THE ANTIOXIDANT ACTIVITY OF THE EXTRACT OF PANGIUM EDULE REINW. (KELUAK) SEED IN COOKED GROUND TURKEY

BY

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THESIS
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Abstract

The effect of Keluak (*Pangium edule* Reinw.) seed extracts (K; 200 and 400 ppm) along with synthetic antioxidant (BHT, 200ppm) on the oxidative stability of cooked ground turkey stored at 2°C for 7 days for the first study and 15 days for the second study was examined. The Keluak seed was extracted with acetone: water: acetic acid (90: 9.5: 0.5, v/v/v) to obtain phenolic extracts and added to the meat system. The meat system included (ground turkey with 15% fat content, 2% salt (by weight) to induce lipid oxidation and 5% (by weight) water to dissolve the salt. Once the antioxidants were added to the meat, the raw mixtures were mixed by hand (wearing plastic gloves) for 5 minutes to ensure homogeneity. The raw mixtures were then formed into patties with diameter of 5.5 cm and thickness of 1.5 cm and cooked on an electric grill to reach internal temperature of 74°C. Meat patties were then overwrapped in commercial plastic wrap and placed over aluminum foil. The progress of lipid oxidation in the first study was assessed after 0, 1, 3, 5, and 7 days using thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and odor sensory analysis. A second study was completed and, TBARS, CD, and peroxide values (PV) were used to determine the extent of lipid oxidation after 0, 1, 3, 5, 7, 10, 13 and 15 days of refrigeration storage. Based on TBARS and CD values, both Keluak extract concentrations (K200 and K400) significantly reduced lipid oxidation and exhibited significant activity comparable to BHT at 200ppm.
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Chapter 1. INTRODUCTION
1.1 General Introduction

Consumer demand for more convenient foods has been increasing rapidly; this includes the demand for precooked meat products. Regardless of the major benefit of consuming poultry meat, cooked meat lipid oxidizes over time which can cause off flavor and odor termed warmed over flavor (WOF). Lipid hydroperoxides and their breakdown products are the major substances responsible for the deterioration and undesired flavor in cooked meat products during storage (1; 2). Therefore, suppression of lipid oxidation is essential to improve the quality and stability of precooked meat products.

Antioxidant addition has been an effective method to prevent oxidation in meat products. Several synthetic antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), tertiary-butylated hydroxyquinone (TBHQ) and propyl gallate have been reported to inhibit lipid oxidation(3; 4). The recent increase in the use of natural ingredients has challenged scientists to discover additional natural source antioxidants. The long-term safety and negative consumer perception of synthetic antioxidants have been questioned and consumers are increasingly demanding additive-free or natural sources. Antioxidants from several natural sources, including honey (1), rosemary extracts (4), and grape seed extracts (5) were reported to be effective. Recent studies on other natural extracts reported their antioxidant activity in meats, basil and galangal extracts (6), and cocoa powder (7) to name a few.

The fermented seed of *Pangium edule* Reinw. or Keluak possesses potential antioxidant activity (11). *Pangium edule* Reinw. is a tall tropical tree that grows primarily in Micronesia, including Indonesia. Keluak is commonly used as a spice for soup in some regions in Indonesia. Previous studies had shown that the Keluak extract has antioxidant activity. Fardiaz and Romlah (11) found that the addition of a Keluak seed extract to cooking oil lengthened the oxidation induction period of the oil. Andarwulan et al. (8; 9) reported that the total phenolic content in the seeds increased with an increase in the duration of
fermentation, although the antioxidant activity remained the same. Chye and Sim (10) reported that an acetone extract containing Keluak phenolics had substantial antioxidant activity as measured by both DPPH radical scavenging and β-carotene bleaching assays. Keluak seems to be a promising and novel natural antioxidant source, but additional investigations regarding its activity in food products are needed.

