was relatively low, as visible spoilage in meats results from TAC levels between 10^5 and 10^6 cfu/g. However, swabbing methods typically give lower numbers than direct sampling (cut, homogenized, and plated) methods.

3.4.6. Myofibrillar fragmentation index (MFI)

MFI is a good indicator of the extent of myofibrillar protein degradation (Olson, Parrish, & Stromer, 1976). The initial MFI was 296.84 ± 1.74 (Figure 4-3). On day 6, a

slight but non-significant increase was observed only in OMAP. On day 12 there was increase of the MFI for NMAP, OMAP and TMAP methods (357.91 ±2.54, 352.79 ± 2.22, and 347.43 ±2.23, respectively). There was no difference in MFI between different packaging methods throughout the entire experiment period (P>0.05). There was no difference in MFI between different packaging methods throughout the entire storage period (P>0.05). Weakened myofibrils cause an increase in MFI during storage postmortem, and increased MFI indicates an increase in tenderness. Clausen, Jakobsen, Ertbjerg, and Madsen (2009) reported that beef steaks stored in OMAP had a lower MFI

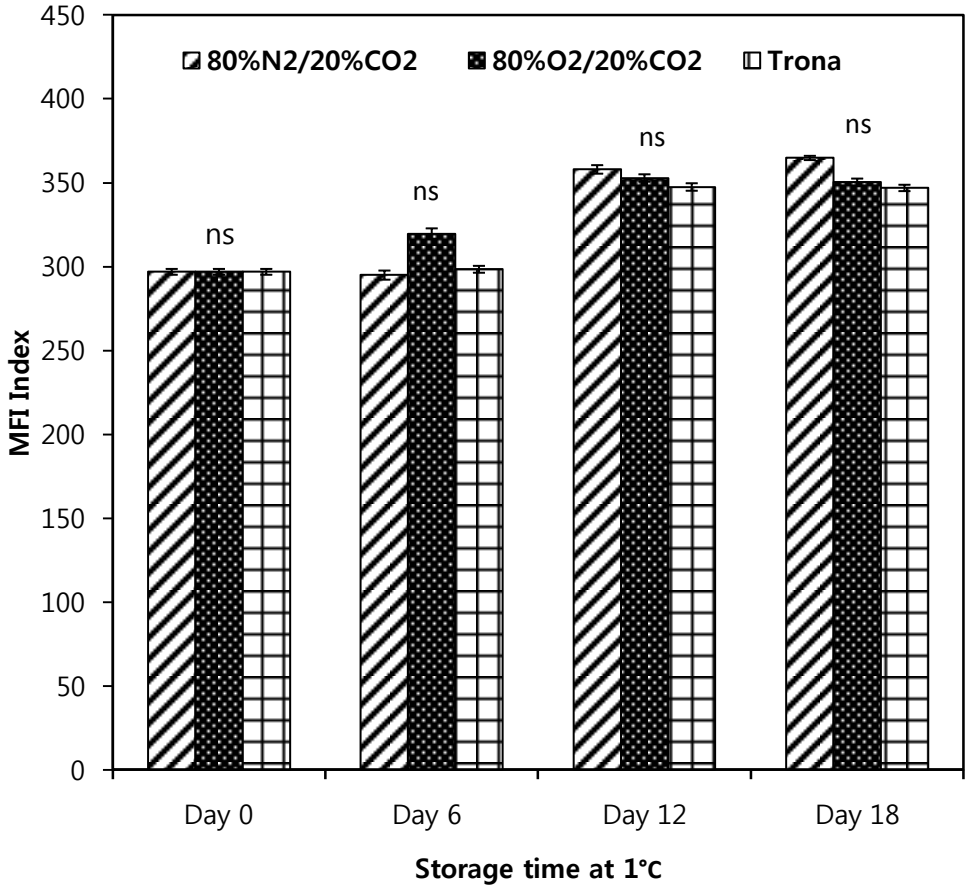

Figure 4-3. MFI index in steaks in three different packaging bags at 1 ℃ for 18 days.
"ns" indicates no significant difference (P>0.05).
Error bars: +/- standard error of the mean at a given time point.

(reduced tenderness) and increased protein oxidation compared to NMAP. They suggested that the decrease in meat tenderness was due to reduced proteolysis and/or protein oxidation.

Lametsch, Hviid, Jensen and Skibsted (2007) reported myofibril fragmentation was reduced in meat stored in high oxygen atmosphere (70% O_2/30% CO_2) indicating less proteolysis and/or cross-linking of proteins. They also found that beef stored in high oxygen atmosphere had decreased tenderness and juiciness at 4℃ compared to skin packaged beef. However, the results from the current study conflict with these findings. Specifically, a high oxygen atmosphere did not result in increased MFI in steaks. MFI values were higher than those typically reported. This is most likely due to the different protein assay used. The Biuret method is most often used in previous studies for MFI measurement, but the more sensitive Bradford method was used in this experiment.

4. Conclusion

Trona mineral was capable of generating more CO_2 than sodium bicarbonate, in both bench top and packaging systems. Trona was able to generate more CO_2 in meat packaging than sodium bicarbonate, which has been used for modified atmosphere packaging, for this reason. Low O_2 packaging delayed lipid oxidation but accelerated surface browning of steaks due to residual oxygen present within the meat tissue itself. There was no difference in pH and tenderness between high O_2 MAP and trona packaging. Continuous CO_2 generation by trona/ acid mixture allowed trona packaging to maintain the headspace concentration of CO_2 above 20% from day 3 to day 12, even though there was no better antimicrobial effect of the higher headspace concentration of CO_2 in trona

packaging compared to high O_2 packaging. Trona packaging can be used as an alternative to high O_2 MAP, which is widely used in industry. Similar color stability of trona packaging to that observed in high O_2 MAP and delayed lipid oxidation of trona packaging than that of high O_2 MAP. Controlling headspace O_2 concentration is necessary to avoid brown pigment formation in trona packaging. Further studies such as sensory tests and texture analysis will help to understand whether this impacts consumers' satisfaction. Additionally, determination of the actual amount of CO_2 absorbed by the meat can be also studied to demonstrate the impact of the absorbed CO_2 in meat on microbial growth.

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CHAPTER 5

USE OF TRONA MINERAL PACKETS IN NON-PACKAGING STORAGE ENVIRONMENTS AND THEIR EFFECT ON FRESH GROUND BEEF SHELF LIFE

Abstract

Trona is an evaporate mineral and the primary source of sodium carbonate in the USA. Trona mineral releases carbon dioxide in the presence of moisture and acid. This study was done to evaluate effects of using mineral packets containing trona and salicylic acid in non-packaging storage environments on pH, color, microbial growth, and lipid oxidation of ground beef under retail (1 ℃) and home (4 ℃) storage conditions. PVC-wrapped patties were held in 4 display conditions: 1) walk-in cooler with 4 mineral packets (66 g each) and intermittent fan-driven air circulation (simulated self-serve retail case); 2) walk-in cooler without mineral packets, and with fan-circulated air; 3) closed polyethelene box (50 x 90 x 18 cm) with 1 mineral packet, sealed with tape (no air circulation); 4) closed polyethelene box, without mineral packet, no air circulation. Total aerobic plate count, pH, Hunter color, and thiobarbituric acid reactive substances were measured after 0, 2, 4, and 7 days of retail storage. After 4 days, some patties from all 4 retail storage conditions were placed into one of two home refrigerators with or without mineral packets. The same analyses were performed after 1, 2, and 4 days of home storage (5, 6, and 8 days total storage time). No significant effects of retail storage were found with mineral packets for color, pH, TAC or TBARS values ($P>0.5$). Exposure to mineral packets for 2 days in home refrigerators slightly but significantly increased TBARS values (0.4 and 0.5 mg MDA/kg for patties with and without mineral packets,

respectively). No other significant effects of mineral packets were observed in home refrigerators. In conclusion, use of mineral packets was not effective in enhancing beef patty color stability or shelf life under the conditions used in this study. This may be due to insufficient amounts of moisture, which is necessary to generate enough CO_2 to affect meat quality. Providing a source of moisture to mineral packets is a possible solution to increase the CO_2 generating capacity packets for retail and home storage applications.

1. Introduction

Meat producers have taken great efforts to seek new technologies to maintain high standards of meat quality that will match consumers' expectations. Color of red meat is important in consumers' purchasing decision. According to Carpenter, Cornforth and Whitter (2001), consumers have a strong preference for red color and reject meats that do not appear red. Surface discoloration can reduce the selling price of beef by nearly 15%, resulting in approximately $1 billion of lost revenue in fresh beef sales every year (Smith, Belk, Sofos, Tatum, & Williams, 2000). Spoilage and discoloration are closely related to shelf life, and are very important in terms of consumers' purchasing decisions (Greene, Hsin, & Zipser, 1971).

According to the Institute of Food Science and Technology guidelines (1993), shelf life is determined by safety, nutrition loss and desirable sensory, chemical, physical and microbiological characteristics. In addition, shelf life is determined by consumers' expectations in qualities such as appearance, taste, texture, and smell (Labuza & Schmidl, 1988). But display life, the possible period of time that a product can be displayed under refrigerated conditions without color change, is equally important. Freshly ground beef at

retail stores is placed in a foam tray and wrapped with oxygen permeable film for display to consumers. Typically, PVC overwrapped fresh beef displayed at retail has 2 to 7 days of color shelf life (Brooks, 2007). Red meat turns brown over time, which is not appealing to consumers (Greene et al., 1971). To attract consumers, improved color stability and protection from spoilage bacteria growth are required. Spoilage due to the growth of non pathogenic bacteria is an essential factor that must be considered, especially in fresh meats.

To extend shelf life, storage conditions must be managed to control oxidation in meat. Retail and home storage temperature can be controlled, but atmospheric control is typically not available in these situations. Trona mineral can produce carbon dioxide in the presence of water and acid. Trona mineral is a potential material for CO_2 generating sachets, as a less expensive alternative packaging or storage method. Placing this mineral with acid in retail or home refrigeration units, or in the packaging itself, may have a positive effect on color stability and help to improve shelf life of meat. However, the question is whether trona mineral can generate at least 20% CO_2 with existing moisture at a given place such as in a cooler or refrigerator, thus providing positive effects on lipid oxidation and color, and affecting aspects of meat other than color and lipid oxidation such as pH, drip loss and microbial growth.

2. Materials and Methods

2.1. Preparation of ground beef

USDA select grade shoulder clods (IMPS:1180) were sliced and coarsely (0.60 cm plate) then finely (0.32 cm) ground through a Hobart grinder model 4125 (Hobart

Mfg. Co., Troy, OH). Thin layer meat patties (20 g) and hamburger patties (100 g) were prepared using a patty mold (d=10 cm), placed in a foam tray, and wrapped with gas permeable PVC film.

2.2. Retail and Home storage conditions

The packaged meat was stored under retail conditions (1 ℃; see Table 5-1) for 0, 2, 4 and 7 days. After 4 days of retail storage meat was pulled from each retail condition and stored in one of two different home storage conditions (4 ℃; see Table 5-1) for 1, 2 and 4 days of home storage (5, 6 and 8 days total storage time). Gas-permeable packets (66 g) containing trona mineral and salicylic acid (14% by the total weight of trona and salicylic acid) were used as follows:

a. Retail conditions. 1) Walk-in cooler (1 ℃) with 4 mineral packets and intermittent fan-driven air circulation (simulated self-serve retail case); 2) Walk-in cooler (1 ℃)

Table 5-1. Experimental design for retail storage and home storage.

Retail Storage			
With Trona Packet		Without Trona Packet	
Open Shelf (OW)	Closed Container (CW)	Open Shelf (OWO)	Closed Container (CWO)
With Trona Packet TY, NY TY-Trona packet at retail storage; Trona packet at home storage NY-No trona storage at retail storage; Trona packet at home storage		Without Trona Packet TN, NN TN-Trona packet at retail storage; No trona packet at home storage NN- No trona packet at retail storage; No trona packet at home storage	
Home Storage			

without mineral packet, and with fan-circulated air; 3) Closed polyethelene box (50 x 90 x 18 cm) with 1 mineral packet, sealed with tape (no air circulation), at 1 ℃; 4) Closed box, without mineral packet, no air circulation, at 1 ℃

b. Home storage conditions. 1) Standard home refrigerator (4 ℃) with mineral packet; 2) Standard home refrigerator (4 ℃) without mineral packet.