The increased consumer focus on natural food ingredients has led to the search for additional natural sources of antioxidant for reducing oxidation in food products. Several studies on the phenolic constituents in numerous plant sources have been conducted; Keluak could be another possible, economical and novel source of natural antioxidants. Even though several studies on the antioxidant activity of Keluak seeds have been conducted (3-7), its efficacy in meat products has not yet been investigated. It is important to explore the use of this plant extract in the meat industry, since traditionally it has been used as a spice flavoring in meat dishes in Southeast Asia. Therefore, the objective of this research was to determine the effectiveness of the phenolic extract of Keluak in cooked ground turkey patties during refrigerated storage.
1.2. References List


Chapter 2. REVIEW OF LITERATURE

2.1 Trend in Poultry Consumption

Consumption of poultry meat has increased in both developed and developing countries (25). In the US, per capita poultry meat consumption increased from 79 pounds in 1990 to 95.4 pounds in 2000, and to just over 100 pounds in 2011 (26). Overall, according to the National Chicken Council (6), per capita consumption of red meat and poultry has not changed significantly, but when beef, pork, and poultry are examined separately, red meat appears to be losing market share to poultry. Chicken and turkey were by far the most widely consumed poultry species at approximately 82.2 and 16.4 pounds per person per year, respectively (26). According to Bilgili (27), it is predicted that poultry will become the overall meat of choice by 2020.

This continued growth and the competitive nature of the poultry industry has been attributed to a variety of factors such as improvements in intensive production and processing; and development of a wide range of value-added, processed products that meet both direct consumer demand and the rapid expansion of fast food outlets. Poultry products are universally popular because they are often not subject to cultural or religious constraints and the meat itself is perceived as wholesome, healthy and nutritious, since it is relatively low in fat and it has a more desirable unsaturated fatty acid composition than other meats. Most importantly, high quality poultry products are available to many people at affordable prices, even though production costs vary widely around the world (25). Precooked meat products have attained a high degree of consumer acceptance because of the relative cost and ease of preparation (28).

Due to the increase in consumption, the world production of poultry has increased the past few decades. The industry will remain responsive to consumer demands, both in the range and nature of the products developed, although the focus on quality will continue. While the term “quality” is defined according to the consumer's own perceptions, goals and personal preferences, however, in practicality, product quality includes scientifically measurable characteristics of color, texture and flavor. One main factor limiting the quality and acceptability of
meat and meat products is lipid oxidation, a process that leads to deterioration of quality characteristics such as color, flavor, texture, nutritive value, and safety. Therefore, the meat industry faces a challenge in developing strategies for preventing lipid oxidation in meat and meat products.

2.2 Lipid Oxidation

2.2.1 Overview

A wide variety of organic molecules are susceptible to chemical attack by oxygen. Lipid oxidation can occur in foods containing considerable amounts of fat (particularly fats containing substantial percentages of unsaturated fatty acids), including nuts, milk and meat products. Oxidation of lipids is initiated by irradiation including exposure to visible light, by enzymes and metal catalysts. Heat and pressure will accelerate lipid oxidation. Quality attributes of foods can be substantially affected by this process. For instance, negative aroma changes in the food can result from volatile oxidation products, the color can darken as a result of condensation reactions between oxidation products and proteins, and lastly, the texture can be modified by the oxidative induction of protein crosslinks. Not unexpectedly, the nutritive value and safety can also be compromised.

2.2.2 Mechanism of Lipid Oxidation

Lipid autoxidation is a free radical chain reaction that once the peroxidation process is initiated, it proceeds as a free-radical mediated chain reaction that can be described in terms of initiation, propagation and termination processes.

2.2.2.1 Initiation

\[
\text{Initiator} \\
R : H \rightarrow R^· + H^·
\] (1)

An initiator reacts with unsaturated lipids (RH) to form a lipid free radical (R·) (reaction 1). Unsaturated lipids (RH) are easily oxidized by one of several reactive oxygen species (ROS), which include oxygen radicals and non-radical derivatives of oxygen such as \( \cdot \text{O}_2 \) and \( \cdot \text{OH} \). Poly-unsaturated fatty acids
(PUFA) are much more susceptible to oxidation than mono-unsaturated fatty acid (MUFA) (46).

The initiation reaction can include transition metals (i.e., iron, copper), oxidants, various hemolysis-prone substances or enzymes (1). This production of free radicals can occur because of the direct thermal dissociation (thermolysis) of hydroperoxides, because of transition metal catalysis or exposure to light (photolysis) with or without the intervention of photosensitizers (30). The spontaneous abstraction of a hydrogen atom from an organic material by molecular oxygen is an endothermic reaction which has a large activation energy and although it might occur to a certain extent, this stage is probably too slow to be of practical importance. Either the organic molecule or the oxygen should be activated before the reaction could take place (1).

2.2.2.2 Propagation

\[ \text{RO}^\cdot + \text{O}_2 \rightleftharpoons \text{ROO}^\cdot \] (2)

\[ \text{ROO}^\cdot + \text{RH} \rightarrow \text{ROOH} + \text{R}^\cdot \] (3)

An unsaturated lipid contains labile hydrogen(s) that can be abstracted relatively easily. This free radical (R·) will react rapidly with molecular oxygen to produce a peroxyl radical (ROO·) (reaction 2). The newly formed hydroperoxyl radical can so forth abstract a hydrogen atom from an adjacent unsaturated fatty acid (reaction 3) and the reaction goes on propagation cycles before termination (29).

2.2.2.3 Termination

\[ \text{ROO}^\cdot + \text{ROO}^\cdot \rightarrow \text{non-radical products [ROOOOR]} \] (4)

\[ \text{RO}^\cdot + \text{R}^\cdot \rightarrow \text{ROR} \]

\[ \text{R}^\cdot + \text{R}^\cdot \rightarrow \text{RR} \]

\[ \text{R}^\cdot + \text{ROO}^\cdot \rightarrow \text{ROOR} \]

Termination can occur by condensation of peroxy, alkoxy, and/or alkyl radicals. Peroxy radicals, at room temperature, can combine to produce peroxy-linked dimers (ROOR). Alkoxy and alkyl radicals, at low oxygen pressure and
high temperatures, can combine to produce ether-linked dimers (ROR) and carbon-carbon linked dimers (RR). Alkyl radicals can combine with peroxy radicals to generate peroxy-linked dimers (ROOR) (30-32).

Hydroperoxides, the primary initial products of lipid oxidation, can breakdown as soon as they are formed. The decomposition can be catalyzed by transition metals, particularly iron or copper or by elevated temperatures, >100°C. Hydroperoxide decomposition can involve several complex pathways, forming a wide variety of volatile and non-volatile secondary products, such as aldehydes, ketones, alcohols, acids and hydrocarbons which can affect flavor greatly. Some of the volatile aldehydes can be extremely potent and can affect flavor of food at concentrations less than 1 ppm (31). Another important termination reaction involves an antioxidant (AH) (30), which will be discussed later in the chapter.

2.3 Lipid Oxidation in Meat

Meat can oxidize very rapidly post-mortem. The rate and degree of lipid oxidation are affected by factors that include meat composition, fatty acid content, processing conditions, and chemical additives in the meat products (3). It is generally agreed that lipid oxidation in muscle foods begins immediately after slaughter in the highly unsaturated phospholipids fraction in the subcellular membranes (25). Botsoglou et al. (25) added that in most cases, fresh meat has an acceptable stability with respect to oxidation, but processing operations such as freezing and thawing, mechanical deboning, mincing, restructuring, the addition of salt, and refrigeration can lead to increased oxidation due to the destruction of cellular structure and functions.

Cooked or pressure-treated meat and meat products can be more susceptible to oxidation, since endogenous antioxidant enzymes may denature and lose their activities, while iron-containing proteins may become a source of catalytic iron.