2.3. pH measurement

A portable pH meter fitted with a semi-solid foods probe (HANNA Instruments, Ann Arbor, MI) was used. The probe was wrapped tightly in 10g of a thin meat patty and held until pH reading was stable.

2.4. Hunter color

Hunter L*, a* and b* were measured on the surface of PVC film by a Miniscan colorimeter (Hunter Labs, Reston, VA) with a 5 mm diameter aperture set to use illumninant D-65 and 2 °observer angle. The colorimeter was standardized with both black and white tiles covered with a single layer of PVC film before use. Three measurements were taken for each sample.

2.5. Thiobabituric acid reactive substances (TBARS)

TBARS assay was performed according to Buege and Aust (1978) with modifications. Thiobarbituric acid (TBA) reagent was made with 15% (W/V) tricholoacetic acid, 0.375% (W/V) thiobarbituric acid, and 0.25 N hydrochloric acid. Ground beef (1 g) was sampled and added to a centrifuge tube. TBA reagent (3 ml) was added to the centrifuge tube, which was then capped loosely and heated for 10 min in a

water bath (100 ℃) to develop pink color. Then, it was cooled with tap water and centrifuged for 25 min at 3200 x g. Two absorbance readings were taken per sample at 532 nm using a Shimadzu UV 2100 U spectrophotometer (Columbia, MD), then averaged. Two replicates were performed per steak. TBA values were calculated according to the following equation:

$$\text{TBA}\left(\frac{\text{mg}}{\text{kg}}\right) =$$

$$A_{532} \times \frac{1\text{ M chromagen}}{156{,}000} \text{ x } \frac{1\text{ mol/L}}{\text{M}} \times \frac{0.004\text{ L}}{1.0\text{ g meat}} \times \frac{72.07\text{ g MDA}}{\text{mole}} \times \frac{1{,}000{,}000\text{ mg}}{\text{kg}}$$

2.6. Total aerobic plate count (TAC)

Sample (10 g) was added to a stomacher bag and mixed with 90 ml of sterile Butterfield's buffer. Butterfield's buffer was prepared by dissolving KH_2PO_4 (34 g) in distilled water (500 ml), then adjusted to pH 7.2 with 1 N NaOH. Volume was brought to 1 liter with distilled water, sterilized at 121 ℃ for 15 min, and refrigerated. This stock solution (1.25 ml) was brought to 1 liter volume with distilled water and sterilized prior to use (Food and Drug Administration, 1998). The stomacher bag was stomached for 30 seconds and 1 ml of mixture was diluted into 9 ml of sterile buffer. The appropriate diluents were plated to 3M aerobic plate count petrifilms (3M Corporation, St. Paul, MN). The films were incubated at 32 ℃ for 48 hr. Colonies were counted according to manufacturer recommendations and expressed as CFU/ml.

2.7. Drip loss measurement

Two identical acrylic display cases were used to simulate retail butcher display conditions. One case contained one mineral packet and the other had no packet. A small

fan was placed in each case to give air circulation. Beef shoulder clods were freshly ground coarsely (0.60 cm plate) then finely (0.32 cm) through a Hobart grinder model 4125 (Hobart Mfg. Co., Troy, OH). Ground beef chubs (2 lbs) were placed on the screen in each display case, with an absorbent pad held to the other side of the screen by steel magnets. Desiccant stones were placed to absorb free moisture in the cases. The weight change of the pad, desiccant and mineral packet was measured. Four complete replicates were performed.

2.8. Statistical analysis

Analysis of variance was performed using the proc mixed function in SAS version 9.1 (SAS Institute, Inc., Cary, NC). Statistical significance was identified at the 95% confidence level, and post-hoc means comparisons were made based on p-values obtained using the Tukey-Kramer adjustment.

3. Results and Discussion

3.1. Retail storage

3.1.1. pH measurement

There were no differences observed for the different storage conditions (mineral packet or none, open or closed storage; $P>0.05$; Figure 5-1; see Appendix D for detailed statistics). The pH of ground beef in the closed system containing a mineral packet was slightly, but not significantly, higher than the other conditions on days 2 and 7. There was no effect of storage with mineral packets on pH.

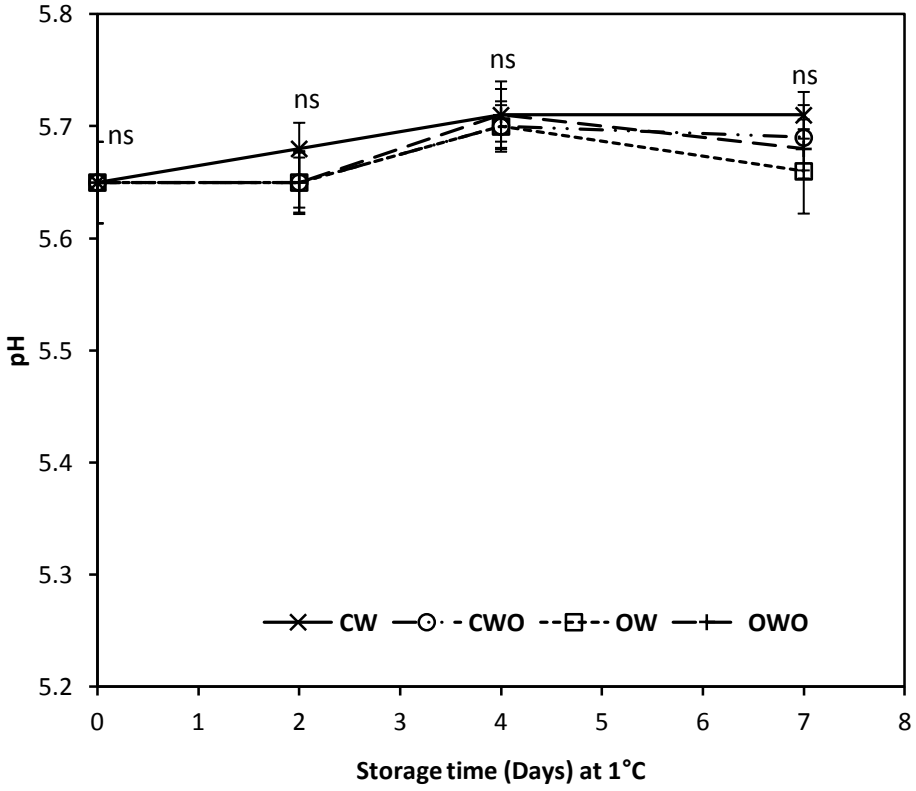

Figure 5-1. pH of ground beef patties under retail storage conditions (1 ℃).
CWO: closed system without trona packet; CW: closed system with trona packet; OWO: open without trona packet; and OW: open with trona packet.
"ns" indicates no significant difference at each time point (P>0.05).
Error bars: +/-standard error of the mean.

3.1.2. Hunter color

Hunter a* and b* values decreased throughout the entire experiment period (Table 5-2). Color stability is often evaluated by a*, hue angle and chroma, since red color is a significant factor in meat color. Redness Redness (a*) and chroma (saturation) decreased, and hue angle (color intensity) during the 7 days of retail storage (Table 5-3). There was no significant difference in Hunter color parameters (a*, b* and L*) between closed and open system regardless of the presence of a mineral packet during retail storage (P>0.05; see Appendix D). In addition, there was no significant difference in hue

Table 5-2. Hunter a*, L* and b* values of ground beef patties under retail storage conditions (1 ℃).

Storage conditions	a*[a]				L*[a]				b*[a]			
	Day 0	Day 2	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7
CWO	19.9 ±0.1 a	17.3 ±0.1 a	14.6 ±0.2 a	12.5 ±0.3 a	41.3 ±0.3 a	42.2 ±0.3 a	40.8 ±0.1 a	42.1 ±0.2 a	16.7 ±0.1 a	15.6 ±0.2 a	14.0 ±0.1 a	14.3 ±0.1 a
CW	19.9 ±0.1 a	17.5 ±0.1 a	15.4 ±0.1 a	12.8 ±0.3 a	41.3 ±0.3 a	41.3 ±0.2 a	41.3 ±0.3 a	40.2 ±0.2 a	16.7 ±0.1 a	15.5 ±0.1 a	14.6 ±0.1 a	13.9 ±0.1 a
OWO	19.9 ±0.1 a	16.5 ±0.1 a	15.3 ±0.2 a	12.8 ±0.3 a	41.3 ±0.3 a	42.7 ±0.2 a	41.4 ±0.2 a	40.1 ±0.2 a	16.7 ±0.1 a	15.4 ±0.1 a	14.5 ±0.1 a	13.6 ±0.1 a
OW	19.9 ±0.1 a	17.5 ±0.1 a	15.9 ±0.1 a	13.0 ±0.3 a	41.3 ±0.3 a	41.4 ±0.3 a	43.0 ±0.3 a	40.6 ±0.3 a	16.7 ±0.1 a	16.0 ±0.1 a	14.9 ±0.1 a	13.2 ±0.1 a

[a]Values represent mean +/- standard error of the mean.
Values sharing letters within, but not between, columns are not significantly different (P>0.05).
CWO: closed system without trona packet; CW: closed system with trona packet; OWO: open without trona packet; and OW: open with trona packet.

Table 5-3. Hue angle and chroma of ground beef patties under retail storage conditions (1 ℃).

Storage conditions	Hue Angle[a]				Chroma[a]			
	Day 0	Day 2	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7
CWO	40.0 ±0.2 a	41.9 ±0.2 a	44.0 ±0.2 a	49.8 ±0.6 a	26.0 ±0.1 a	23.3 ±0.1 a	20.3 ±0.2 a	19.1 ±0.2 a
CW	40.0 ±0.2 a	41.4 ±0.1 a	43.4 ±0.2 a	48.0 ±0.6 a	26.0 ±0.1 a	23.4 ±0.1 a	21.3 ±0.2 a	19.0 ±0.2 a
OWO	40.0 ±0.2 a	42.9 ±0.3 a	43.5 ±0.2 a	47.5 ±0.6 a	26.0 ±0.1 a	22.6 ±0.2 a	21.1 ±0.2 a	18.8 ±0.2 a
OW	40.0 ±0.2 a	42.5 ±0.3 a	43.1 ±0.2 a	48.3 ±0.6 a	26.0 ±0.1 a	23.7 ±0.1 a	21.8 ±0.1 a	19.4 ±0.2 a

[a]Values represent mean +/- standard error of the mean.
Hue angle (true redness) was calculated as [arctangent (b*/a*)]. Chroma (color saturation) was calculated as $\sqrt{(a^{*2}+ b^{*2})}$.
Values sharing letters within, but not between, columns are not significantly different (P>0.05).
CWO: closed system without trona packet; CW: closed system with trona packet; OWO: open without trona packet; and OW: open with trona packet.

angle and chroma between closed and open system regardless of the presence of a mineral packet during retail storage (P>0.05).