Poultry meat is particularly susceptible to lipid oxidation due to its high content of polyunsaturated lipids, pigments, metal catalysts, and various other
oxidizing agents (25). This implies that even low-fat poultry meat products are susceptible to lipid oxidation since fat reduction primarily reflects a reduction in triacylglycerides while the phospholipid fraction, which is high in polyunsaturated fat, is much less affected (34). Another factor is that species differences in susceptibility to lipid oxidation are largely determined by the level of poly-unsaturated fatty acid (PUFA) present in meat. Thus, the extent of lipid oxidation in cooked meat is directly related to the level of unsaturated fatty lipids, with susceptibility to oxidation decreasing in the order of chicken > pork > beef > lamb (4). Chicken has fewer tendencies to develop oxidized flavor than turkey because the higher level of vitamin E in chicken fat retards oxidation. Oxidation proceeds faster in turkey thigh meat than in breast meat, as the darker meat contains more lipid and heme iron (17).

Hydroperoxides formed during lipid peroxidation undergo decomposition to form secondary oxidation products. Those secondary products include volatile aldehydes, ketones, acids, alcohols, and hydrocarbon compounds. Aldehydes (i.e., hexanal) are one of the primary causes of rancid and stale flavors in precooked meat products (3).

2.3.1 Factors Affecting the Extent of Lipid Oxidative Stability in Meat

There are factors that can affect lipid stability in meat and meat products such as fatty acid composition, endogenous prooxidative or antioxidative components, meat processing operations and various additives.

2.3.1.1 Fatty Acid Composition of Meat

It is generally agreed that unsaturated fatty acids (UFA) are responsible for lipid oxidation in meat. Also, as the concentration of polyunsaturated fatty acids (PUFA) increases, lipid oxidation increases in muscle foods (4). The amount of UFA in muscle foods varies depending on the animal species, genetic origin, diet, sex, age at which the animal is slaughtered, type of muscle, and processing factors such as fat addition to ground meat (5).

Turkey meat showed low values for total lipid and cholesterol, but greater values than beef, pork and lamb for its PUFA content. The content of n3
fatty acids was the greatest in turkey among meats that included beef, pork and lamb. The species is the major source of variation in fatty acid composition. The fatty acid composition of turkey meat is shown in Table 2.1 (50). Ruminant meats contain a greater percentage of saturated fat compared with the meat of monogastric animals (6).

2.3.1.2 Endogenous prooxidative and antioxidative constituents

Endogenous prooxidative constituents in meat systems consist of iron-containing proteins, transition metals, and endogenous enzymes. The catalytic effect of iron-containing protein on the oxidative deterioration of PUFA was first described by Robinson (35). Since then heme catalyzed lipid oxidation has been studied extensively, and it has become a generally accepted phenomenon that lipid oxidation is accelerated by heme compounds and transition metals in meat systems.

Lipoxygenase, peroxidase, and microsomal enzymes, identified in various animal tissues, catalyze the reaction of PUFA oxidation with the formation of unconjugated dienes (7). However, enzymatic lipid oxidation catalysts are normal components in blood and, consequently, are not a primary problem in most muscle foods (8).

2.3.1.3 Meat Processing

According to McMillin (33), quality of raw materials, time postmortem, extent of heating, and comminution or particle size reduction and the ingredients added are processing factors that can influence the rate of lipid oxidation. Even though lipid oxidation varies among different animal species, such species effects have been inconsistent, and may be due to differences in anatomical location, diet, environmental temperature, sex, age, phospholipid content, body composition, and experimental conditions such as cooking temperature, sample handling and method for assessing lipid oxidation.

Heating encourages the development of lipid oxidation by affecting the distribution of the iron in the muscle food (9). During cooking, a variety of volatiles producing off-odors and flavors are generated as PUFA oxidize (10).
Heat quickly decomposes hydroperoxides produced, resulting in the production of desirable flavor in cooked products. The shelf-life of meat products is usually prolonged by frozen storage. Under freezing conditions, the rate of oxidation can be reduced, but not completely prevented (1).

Lipid oxidation in cooked meat is commonly referred to as warmed-over-flavor (WOF). WOF often develops within 1-3 days of refrigerated storage and is characterized by the loss of fresh cooked meat aroma and a simultaneous increase in undesirable odors which are commonly described as “stale”, “wet cardboard”, “painty”, “grassy” or “rancid” (11). The most important contributors to the development of WOF are the secondary oxidative compounds such as hexanal, pentanal, and 2, 3- octanedione (12). According to Kerler and Grosch (59) identified (E, E)-2, 4-decadienal, butyric acid, and furaneol as key compounds in fresh boiled chicken aroma, while hexenal, butyric acid, and 1-octen-3-one were the main compounds in chicken reheated after 48 hours storage at 4°C.

The tendency of precooked meat products to oxidize has led food scientists, processors and the industries to develop techniques that allow extended shelf life of these products. In addition to antioxidants, the appropriate packaging and proper storage conditions are useful techniques to achieve extended storage times.

2.3.1.4 Non-meat Additives

Non-meat additives can be prooxidative or antioxidative agents. The addition of salt, NaCl, commonly used in meat products to add flavor and functionality at 0.5-2.5% (4) is a prooxidant. Salt has been shown to accelerate oxidation in pork patties (13) by enhancing the activity of iron atoms, which then promotes iron-catalyzed oxidation and flavor deterioration (1). Transitions metals, other than iron (copper, especially) supplied by the water, from processing equipment and/or spices can also promote oxidation (36).

The use of antioxidants, synthetic or natural, has been used to prevent lipid oxidation in meat products. The addition of nitrites, phosphates, citric acid, phytic acid, and EDTA has been reported to inhibit lipid oxidation in meat.
products (10, 14). In addition, synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), tertiary-butylated hydroquinone (TBHQ) and propyl gallate has been widely used for more than 50 years to inhibit lipid oxidation in meat products (12).

Several water-soluble compounds including ascorbic acid, uric acid, cysteine, and glutathione are also known to scavenge radicals and delay oxidation (37). In meat, the functions of ascorbic acid include maintenance of cured color and prevention of off-flavors (15). Ascorbic acid becomes a prooxidant in meat products at concentrations greater than 300 mg/kg (8).

In the last 30 years natural antioxidant use has increased. Various plant materials and extracts containing polyphenolic compounds have been proven to be effective antioxidants in meat systems. Rosemary and rosemary extracts at concentrations between 0.02 to 0.03% are one of the many natural antioxidants being studied (3, 16-18)

2.3.2 Measurement of rancidity in meat

Hydroperoxides, the primary products of lipid oxidation, are colorless, tasteless and odorless. It is the breakdown of these peroxides that produce a complex mixture of low molecular weight volatile compounds including alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids, many with distinctive odor and flavor characteristics (19; 38). These compounds convey rancid, cardboard, oxidized pungent and other off-flavors characteristic of oxidized meat (39).

Since oxidative decomposition products are of great importance to product acceptability and nutritional quality, a wide variety of assays from subjective analyses (i.e. sensory evaluation) to physical and chemical analyses of various components have been developed to detect and quantify lipid oxidation in foods. However, there is no single effective method to measure all the oxidative products that are generated during lipid oxidation. There is no good method useful for all stages of oxidation. An assay can only monitor only a few changes or specific reactants when applied to a food system under specific conditions. Therefore, a combination of assays would be required to monitor the extent of
lipid oxidation in most foods. It is also common to apply methods that are based on measuring one of more of the key primary or secondary oxidation products.

2.3.2.1 Peroxide Value

Since the primary product of lipid oxidation are hydroperoxides, it is proper to determine their concentration as one measure of oxidation. The peroxide value (PV) assay represents the total concentration of peroxides and hydroperoxides present at a certain time in the food product. However, this test is restricted by the chemical instability of these compounds; after their concentration reaches a maximum level, they decay as a function of temperature and the presence of other food components, particularly transition metals, such as iron and copper (1).