3.1.3. Thiobarbituric Acid Reactive Substances (TBARS)

TBARS values are presented in Figure 5-2. TBARS values for all treatments increased throughout the 7 day storage period. The TBARS values in samples held with mineral packets was slightly, though not significantly, lower than those without mineral

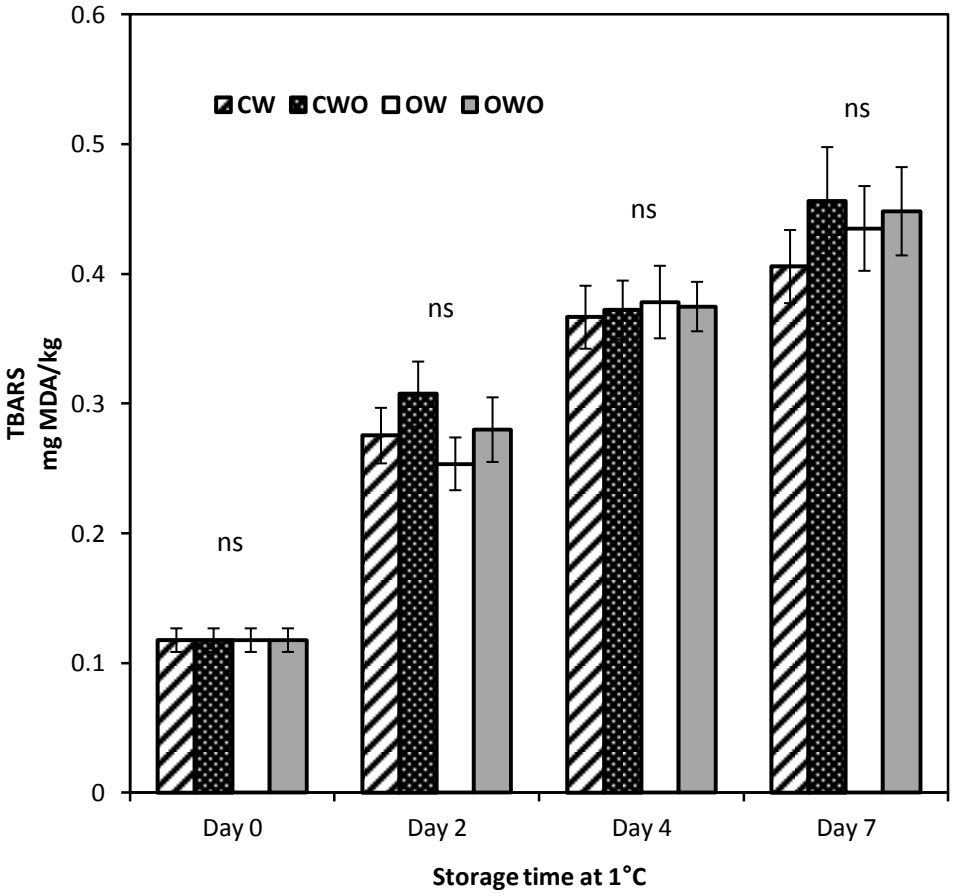

Figure 5-2. TBARS values of ground beef patties under retail storage conditions (1 ℃). CWO: closed system without trona packet; CW: closed system with trona packet; OWO: open without trona packet; and OW: open with trona packet.
"ns" indicates no significant difference (P>0.05).
Error bars: +/-standard error of the mean.

packets (P>0.05). Ground beef patties held in a closed system without mineral packets had higher TBARS values than those held in the other conditions on days 2 and day 7. However, this difference was not statistically significant (P>0.05). TBARS values for all retail storage conditions stayed under the threshold typically associated with sensory acceptability (0.5 mg MDA/kg; Gray & Pearson, 1987) through the entire course of retail storage.

3.1.4. Total Aerobic plate Count (TAC)

Aerobic plate counts of patties increased from 10^3 to 10^5 throughout 7 days storage at 1 ℃ (Figure 5-3). No significant effect of retail storage condition on TAC was seen (P>0.05). In closed storage conditions, the patties with mineral packets had slightly, but not significantly, lower counts. The maximum growth was found in closed systems without mineral packets (5.0 log cfu/g) on day 7. Aerobic plate counts for all systems remained below the level typically associated with visible spoilage for the entire course of retail storage (< 10^7 cfu/g; Sofos, 1994).

3.2. Home storage

Since there were no significant effects of retail storage system (open shelf/closed case) on the values of TBARS, color, pH and TAC, values were pooled by the presence or absence of mineral packets during retail storage to examine the effects of home storage conditions.

3.2.1. pH measurement

pH change is shown in Figure 5-4. pH of all the treatments increased slightly on

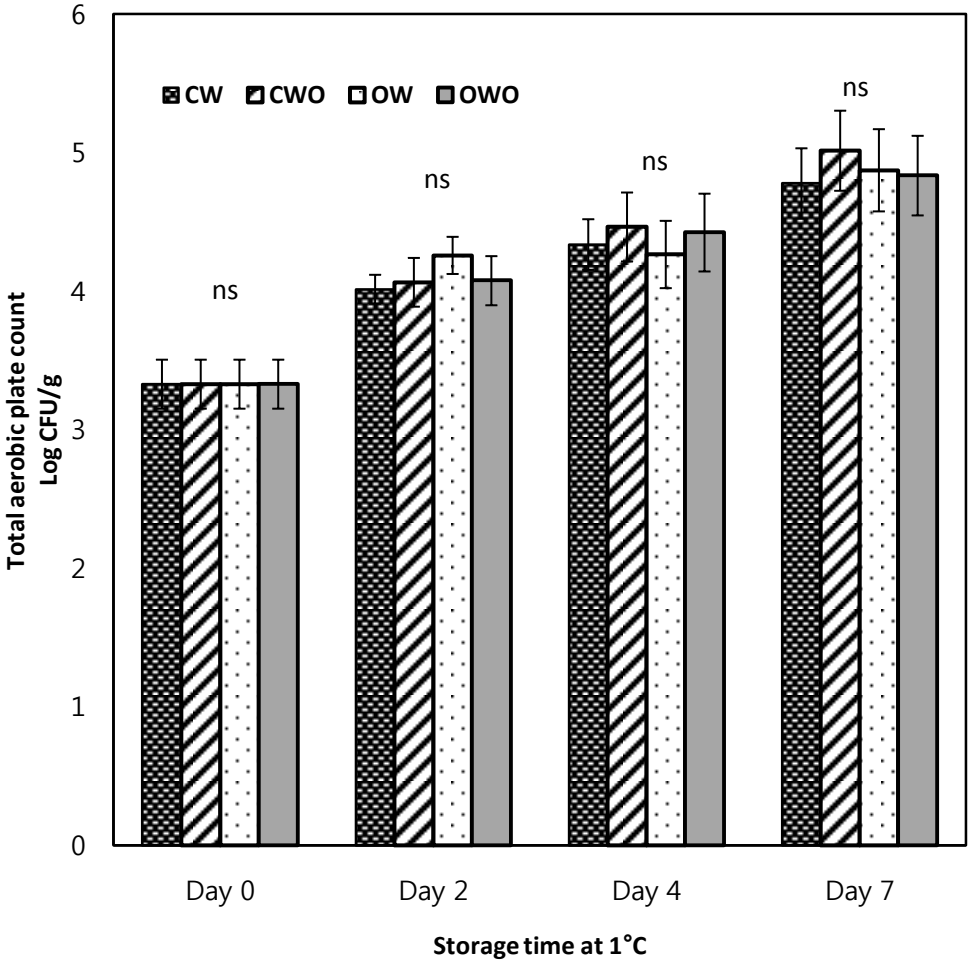

Figure 5-3.Total aerobic plate counts of ground beef patties under retail storage conditions (1 ℃).
CWO: closed system without trona packet; CW: closed system with trona packet; OWO: open without trona packet; and OW: open with trona packet.
"ns" indicates no significant difference (P>0.05).
Error bars: +/-standard error of the mean.

day 2 and decreased on day 4, but there was no significant difference among the treatments (P>0.05). In addition, the presence of mineral packets during retail and/or home storage did not affect the pH of ground beef. Thus, there was no significant effect of mineral packets.

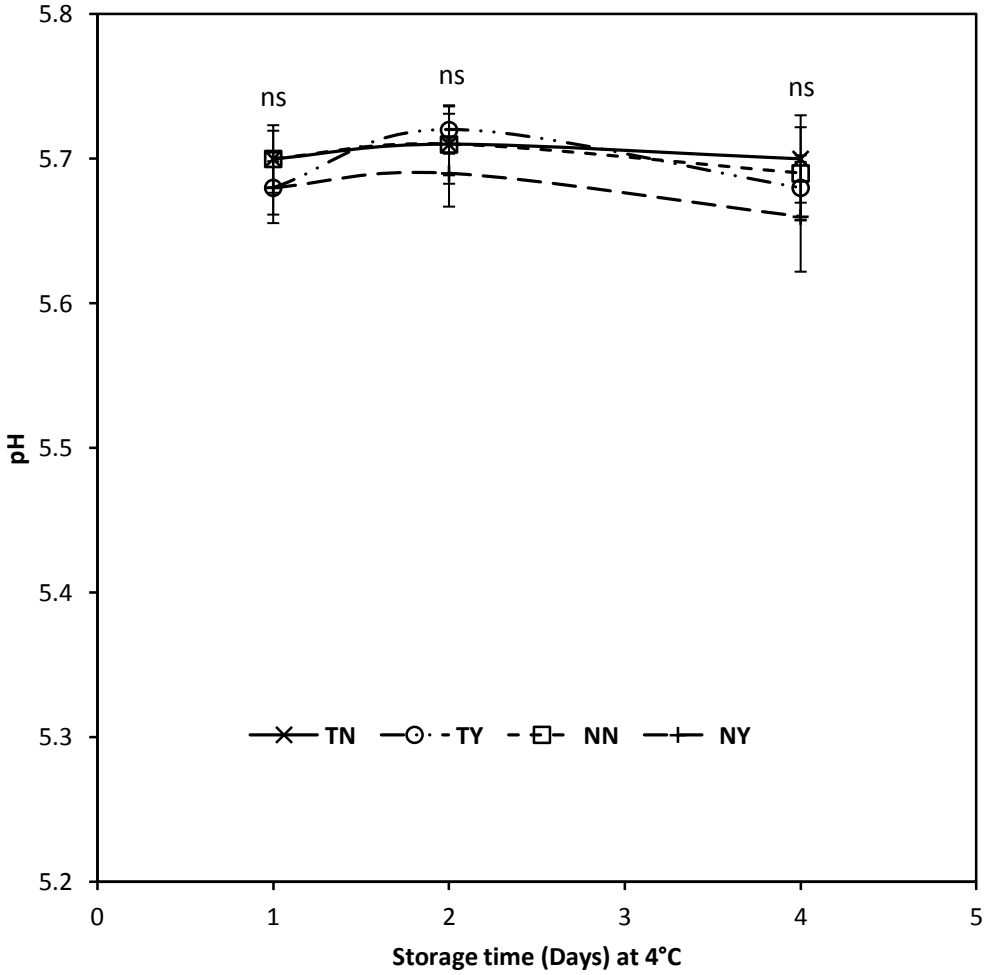

Figure 5-4. pH of ground beef patties under home storage conditions (4 ℃).
See table 5-1 for storage condition descriptions.
"ns" indicates no significant difference. Error bars: +/-standard errors of mean.

3.2.2. Hunter color

Hunter a* and b* values decreased throughout the entire experiment period (Table 5-4). There was no significant effect of mineral packets on Hunter color parameters (a*, b* and L*) during home storage (P>0.05). Redness (a*) and chroma (saturation) decreased, and hue angle (color intensity) increased during home storage (Table 5-5). There was no significant difference between chroma, and hue angle during home storage (P>0.05).

Table 5-4. Hunter a*, L* and b* values of ground beef patties under home storage conditions (4 ℃).

Storage conditions	a*[a]		
	Day 1	Day 2	Day 4
TN	14.6 ±0.1 a	14.3 ±0.1 a	11.3 ±0.1 a
TY	14.8 ±0.1 a	13.3 ±0.1 a	11.5 ±0.1 a
ON	14.8 ±0.1 a	13.9 ±0.1 a	10.9 ±0.1 a
OY	14.5 ±0.1 a	13.2 ±0.1 a	11.2 ±0.2 a
Storage conditions	L*[a]		
	Day 1	Day 2	Day 4
TN	41.2 ±0.1 a	40.2 ±0.1 a	42.3 ±0.2 a
TY	41.4 ±0.1 a	41.5 ±0.2 a	41.8 ±0.2 a
ON	42.3 ±0.1 a	41.4 ±0.1 a	43.3 ±0.2 a
OY	42.9 ±0.1 a	41.2 ±0.1 a	41.0 ±0.3 a
Storage conditions	b*[a]		
	Day 1	Day 2	Day 4
TN	15.7 ±0.2 a	13.9 ±0.1 a	13.9 ±0.0 a
TY	14.4 ±0.1 a	14.1 ±0.1 a	13.9 ±0.0 a
ON	14.9 ±0.0 a	14.1 ±0.0 a	13.9 ±0.0 a
OY	14.5 ±0.1 a	14.2 ±0.0 a	13.5 ±0.1 a

[a]Values represent mean +/- standard error of the mean. Values sharing letters within, but not between, columns are not significantly different (P>0.05).
See table 5-1 for storage condition descriptions.