In muscle tissue, the lipid is extracted with a mixture of solvents without decomposition of the hydroperoxides. One of the most common extraction methods was developed by Folch, Lees, and Stanley (20). Several methods to determine the PV have been developed that include iodometric titration method, a ferric thiocyanate method, and other colorimetric methods (21; 40). PV can be expressed in several units, such as meq iodine/kg fat (40), or as meq of peroxide/kg fat (45).

2.3.2.2 Conjugated Dienes

Conjugated diene hydroperoxides (CDs) are one of the primary products of the oxidation for linoleate (18:2) and PUFAs with 3 or more double bonds. CDs can be determined quantitatively by their absorbance at 232-234 nm (22). In meat samples, the CD are first extracted from homogenized sample with an extracting solution, such as 3:1 hexane: isopropanol (23; 24)

The greater the amount of PUFA in a fat, the greater the potential rise in the CD values. However, the CD values plateau at a certain concentration, at which the breakdown roughly equals the formation of new CDs. At this point in the oxidation process, no additional changes can be measured by the CD assay (41).
2.3.2.3 Thiobarbituric Acid Reactive Substances (TBARS)

An alternative approach to the determination of the extent of oxidation is the measurement of selected secondary oxidation products. In contrast to the PV assay, those assays are not limited to the early stages of oxidation and may reflect the formation of volatile products, such as carbonyl compounds, which actually contribute to the rancid and objectionable flavor. The application of methods based on this approach requires a detailed knowledge of the chemistry involved and an understanding of the stability of the compounds assayed, etc. Among these methods, the thiobarbituric acid test (TBA) is one of the most common, in spite of criticisms of its reproducibility and reliability. The test is based on the color product resulting from the condensation of TBA with malonaldehyde which is presumably generated in the oxidized fats. However, a large body of evidence suggests that other food components can react with TBA to generate the same chromophore and even the formation of malonaldehyde is dependent on the composition of the initial lipid (1). For example, other products of lipid oxidation such as alka-2, 4-dienals, also react with TBA to form a red complex with the same absorption maximum as the malonaldehyde-TBA complex shown in the diagram below (42). For this reason, Gray suggested that the TBA procedure should be used to assess the extent of lipid oxidation in general, rather than to quantify malonaldehyde, and the term 'thiobarbituric acid-reactive substances' (TBARS) is now commonly used in place of the TBA number or value (10).

The extent of lipid oxidation is reported as the 'TBA number' or 'TBA value' and is expressed as milligrams of malonaldehyde equivalents per kilogram of sample. Malonaldehyde is a relatively minor lipid oxidation product formed
during the oxidation of polyunsaturated fatty acids, and reacts with TBA to produce a colored complex with an absorption maximum at 530-532 nm. Malonaldehyde has been reported to correlate well with sensory scores of oxidized flavors in muscle foods (38). This assay is not recommended for monitoring the extent of oxidation of food oils.

![Malonaldehyde and TBA reaction](http://mnh20.wordpress.com/2010/07/19/518/)

2.3.2.4 Sensory Evaluation

A wide range of methods have been used to examine the effects of lipid oxidation on the quality of stored turkey meat. In addition to the chemical or instrumental techniques, sensory evaluation is very useful for assessing the quality and stability of meat and meat products from lipid oxidation. Most of these analyses, such as the PV, TBARs, CDs or gas chromatographic analysis of specific volatiles can be correlated to sensory analysis, but none of these chemical or physical analyses can effectively replace the human nose, taste buds and sensory receptors (43).

According to Warner (43), sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to food and material characteristics as they are perceived by sense of sight, smell, taste, touch, and hearing. Although sensory evaluation can have a low reproducibility relative to gas chromatography, well-trained and experienced sensory panelists operating under controlled conditions can provide accurate, reliable data. The American Meat Science Association (44) has developed standard guidelines for sensory analyses. The AMSA recommendations include sample preparation, cooking methods and procedures, facilities, preparation and presentation of samples,
trained sensory panels, test forms, consumer panels, and instrumental measurement of tenderness.