Table 5-5. Hue angle and chroma values of ground beef patties under home storage conditions (4 ℃).

Storage conditions	Hue Angle[a]		
	Day 1	Day 2	Day 4
TN	46.0 ±0.2 a	44.3 ±0.1 a	51.7 ±0.3 a
TY	44.3 ±0.1 a	47.2 ±0.3 a	51.4 ±0.3 a
ON	45.3 ±0.2 a	45.7 ±0.1 a	52.8 ±0.4 a
OY	45.7 ±0.2 a	47.7 ±0.3 a	51.0 ±0.5 a
Storage conditions	Chroma[a]		
	Day 1	Day 2	Day 4
TN	21.7 ±0.2 a	19.9 ±0.1 a	18.1 ±0.1 a
TY	20.7 ±0.1 a	19.5 ±0.1 a	18.2 ±0.1 a
ON	21.0 ±0.1 a	19.9 ±0.1 a	17.9 ±0.1 a
OY	20.4 ±0.1 a	19.5 ±0.1 a	17.8 ±0.1 a

[a]Values represent mean +/- standard error of the mean. Values sharing letters within, but not between, columns are not significantly different (P>0.05).
See table 5-1 for storage condition descriptions. Hue angle (true redness) was calculated as [arctangent (b*/a*)]. Chroma (color saturation) was calculated as $\sqrt{(a^{*2} + b^{*2})}$.

3.2.3. Thiobaribituric acid reactive substances (TBARS)

TBARS values are presented in Figure 5-5. The TBARS values increased throughout the 4 days of home storage. No significant difference was found between samples stored with or without mineral packets during home storage (P>0.05). There was no obvious trend observed, however, for the whole storage period, (including 7 days of retail storage and 4 days of home storage). TBARS values of beef patties remained under 0.5 mg MDA/kg, the threshold typically associated with sensory acceptability (Gray & Pearson, 1987).

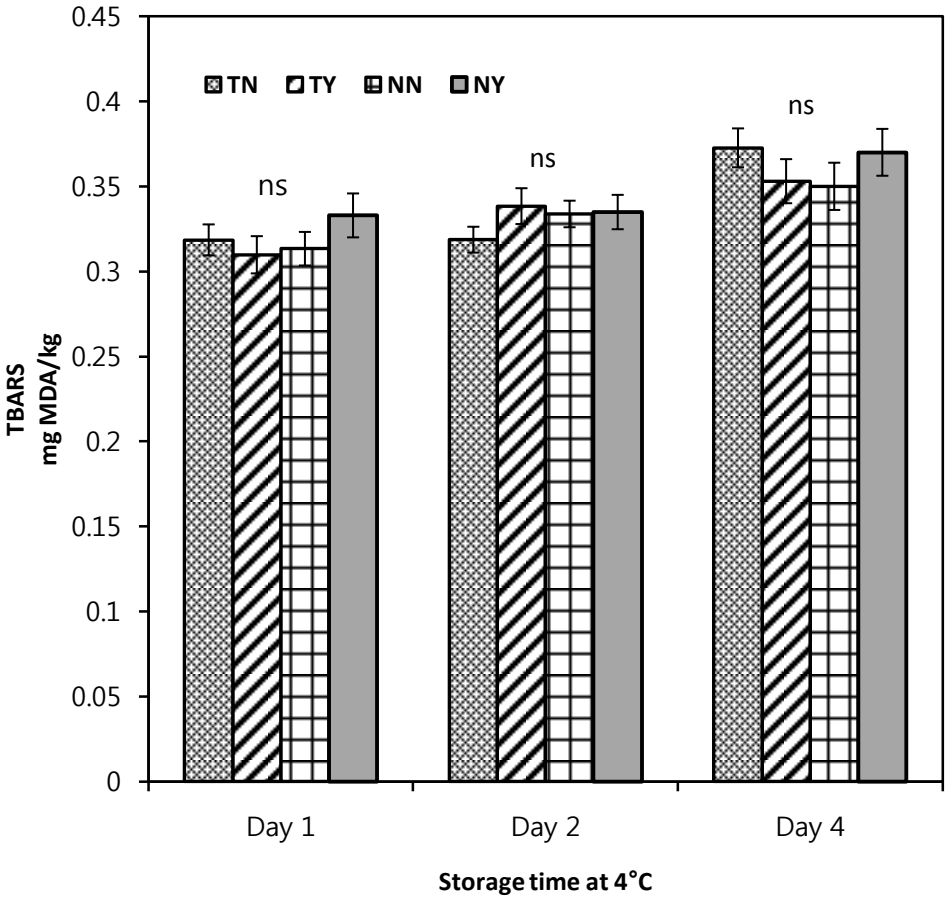

Figure 5-5. TBARS values of ground beef patties under home storage conditions (4℃). See table 5-1 for storage condition descriptions. "ns" indicates no significant difference (P>0.05). Error bars: +/-standard errors of the mean.

3.2.4. Total aerobic plate count (TAC)

The growth of aerobic psychrotrophic bacteria is shown in Figure 5-6. The patties with mineral packets at retail storage had slightly lower TAC values overall as compared to home storage, but there was no significant difference in TAC between patties stored with or without mineral packets during the initial retail storage (P>0.05). The highest TAC values were found on day 4 of home storage, though they still had not reached unacceptable levels (< 10^7 cfu/g; Sofos, 1994).

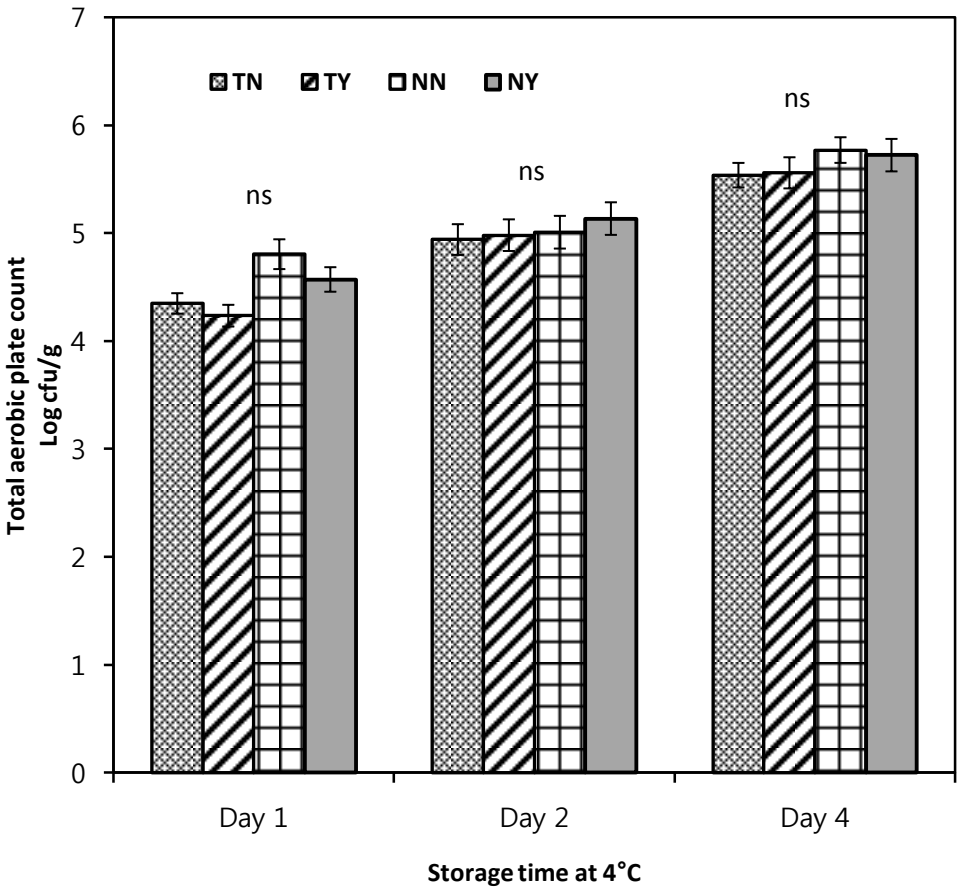

Figure 5-6. Total aerobic plate counts in ground beef patties under home storage conditions (4 ℃).
See table 5-1 for storage condition descriptions. "ns" indicates no significant difference (P>0.05). Error bars: +/- standard errors of the mean.

3.3. Drip loss

Figure 5-7 shows the cumulative moisture losses of freshly ground beef. Ground beef chubs held under both storage conditions lost moisture, and the levels of loss were almost identical for the first several hr. From 24 hr to 48 hr the water loss of ground beef stored with a mineral packet was higher than that without the packet ($P<0.05$, see Apendix). Seideman, Carpenter, Smith, Dutson, and Dill (1979) reported an increased weight loss in meat stored in 100% CO_2 atmosphere as compared to 100% N_2. They suggested that CO_2 may bind to meat proteins, thus decreasing their ability to retain water. In addition, O'Keeffe and Hood (1981) reported that CO_2 dissolved in meat lowers the pH, which in turn decreases water holding capacity.

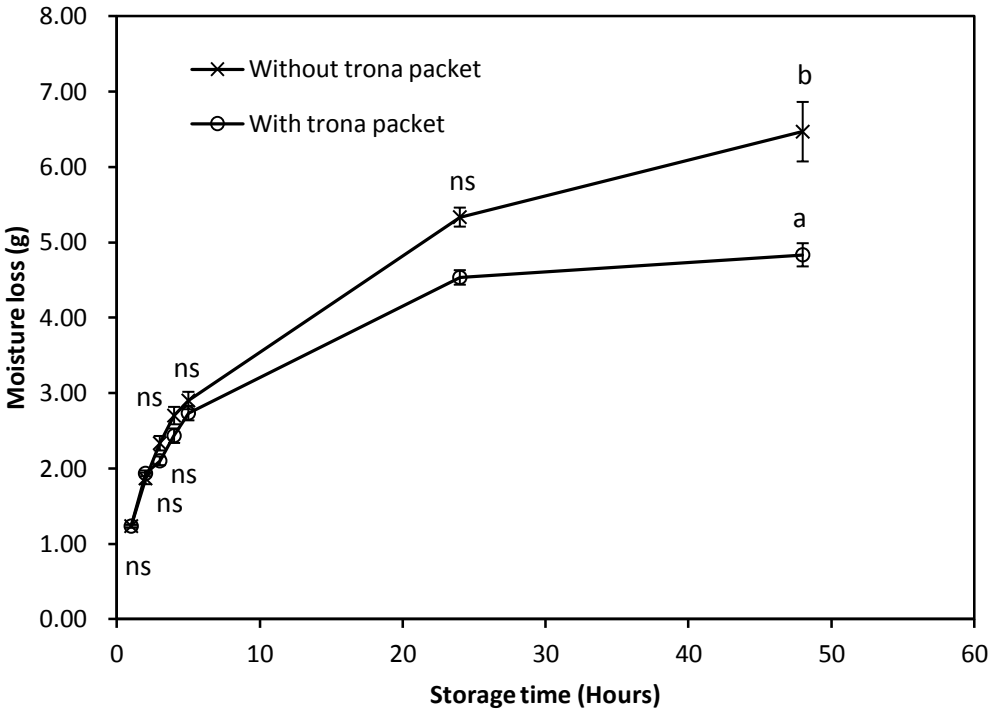

Figure 5-7. Drip loss of ground beef chubs (2 lb=908 g) stored at 7 ℃.
Values sharing letters are not significantly different at each time point ($P<0.05$). "ns" indicates no significant difference. Error bars: +/- standard errors of the mean.

4. Conclusion

Use of mineral packets containing trona and salicylic acid in different storage systems (open shelf, closed case) did not affect color, pH, microbial growth, or TBA values of ground beef patties held under retail (1 ℃) or home storage (4 ℃) conditions. This was most likely because humidity within the storage coolers and/or refrigerators was not high enough to allow the CO_2 generating reaction to occur. As mentioned in Chapter 4, the amount of moisture is an important factor in the ability of the chemicals contained in the mineral packet to react. To increase effectiveness of mineral packets, it is recommended to add a readily available source of water to the packet or to the storage cooler.

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CHAPTER 6

THE EFFECT OF MINERAL PACKETS ON PH, COLOR, MICROBIAL GROWTH, AND LIPID OXIDATION OF CHICKEN IN EXTENDED STORAGE

Abstract

Fresh chicken breast and thigh portions packaged in a PVC overwrapped tray with a mineral packet containing trona and salicylic acid (0, 2 or 4 g total) were stored at 1 ℃ and tested to evaluate the effect of the mineral mixture on pH, Hunter color, thiobarbituric acid reactive substances (TBARS), total aerobic count (TAC) and texture after extended storage (0, 15, 18, 21, 24, and 27 days). Negative a* values (less red; more green) observed in both breast and thigh portions decreased after day 0. Slight increases from initial pH were observed in both chicken portions. Decrease in TBARS values were possibly due to diffusion of malondialdehyde and other secondary oxidation products during the extended storage. Chicken breast and thigh portions stored with mineral packets (2 g and 4 g) had significantly lower aerobic plate counts than those without the mineral mixture (0 g) on day 15 ($P<0.05$). Mineral packets had no antimicrobial effect during the remaining storage period. After 15 days of storage, sufficient bacterial growth had occurred in both chicken portions to be considered spoiled. There was no significant effect of mineral packets on pH, color, or TBARS values. In conclusion, trona packets did not prolong chicken meat shelf life during extended storage. The amount of absorbed CO_2 in meat may help to understand effectiveness of CO_2 generating mineral packets in chicken storage.

1. Introduction

Poultry is often stored under modified atmosphere to control its color, microbial growth and odor. CO_2 is often used to control microbial growth in fresh poultry. For instance, chicken stored in 25% CO_2 showed an extended shelf life (Ogilvy & Ayres, 1951). Lower aerobic plate counts were observed after 14 days for chicken breast and leg portions stored in 80% CO_2 compared to those held in air (Hotchkiss, Baker, & Qureshi 1985). The role of O_2 is important on microbial growth during storage of chicken since the predominant spoilage microbes growing on meat are dependent on the presence of O_2. Under aerobic conditions, the predominant spoilage organism in chicken breast meat is *Pseudomonas* (Hart, Mead, & Norris, 1991; Kakouri & Nychas, 1994), and growth of *Pseudomonas* on cut up chicken is closely related to off-odor and slime development (Ayres, Ogilvey, & Stweart, 1950). According to Kakouri and Nychas (1994), lactic acid bacteria (LAB) and *Bronchothrix themosphacta* were the predominant organisms found in samples stored in the absence of O_2 (e.g. vacuum packaging and CO_2/N_2 packaging). LAB are responsible for a souring smell in poultry (Dainty & Mackey, 1992; Stiles, 1991).

The color of raw poultry varies from grey to yellow. Color of poultry is important for consumers purchasing decision. Pale, soft and exudative (PSE) meat has been one of problems in the poultry industry due to economical reasons (De Lourdes Perez-chabela, & Totosaus, 2012). The color of broiler breast was strongly correlated to pH, and the values of redness (a*) increased but that of lightness (L*) and yellowness (b*) decreased, as the pH increased (Allen, Russell, & Fletcher, 1997). A pale yellowish-brown color, which is considered as discoloration, is related to oxidative processes

(Fraqueza & Barreto, 2011). Pale green meat color can be caused in the presence of H_2O_2 by acids from the metabolism of lactic acid bacteria or fat oxidation induces myoglobin oxidation with colemyoglobin production (Lawrie, 1998; Ranken, 2000). Increased L* and b* values can be related to discoloration during storage time in turkey meat under aerobic packaging (Santé, Lebert, Le Pottier, & Ouali, 1996; Santé, Renerre, & Lacourt, 1994). Ultimate pH of meat is important since a low ultimate pH can result in meat that appears less red and more yellow (Castellin, Mungai, & Dal Bosco, 2002). Poultry meat contains less myoglobin as compared to red meats (Millar, Willson, Moss, & Ledward, 1994). As a result, poultry muscle exposed to air does not display the bright red color associated with oxymyglobin (Gill & Gill, 2005). More red color (higher a* value) was observed in ground chicken held under 80%N_2/20% CO_2 as compared to those held under a 62% CO_2, 8% O_2, 30% N_2 gas mixture (Saucier, Gendron, & Gariepy, 2000).

In previous studies, it was found that mineral packets containing trona and salicylic acid are able to release carbon dioxide (CO_2) in the presence of moisture (see Chapter 4). However, it is not known if CO_2 generated from mineral packet is an effective method to control quality of chicken. Thus, the aim of this study was to evaluate the effectiveness of mineral packets containing trona and salicylic acid on quality of PVC packaged chicken during the extended storage. Since this experiment was designed for extended storage life of chicken, the tests were not performed until later in the storage time (after 15 days of storage). Different amounts of trona/acid mixture (0, 2, or 4 g) were used to assess whether low amounts of mineral can affect chicken quality. Two different cuts of chicken (boneless breast and thigh portions) were used since differences in the effect of CO_2 on color and lipid oxidation were expected due to different myoglobin and

fat contents between chicken breast and thigh meat.

2. Materials and Methods

2.1. Chicken preparation

Boneless chicken breasts and thighs were obtained directly from the harvest facility on three different harvest dates. Chicken portions (18 breasts and 18 thighs) from each harvest date were considered as one replicate, thus there were three replicates. Skinless chicken (one breast and one thigh per package) was packaged in a foam tray with an absorbent pad and mineral packets (totaling 0, 2, or 4 g of trona/acid mixture), and over-wrapped with PVC film (Koch, Kansas City, MO; O_2 permeability = 8400 $cm^3/(24\ hr \times m^2 \times atm)$ at 23 ℃; water vapor transmission = 83 $g/(24\ hr \times m^2)$ at 23 ℃ and 50% relative humidity). Each chicken breast and thigh weighed about 204 g and 108 g, respectively. The mineral packets contained trona mineral and salicylic acid (salicylic acid present at 14% of the total weight of the mixture). Chicken was stored in a walk-in cooler (1 ℃) for 27 days to simulate retail storage conditions. Chicken samples were tested on days 0, 15, 18, 21, 24, and 27 for TBARS, pH, TAC, texture and Hunter color.

2.2. pH measurement

pH was measured directly on samples. A portable pH meter fitted with a semi-solid foods probe (HANNA Instruments, Ann Arbor, MI) was used. The probe was inserted directly into the samples and held until a stable reading was observed.

2.3. Hunter color

Hunter L*, a* and b* were measured on the surface of PVC film by a Miniscan

colorimeter (Hunter Labs, Reston, VA) with a 5 mm diameter aperture set to use illumninant D-65 and 2 °observer angle. The colorimeter was standardized with both black and white tiles covered with a single layer of PVC film before use. Three measurements were taken for each sample.

2.4. Thiobabituric Acid Reactive Substances (TBARS)

Chicken samples were collected with a coring device (1.5 cm diameter) from the center of chicken breasts and thighs. The collected samples were minced then used for TBARS assay. TBARS assay was performed according to Buege and Aust (1978) with modifications. Thiobarbituric acid (TBA) reagent was made with 15% (W/V) tricholoacetic acid, 0.375% (W/V) thiobarbituric acid, and 0.25 N hydrochloric acid. Minced core samples (1 g) were added to a centrifuge tube. TBA reagent (3 ml) was added to the centrifuge tube, which was then capped loosely and heated for 10 min in a water bath (100 ℃) to develop pink color. Then, it was cooled with tap water and centrifuged for 25 min at 3,200 x g in a Beckman coulter centrifuge (Palo Alto, CA). Two absorbance readings were taken per sample at 532 nm using a UV 2100 U spectrophotometer (Columbia, MD) then averaged. Two replicates were performed per sample. TBA values were calculated according to the following equation:

$$\text{TBA}\left(\frac{\text{mg}}{\text{kg}}\right) =$$

$$A_{532} \times \frac{1\text{ M chromagen}}{156{,}000}\ \text{x}\ \frac{1\text{ mol/L}}{\text{M}} \times \frac{0.004\text{ L}}{1.0\text{ g meat}} \times \frac{72.07\text{ g MDA}}{\text{mole}} \times \frac{1{,}000{,}000\text{ mg}}{\text{kg}}$$

2.5. Total aerobic plate count (TAC)

Swabbing method was used for microbial growth test. A 5 x 5 cm^2 area on the

surface of each breast and thigh was swabbed with a sterile cotton swab in three different directions. The swab was placed in 9 ml of sterile Butterfield's phosphate buffer. Butterfield's buffer was prepared by dissolving KH_2PO_4 (34 g) in distilled water (500 ml), then adjusted to pH 7.2 with 1 N NaOH. Volume was brought to 1 liter with distilled water, sterilized at 121 ℃ for 15 min, and refrigerated. This stock solution (1.25 ml) was brought to 1 liter volume with distilled water and sterilized prior to use (Food and Drug Administration, 1998). Stomacher bag was homogenized for 30 seconds, then diluted to subsequent concentrations and plated to Petrifilm^{TM} aerobic count plates (3M Corporation, St. Paul, MN) in duplicate. Plates were incubated at 35 ℃ for 48 hr. Colonies were counted according to manufacturer recommendations and expressed as CFU/cm^2.

2.6. Texture analysis

Chicken samples were collected with a coring device (1.5 cm diameter) from the center of chicken breasts. A TMS-Pro texture analyzer (Food Technology Corp., Sterling, VA) was used for compression testing in 500 g load cell using a 1.5 inch wide circular plate at 200 mm/min crosshead speed for compression and at -200 mm/min for withdrawing. The peak force (mN) of the first compression cycle was used for evaluation of firmness. The first area where the force curve dropped under 0 mN during crosshead withdrawal was used for evaluation of adhesiveness (mN Sec).

2.7. Statistical analysis

Analysis of variance was performed using the proc mixed function in SAS version 9.1 (SAS Institute, Inc., Cary, NC). For texture analysis the data was pooled across storage time. Statistical significance was identified at the 95% confidence level,

and post-hoc means comparisons were made based on p-values obtained using the Tukey-Kramer adjustment. Three replicates were performed.

3. Results and Discussion

3.1. pH measurement

pH values of breast and thigh portions are shown in Figure 6-1. A slight increase in pH was observed in both breast and thigh portions after day 0, but the increase was not significant. No significant differences were found in pH at each individual time point ($P>0.05$; see Appendix E for detailed statistics), regardless of storage conditions.

3.2. Hunter color

The initial Hunter a* readings were negative for both chicken breast (Table 6-1) and thigh (Table 6-2) portions. The Hunter a* values of chicken breast decreased (less red and more green) throughout the entire storage time. Significantly higher a* values were seen in chicken breast portions stored with 2 g of mineral mixture on day 27, and higher b* values on day 21. A decrease in a* values was observed in chicken thigh portions (Table 6-2), similar to that seen in chicken breasts. Hunter b* values increased from the initial b* value, but no significant changes were observed during the remaining storage days. Significant lower a* values were observed in chicken thighs stored with 0 g of mineral mixture on day 18. Significantly higher b* values were seen in chicken thigh portions stored with 2 g on day 21. Chicken thighs stored with 2 g of mineral mixture had significant lower L* values on day 18 but higher values on day 21. Overall, there was no obvious trend in chicken thigh color. No significant differences were observed in Hunter

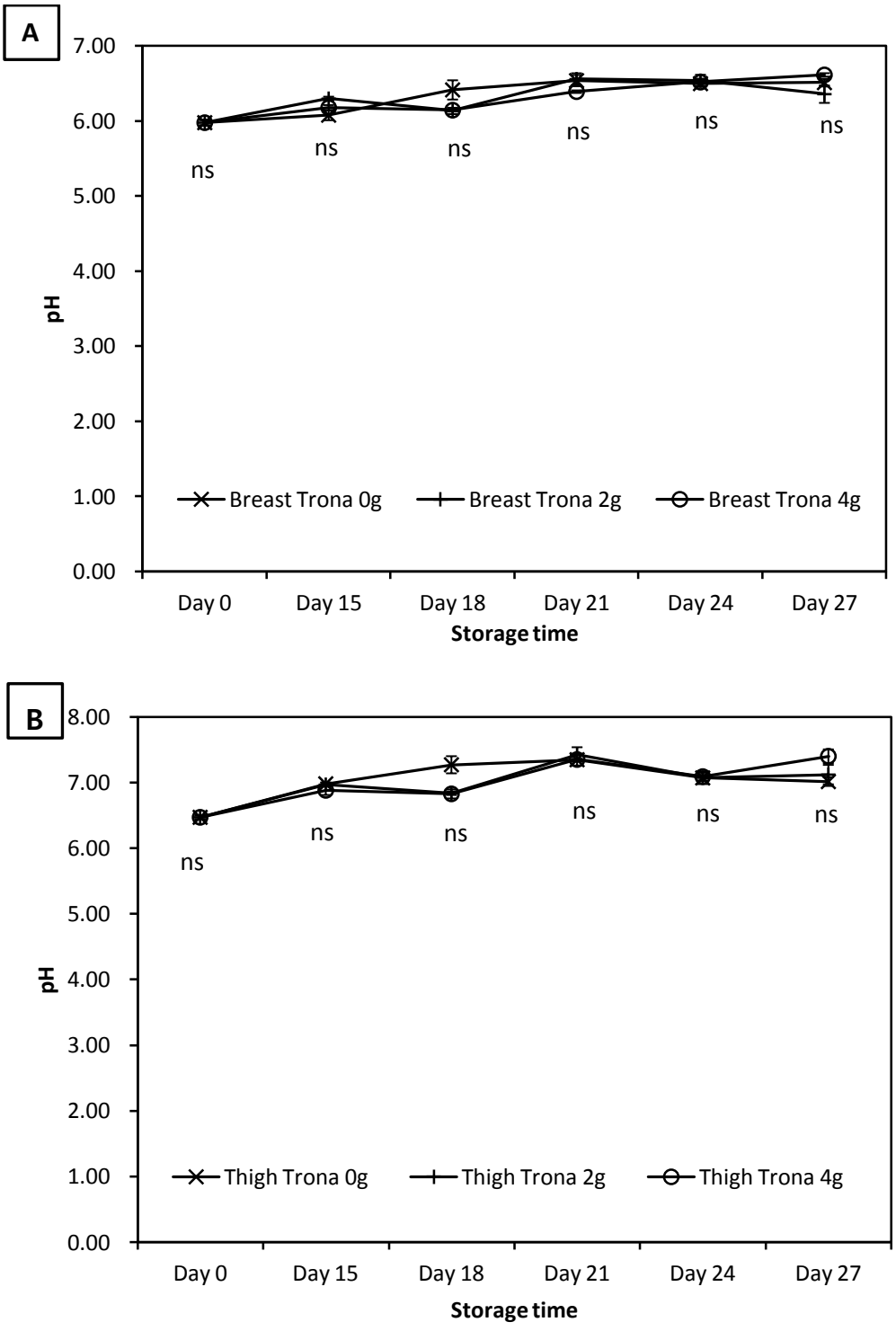

Figure 6-1. pH of chicken breast (A) and thigh (B) stored with different amounts of mineral mixture (1 ℃).
"ns" indicates no significant difference (P>0.05). Error bars: +/- standard error of the mean.

Table 6-1. Hunter a*, b* and L* values of chicken breast stored at 1°C.

a*[a] (redness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	-1.44 ±0.08 a	-1.84 ±0.06 a	-2.07 ±0.05 a	-1.99 ±0.07 a	-2.08 ±0.05 a	-1.99 ±0.05 ab
2 g	-1.44 ±0.08 a	-1.87 ±0.06 a	-2.22 ±0.04 a	-1.72 ±0.10 a	-2.28 ±0.06 a	-1.81 ±0.06 a
4 g	-1.44 ±0.08 a	-1.75 ±0.05 a	-2.08 ±0.04 a	-1.72 ±0.08 a	-2.09 ±0.04 a	-2.33 ±0.05 b
b*[a] (yellowness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	0.15 ±0.33 a	0.78 ±0.20 a	0.16 ±0.11 a	1.75 ±0.12 ab	1.61 ±0.24 a	1.39 ±0.05 a
2 g	0.15 ±0.33 a	0.90 ±0.14 a	1.49 ±0.11 a	3.11 ±0.18 a	0.66 ±0.12 a	0.58 ±0.21 a
4 g	0.15 ±0.33 a	1.38 ±0.21 a	0.51 ±0.17 a	0.89 ±0.17 b	0.99 ±0.17 a	-0.92 ±0.31 a
L*[a] (lightness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	42.37 ±0.40 a	43.35 ±0.36 a	41.75 ±0.19 a	42.68 ±0.28 a	43.49 ±0.11 a	42.74 ±0.19 a
2 g	42.37 ±0.40 a	42.94 ±0.38 a	42.28 ±0.10 a	42.86 ±0.43 a	42.63 ±0.19 a	37.08 ±0.21 a
4 g	42.37 ±0.40 a	41.76 ±0.56 a	43.09 ±0.23 a	41.82 ±0.17 a	42.59 ±0.19 a	42.52 ±0.31 a

[a]Values represent mean +/- standard error of the mean. Values sharing letters within, but not between, columns are not significantly different (P>0.05).

Table 6-2. Hunter a*, b* and L* values of chicken thigh stored at 1°C.

a*[a] (redness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	-0.99 ±0.10 a	-1.94 ±0.07 a	-2.83 ±0.06 a	-2.01 ±0.06 a	-1.87 ±0.08 a	-1.90 ±0.08 a
2 g	-0.99 ±0.10 a	-1.08 ±0.02 a	-1.21 ±0.17 b	-1.78 ±0.09 a	-2.35 ±0.07 a	-1.48 ±0.15 a
4 g	-0.99 ±0.10 a	-1.99 ±0.06 a	-1.17 ±0.19 b	-1.43 ±0.08 a	-1.67 ±0.14 a	-1.51 ±0.11 a
b*[a] (yellowness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	0.36 ±0.25 a	3.22 ±0.20 a	4.53 ±0.20 a	3.23 ±0.21 a	3.72 ±0.28 a	3.62 ±0.08 a
2 g	0.36 ±0.25 a	2.80 ±0.17 a	2.42 ±0.12 a	7.92 ±0.23 b	1.88 ±0.13 a	3.67 ±0.40 a
4 g	0.36 ±0.25 a	2.35 ±0.13 a	2.63 ±0.30 a	3.42 ±0.12 a	3.20 ±0.34 a	3.21 ±0.22 a
L*[a] (lightness)						
Trona	DAY 0	DAY 15	DAY 18	DAY 21	DAY 24	DAY 27
0 g	40.06 ±0.75 a	48.10 ±0.36 a	48.10 ±0.36 a	44.88 ±0.36 a	44.43 ±0.29 a	42.05 ±0.02 a
2 g	40.06 ±0.75 a	42.90 ±0.38 a	42.90 ±0.38 b	51.69 ±0.45 b	43.35 ±0.10 a	44.67 ±0.43 a
4 g	40.06 ±0.75 a	48.08 ±0.56 a	48.08 ±0.56 a	46.77 ±0.37 a	45.21 ±0.41 a	44.94 ±0.50 a

[a]Values represent mean +/- standard error of the mean. Values sharing letters within, but not between, columns are not significantly different (P>0.05).

a*, b*, and L* values among breasts or thighs corresponding to the different amounts of mineral mixture. b* values increased with decreasing a* values during the 27 days of storage in the current study. Both chicken thigh and breast portions became more yellow and slightly green. In a previous study, however, decrease in a* and b* values were observed in chicken fillet during 24 days of storage (Kenawi, Abdel-Aal, & Abbas, 2007). With decreasing a* and b* values, chicken would turn dull grey or greenish grey.

3.3. Thiobarbituric acid reactive substances (TBARS)

TBARS values decreased throughout the entire 27 days of storage period in chicken breast and stayed under 0.5 mg malondialdehyde (MDA)/ kg regardless of the amount of mineral mixture used (Figure 6-2A). A decrease in TBARS values was also observed in chicken thighs, with the exception of thigh portions stored with 2 g of mineral mixture at day 27 (Figure 6-2B). A decrease in TBARS values has been observed in multiple long term poultry storage studies (Grau, Guardiola, Grimpa, Barroeta, & Codony, 2001; King, Uijttenboogaart, & Vries, 1995; Wen, Morrisey, Buckley, & Sheehy, 1996). Cortinas et al. (2005) suggested that TBARS measurement might not be a good indicator of lipid oxidation in extended storage situations since there tends to be a decrease in the amount of MDA available for the reaction with thiobarbituric acid. Moreover, MDA reacts with various other compounds and can form MDA dimers or trimers (Aubourg, 1993; Esterbauer, Schaur, & Zollner, 1991; Gutteridge, 1975). A decrease in TBAR values does not equate to a reduction in total volatile compounds (Ajuyah, Hardin, & Sim, 1993) or lipid hydroperoxides (Grau et al., 2001), both of which can contribute to sensory perception of rancidity. Even though there was a significant

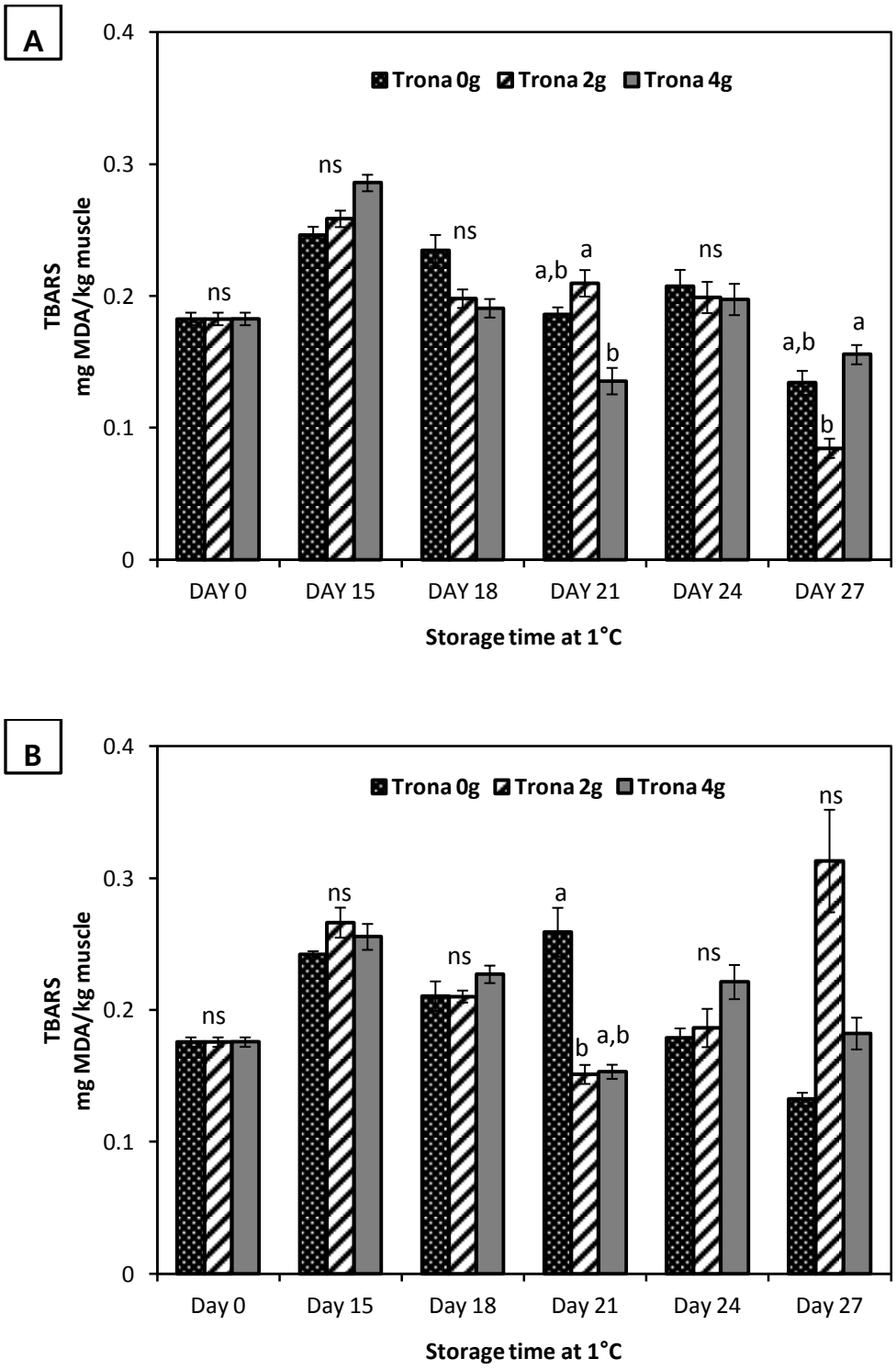

Figure 6-2. TBARS values of chicken breast (A) and thigh (B) at 1 ℃.
Values sharing letters within each time points are not significantly different (P>0.05). "ns" indicates no significant difference (P>0.05). Error bars: +/- standard error of the mean.

difference in TBARS among chicken breasts with different amounts of mineral mixture on day 27, the difference may not be important from a practical standpoint.

3.4. Total aerobic plate count (TAC)

An increase in TAC was observed in both chicken breast (Figure 6-3A) and thigh (Figure 6-3B) portions. A significantly higher aerobic count was observed in both chicken breast and thigh portions packaged without mineral packets (0 g) as compared to those with mineral packets (2 g and 4 g) on day 15. There was no significant difference in the TAC values of chicken between 2 g and 4 g of mineral mixture ($P>0.05$). Bacterial spoilage occurs when the number of bacteria reaches 10^7 logs of cfu/cm^2 (Dainty & Mackey, 1992). The counts for all storage conditions were above 7 log cfu/cm^2 on day 18. At this point, all samples were spoiled, making it difficult to observe any additional differences in TAC. Off odors were detected on day 15 for both chicken portions, and the odor became stronger after day 15. Off odor development of poultry is closely related to the level of microbial growth during storage (Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006). The reported levels of microbial populations to develop off odors in poultry vary. Off odor was detected in aerobic plate counts between 1.6×10^5 to 10×10^8 cfu/cm^2 (Elliott & Michener, 1961). Ayres et al. (1950) reported that ammoniacal odor was detected when microorganism population exceeded 1.0×10^8 cfu/cm^2. Thornley, Ingram, and Barnes (1960) also reported a putrid ammoniacal odor development from chicken carcasses when counts exceeded 1.0×10^8 cfu/cm^2 on chicken skin.

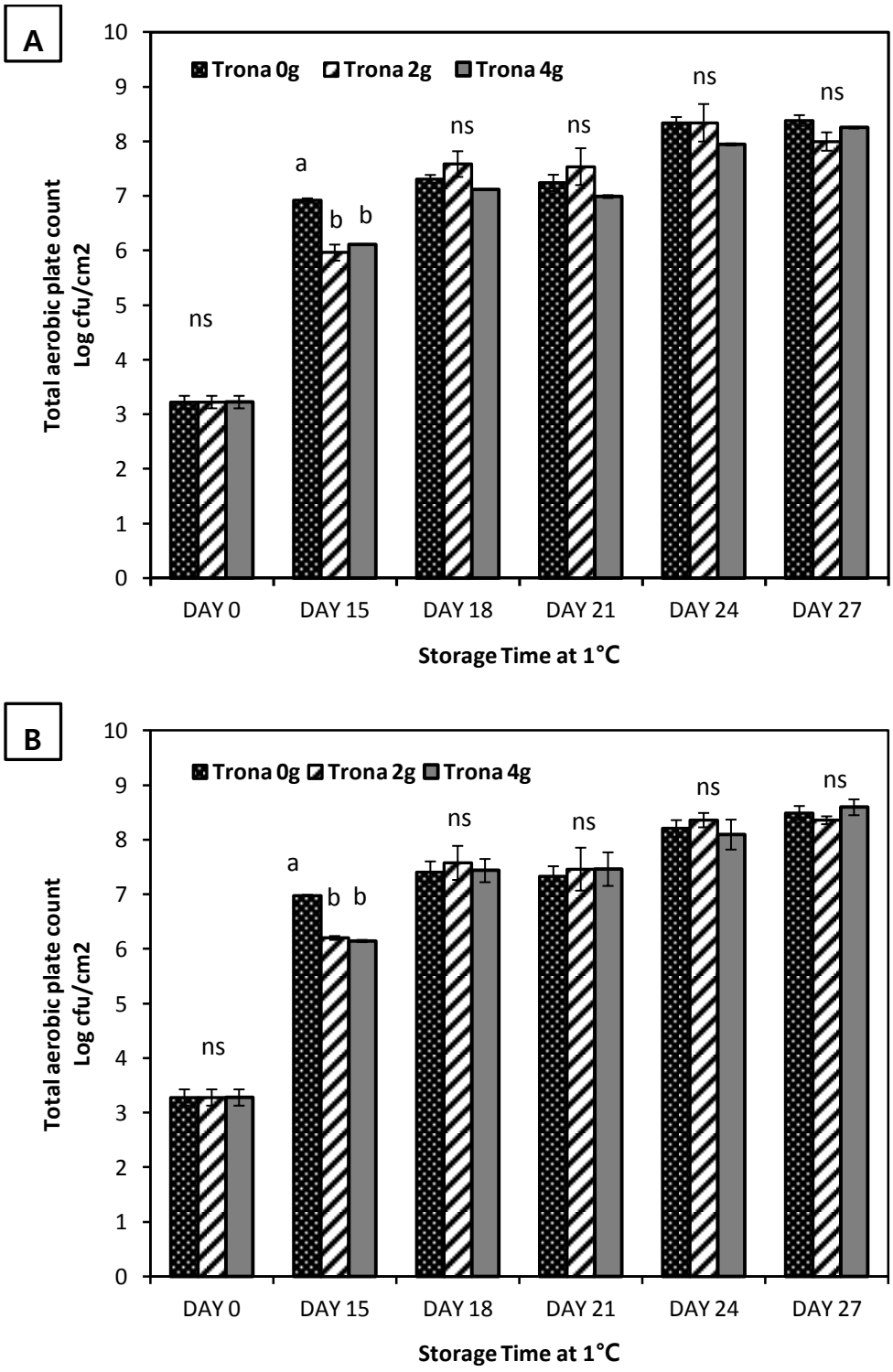

Figure 6-3. Total aerobic plate count of chicken breast (A) and thigh (B) at 1 ℃.
Values sharing letters within each time point are not significantly different (P>0.05).
”ns” indicates no significant difference (P>0.05).
Error bars: +/- standard error of the mean.

3.5. Texture Analysis

The effect of different amounts of mineral mixture on texture of chicken breast portions is shown in Table 6-3. There was no significant effect of mineral packets on firmness or adhesiveness. Additionally, there was no correlation between adhesiveness and firmness of chicken breasts (see Appendix E).

4. Conclusion

There was no significant effect of storage with mineral packets on color stability, lipid oxidation, pH or texture of raw chicken portions. Total aerobic plate counts were significantly lower on day 15 for samples stored with mineral packets, regardless of the amount used. Because this experiment was designed for extended storage life of chicken, the tests were not performed until later in the storage time (after 15 days of storage). The chicken portions had already begun to exhibit signs of spoilage at this point, so it was impossible to see the actual effect of CO_2 generated by the mineral packets after 15 days. Additional studies examining the effect of mineral packets on chicken breast and thigh portions would be useful to determine whether there is a limiting effect on microbial growth during the early stages of storage. Additionally, measuring the amount of CO_2 absorbed in meat may help to understand the effects of CO_2 generating mineral packets on microbial growth in chicken portions. Furthermore, tests to identify specific microbes,

Table 6-3. Pooled Means for firmness and adhesiveness of chicken breasts.

Trona	Firmness[a](mN)	Adhesiveness [a](mN sec)
0	4177.13 ± 199.60 a	322.82 ± 8.83 a
2	4312.93 ± 168.97 a	303.19 ± 8.92 a
4	3874.60 ± 110.49 a	318.47 ± 4.95 a

[a]Values represent mean +/- standard error of the mean pooled over time (15, 18, 21, 24, and 27 days).Values sharing letters are not significantly different (P>0.05).

such as lactic acid bacteria, *E.coli* or *Salmonella* ssp. may help optimize the use of CO_2 generating mineral packets in fresh chicken storage.

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CHAPTER 7

OVERALL SUMMARY

Processing and storage conditions influenced quality of oils and meats such as appearance and oxidative stability. Soybean oil (SBO) was more resistant to oxidation than interesterified soybean oil (IESBO). IESBO had higher initial peroxide values (PV) and showed a shorter induction period than SBO. This could be because of loss of antioxidants in IESBO through interestserification process. However, SBO had two times higher oxidation rate than IESBO after its induction period at room temperture. The higher content of polyunsaturated fatty acids (linoleic and linolenic) and linoleic and linolenic fatty acid at triacylglycerol sn-2 position in SBO might be the reason for the higher oxidation rate oxidation. High intensity ultrasound (HIU) did not increase oxidation in SBO and IESBO until the oils were already highly oxidized (PV>10mEq/kg). In this study, it was difficult to compare oxidative stability of IESBO or SBO due to low TBARS values (less than 0.1 mg MDA/kg) and fluctuations were observed throughout the entire storage period, regardless of HIU application, at both room temperature (25 ℃) and elevated temperature (40 ℃). Headspace volatile analysis can be useful to measure secondary oxidative products that are responsible for rancid flavors and odors. HIU improved the consistency of IESBO as reported in previous studies. Sonicated IESBO had a thick creamy consistency, while a separation of liquid and crystals was observed in non sonicated IESBO. SBO did not show any change on appearance regardless of HIU application.

Storage condition of meat had effects on lipid oxidation and other quality factors.

The CO_2 generating capacity of trona mineral and the feasibility of trona as a CO_2 producing product in modified atmosphere packaging (MAP) of steaks were evaluated in this study. Trona could generate carbon dioxide (CO_2) in the presence of moisture and acid and was able to generate more CO_2 than sodium bicarbonate. When the mixture (4 g) of trona mineral and salicylic acid was placed in soaker pad for CO_2 generation with a 113.4 g steak (no gas flushing) in a master bag (internal size: 17.8 cm x 17.8 cm), headspace CO_2 level exceeded 20%, the minimum level to have an antibacterial effect. On the other hand, headspace CO_2 level in low oxygen MAP (gas flushed 80% N_2/20% CO_2) and high oxygen MAP (gas flushed 80% O_2/20% CO_2) declined and stayed under 20%. However, steaks in high oxygen MAP had lower aerobic counts than those in trona packaging despite higher headspace CO_2 level of trona packaging. The steaks in trona packaging maintained red color through 12 days, but small discoloration (brown) spots started to appear on the surface of the steaks in trona MAP after 6 days. High residual oxygen level (>0.05%) accelerated metmyoglobin formation on the steaks in low oxygen MAP. High oxygen MAP

Mineral packets containing the mixture (66 g) of trona and acid were not able to generate CO_2 effectively without a source of moisture. Mineral packets placed in simulated self serve retail case and closed butcher case or home refrigerator did not have an effect on color, lipid oxidation, pH or microbial growth on ground beef hamburger patties when mineral packets were placed without water source. Providing a readily available source of moisture to mineral packets is a possible solution to improve the CO_2 generating capacity packets for retail and home storage applications.

There was no significant effect of storage with mineral packets (0, 2 and 4 g) on

color stability, lipid oxidation, pH or texture of raw chicken portions after extended storage. Total aerobic plate counts were significantly lower on day 15 for samples stored with mineral packets, regardless of the amount used. The aerobic counts for all storage condition exceeded 7 log cfu/cm^2 after day 15, indicating all chicken samples were spoiled. It was impossible to see the actual effect of CO_2 generated by the mineral packets after 15 days. Additional studies examining the effect of mineral packets on chicken breast and thigh portions would be useful to determine whether there is a limiting effect on microbial growth during the early stages of storage.

In summary, HIU is an effective method to improve consistency of semi solid oils, and it does not retard or improve oxidative stability of the oils. The effect of HIU in oils on oxidative stability can be dependent on the processing conditions such as HIU application period and power setting. Thus, further studies are required to determine the effect of different HIU application conditions on oxidative stability of oils. In meat packaging, mixture of trona and salicylic acid can be an effective source of CO_2 in packaging headspace. However, sufficient amount of moisture is required for CO_2 generation because CO_2 generating capacity of the mixture is dependent on moisture availability. When mineral packets are used in beef steak packaging without gas flushing or oxygen scavengers, small discoloration spots appear on the surface of steaks. To improve color stability of red meat, oxygen level has to be controlled. The amount of CO_2 absorbed in meat may help to understand the effects of CO_2 generating mineral packets on microbial growth since the amount of CO_2 absorbed in meat is a possible factor that is responsible for its microbial inhibitory effect.

APPENDICES

APPENDIX A

A PICTURE OF IESBO IN CHAPTER 3

Figure E1.Separation of liquid and crystals in non-sonicated IESBO stored at 25 ℃.

APPENDIX B

STATISTICS FOR CHAPTER 3

Table B1. Type 3 tests fixed effects for PV of oils at 25 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
Type	1	158	2657.27	<.0001
HIU	1	158	63.56	<.0001
Type*HIU	1	158	694.74	<.0001
Time	19	158	132848	<.0001
Type*Time	19	158	8482.70	<.0001
HIU*Time	19	158	119.94	<.0001
Type*HIU*Time	19	158	206.28	<.0001

Table B2. Type 3 tests fixed effects for PV of oils at 25 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	8	701.06	<.0001
Time	19	152	136847	<.0001
System*Time	57	152	3024.70	<.0001

Table B3. Type 3 tests fixed effects for PV of oils at 40 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
Type	1	54	38062.8	<.0001
HIU	1	54	210.44	<.0001
Type*HIU	1	54	1150.20	<.0001
Time	6	54	61653.3	<.0001
Type*Time	6	54	10254.6	<.0001
HIU*Time	6	54	108.50	<.0001
Type*HIU*Time	6	54	379.81	<.0001

Type 3 tests fixed effects for PV of oils at 40 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	8	8203.08	<.0001
Time	6	48	63891.5	<.0001
System*Time	18	48	3710.96	<.0001

Table B5. Type 3 tests fixed effects for TBARS of oils at 25 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
Type	1	6.43	37.05	0.0007
HIU	1	6.43	48.38	0.0003
Type*HIU	1	6.43	1.33	0.2896
Time	19	238	22.85	<.0001
Types*Time	19	238	32.68	<.0001
HIU*Time	19	238	5.77	<.0001
Types*HIU*Time	19	238	8.02	<.0001

Table B6. Type 3 tests fixed effects for TBARS of oils at 25 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	7.71	29.45	0.0001
Time	19	130	21.24	<.0001
System*Time	57	127	14.51	<.0001

Table B7. Type 3 tests fixed effects for TBARS of oils at 40 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
Type	1	5	1.16	0.3298
HIU	1	130	6.11	0.0148
Type*HIU	1	130	6.98	0.0093
Time	6	130	20.18	<.0001
Type*Time	6	130	17.18	<.0001
HIU*Time	6	130	67.14	<.0001
Type*HIU*Time	6	130	5.22	<.0001

Table B8. Type 3 tests fixed effects for TBARS of oils at 40 ℃.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	20	3.36	0.0391
Time	6	120	20.00	<.0001
System*Time	18	120	29.58	<.0001

APPENDIX C

STATISTICS FOR CHAPTER 4

Table C1. Type 3 tests fixed effects for CO_2 generation in bench experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	5	192	288.71	<.0001
Time	7	192	206.88	<.0001
Trt*Time	35	192	6.54	<.0001

Table C2. Type 3 tests of fixed effects for CO_2 generation in packaging experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	4	120	135.21	<.0001
Time	5	120	258.15	<.0001
Trt*time	20	120	5.73	<.0001

Table C3. Headspace CO_2 level in the bags containing trona mixture.

Replicates	% CO_2				
	Day 3	Day 6	Day 9	Day 12	Day 18
1	23	29.8	27.2	20.9	13.6
1	19.7	29.8	26.5	24.8	
1	18.2	28.2			
2	19.9	31.7	31.8	27.5	19.5
2	21.6	31.1	31.2	29.5	
2	18.9	28.9			
3	22.9	29.6	29.7	27.5	14.8
3	21.9	30.7	29.4	27.6	
3	24.7	32.2			
Mean	21.2	30.22222	29.3	25.8	15.96667
SD	2.158124	1.307457	2.110924	2.9	3.118226

Table C4. Type 3 tests of fixed effects for Hunter L* of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	96	0.21	0.8142
Time	3	96	4.86	0.0034
Trt*Time	6	96	1.49	0.1888

Table C5. Type 3 tests of fixed effects for Hunter a* of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	96	44.96	<.0001
Time	3	96	71.67	<.0001
Trt*Time	6	96	10.39	<.0001

Table C6. Type 3 tests of fixed effects for Hunter b* values of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	96	39.36	<.0001
Time	3	96	8.04	<.0001
Trt*Time	6	96	8.11	<.0001

Table C7. Type 3 tests of fixed effects for hue angle of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	96	29.22	0.0356
Time	3	96	94.99	<.0001
Trt*Time	6	96	6.29	0.0220

Table C8. Type 3 tests of fixed effects for chroma of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	96	54.21	<.0001
Time	3	96	35.97	<.0001
Trt*Time	6	96	11.88	0.0013

Table C9. Type 3 tests of fixed effects for TBARS of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	60	8.41	0.0006
Time	3	60	8.74	<.0001
Trt*Time	6	60	2.03	0.0759

Table C10. Type 3 tests of fixed effects for TAC of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	22	5.95	0.0086
Time	3	22	19.62	<.0001
Trt*Time	6	22	1.48	0.2325

Table C11. Type 3 tests of fixed effects for MFI of steaks in steak storage experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	132	1.12	0.3294
Time	3	132	42.06	<.0001
Trt*time	6	132	1.35	0.2398

APPENDIX D

STATISTICS FOR CHAPTER 5

Table D1. Type 3 tests of fixed effects for pH of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	30	0.26	0.8544
Time	3	30	2.97	0.0475
System*Time	9	30	0.13	0.9985

Table D2. Type 3 tests of fixed effects for Hunter a* value of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	176	0.90	0.4420
Time	3	176	86.46	<.0001
System*Time	9	176	0.34	0.9587

Table D3. Type 3 tests of fixed effects for Hunter b* value of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	176	0.98	0.4018
Time	3	176	19.24	<.0001
System*Time	9	176	0.89	0.5348

Table D4. Type 3 tests of fixed effects for Hunter L* value of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	176	0.08	0.9703
Time	3	176	3.04	0.0303
System*Time	9	176	1.13	0.3414

Table D5. Type 3 tests of fixed effects for hue angle of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	176	0.16	0.9227
Time	3	176	38.70	<.0001
System*Time	9	176	0.34	0.9605

Table D6. Type 3 tests of fixed effects for chroma of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	176	1.27	0.2858
Time	3	176	75.98	<.0001
System*Time	9	176	0.60	0.7928

Table D7. Type 3 tests of fixed effects for TBARS of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	112	0.08	0.9705
Time	3	112	16.22	<.0001
System*Time	9	112	0.04	1.0000

Table D8. Type 3 tests of fixed effects for TAC of ground beef at retail storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	84	0.41	0.7485
Time	2	84	9.64	<.0001
System*Time	6	84	0.10	0.9964

Table D9. Type 3 tests of fixed effects for pH of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	33	0.23	0.8717
Time	2	33	0.98	0.3855
System*Time	6	33	0.26	0.9529

Table D10. Type 3 tests of fixed effects for Hunter a* value of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	276	0.50	0.6830
Time	2	276	40.92	<.0001
System*Time	6	276	0.41	0.8701

Table D11. Type 3 tests of fixed effects for Hunter b* value of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	276	0.44	0.7242
Time	2	276	9.98	<.0001
System*Time	6	33276	0.41	0.8736

Table D12. Type 3 tests of fixed effects for Hunter L* value of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	276	1.15	0.3294
Time	2	276	1.682.00	0.1371
System*Time	6	276	0.891.06	0.3895

Table D13. Type 3 tests of fixed effects for hue angle of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	276	0.49	0.6879
Time	2	276	29.70	<.0001
System*Time	6	276	0.77	0.5944

Table D14. Type 3 tests of fixed effects for chroma of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	276	0.37	0.7715
Time	2	276	36.41	<.0001
System*Time	6	276	0.15	0.9887

Table D15. Type 3 tests of fixed effects for TBARS of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	179	0.05	0.9855
Time	2	179	0.87	0.4191
System*Time	6	179	0.05	0.9996

Table D16. Type 3 tests of fixed effects for TAC of ground beef at home storage.

Effect	Num DF	Den DF	F Value	Pr > F
System	3	84	0.41	0.7485
Time	2	84	9.64	<.0001
System*Time	6	84	0.10	0.9964

Table D17. Type 3 tests of fixed effects for drip loss.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	1	28	11.33	0.0022
Time	6	28	91.42	<.0001
Trona*Time	6	28	3.09	0.0189

APPENDIX E

STATISTICS FOR CHAPTER 6

Table E1. Type 3 tests of fixed effects (ANOVA) for pH of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	36	0.15	0.8622
Time	5	36	12.55	<.0001
Trt*Time	10	36	1.05	0.4265

Table E2. Type 3 tests of fixed effects (ANOVA) for pH of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	36	0.16	0.8541
Time	5	36	16.00	<.0001
Trt*Time	10	36	1.23	0.3081

Table E3. Type 3 tests of fixed effects (ANOVA) for Hunter a* of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	144	0.01	0.9941
Time	5	144	6.20	<.0001
Trt*Time	10	144	0.68	0.7455

Table E4. Type 3 tests of fixed effects (ANOVA) for Hunter a* of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	2	144	3.49	0.0331
Time	5	144	2.83	0.0181
Trona*Time	10	144	1.73	0.0796

Table E5. Type 3 tests of fixed effects (ANOVA) for Hunter L* of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	144	0.30	0.7445
Time	5	144	0.19	0.9665
Trt*Time	10	144	0.44	0.9219

Table E6. Type 3 tests of fixed effects (ANOVA) for Hunter L* of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	2	144	0.45	0.6380
Time	5	144	10.56	<.0001
Trona*Time	10	144	2.64	0.0056

Table E7. Type 3 tests of fixed effects (ANOVA) in for Hunter b* of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	2	144	1.65	0.1955
Time	5	144	2.92	0.0154
Trona*Time	10	144	1.35	0.2077

Table E8. Type 3 tests of fixed effects (ANOVA) in for Hunter b* of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	2	144	1.61	0.2043
Time	5	144	13.55	<.0001
Trona*Time	10	144	3.70	0.0002

Table E9. Type 3 tests of fixed effects (ANOVA) in for TBARS of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	90	0.35	0.7053
Time	5	90	13.92	<.0001
Trt*Time	10	90	1.62	0.1127

Table E10. Type 3 tests of fixed effects (ANOVA) in for TBARS of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	90	0.52	0.5947
Time	5	90	2.31	0.0507
Trt*Time	10	90	2.55	0.0093

Table E11. Type 3 tests of fixed effects (ANOVA) in for TAC of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	36	2.70	0.0811
Time	5	36	189.96	<.0001
Trt*Time	10	36	1.29	0.2732

Table E12. Type 3 tests of fixed effects (ANOVA) in for TAC of chicken thighs.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	36	0.48	0.6221
Time	5	36	259.23	<.0001
Trt*Time	10	36	1.16	0.3507

Table E13. Type 3 tests fixed effects for firmness of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trona	2	30	0.16	0.8538
Time	4	30	3.34	0.0224
Trona*Time	8	30	1.25	0.3033

Table E14. Type 3 tests fixed effects for adhesiveness of chicken breasts.

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	30	0.12	0.8877
Time	4	30	2.23	0.0898
Trt*Time	8	30	0.51	0.8391

Table E15. Correlation between adhesiveness and firmness of chicken breasts.

	Adhesiveness
Firmness	0.25650 p=0.0890