2.4 Control of Oxidation: Antioxidants

Antioxidants are substances that can delay the onset of lipid oxidation and extend the induction period. They must be added as early as possible in the manufacturing process or to finished food products to be effective in reducing lipid oxidation. Antioxidants cannot reverse the oxidation of rancid food products. Moreover, antioxidants are not effective in controlling hydrolytic rancidity which is enzyme-catalyzed hydrolysis of fats. Antioxidants can control lipid peroxidation in six ways: reducing localized O\(_2\) concentrations, scavenging species that initiate peroxidation, quenching singlet O\(_2\) to prevent the formation of peroxides, binding free metal ions (chelating agent) into a form that will not generate reactive species or decompose lipid peroxides to peroxy or alkoxyl radicals, peroxide removal, and chain breaking to prevent more hydrogen abstraction from fatty acid side chains (46). There are three types of antioxidants based on their mechanism (46; 47):

2.4.1 Type I - Also called the primary or free-radical chain stopper antioxidants:

Type I antioxidants inhibit the chain reaction by acting as hydrogen donors or free-radical acceptors (AH) (46). Compounds in this category stop the free radical chain reactions by converting the radicals into more stable products (47) by reacting primarily with ROO• and not with R radicals. Basically, the mechanism is a competition between “the inhibitor reaction” (46):

\[
\text{ROO}^\cdot + \text{AH} \rightarrow \text{ROOH} + \text{A}^\cdot
\]

And “the chain propagation reactions”: 

\[
\text{ROO}^\cdot + \text{RH} \rightarrow \text{ROOH} + \text{R}^\cdot
\]

The effectiveness of a primary antioxidant is dependent on several factors such as activation energy, rate constants, oxidation-reduction potential, ease of antioxidant loss or destruction, and solubility properties (46). Labuza pointed out that another important characteristic of an efficient antioxidant is that the
resulting antioxidant free radical must not, itself, initiate new free radicals or be rapidly oxidized by a chain reaction (46). Type I antioxidants are primarily phenolic compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (47) and tert-butylhydroquinone (TBHQ) commonly present in foods. These synthetic food additives are permitted to be used alone or in combination. However, the antioxidant content of a food containing these additives must not exceed 0.02% of the oil or fat content of the food according to the Code of Federal Regulations (58).

Phenolic compounds, synthetic or natural, are excellent antioxidants since they possess benzene rings containing hydroxyl groups and are relatively reactive. Phenols produce stable antioxidant radicals A· that are sufficiently unreactive to propagate the chain and compete with the lipid substrate (RH), which is present at a much greater concentration (46):

\[
A^\cdot + ROO^\cdot \rightarrow \text{Non-radical products}
\]

\[
A^\cdot + A^\cdot \rightarrow \text{Non-radical products}
\]

Since synthetic antioxidants such as BHT and BHA have restrictions for use in food due to their toxicological effects on several species and they are suspected to be potential carcinogens (48) the search for natural and safe antioxidants, especially those of plant origin, has greatly increased in recent years.
2.4.2 Type II- Secondary antioxidants or Free-radical production preventer

This category includes compounds that reduce the rate of chain initiation by metal chelation, oxygen scavenging, or absorption of UV radiation (47). The common chelating agents include ethylenediamine tetraacetic acid (EDTA), citric acid and phosphates. EDTA is used for chelation of many metals and it can be an effective oxidation inhibitor in foods, especially those with intermediate to high moisture content, but not in protein-type food such as meats which contain substantial amounts of bound form of transition metals (46). In addition, EDTA does not protect food during heating because it is less stable at higher temperatures; therefore, it is restricted to foods held or processed at cool temperatures (49). Citric acid is especially useful in oils and intermediate moisture foods (46). Ascorbic acid is commonly used as a food antioxidant due to its oxygen scavenging and reducing properties (47); it is especially effective when it is used in combination with iron chelators (46). Polynphosphates are more effective chelators and antioxidants than monophosphates. However, their antioxidant activity could be reduced or eliminated depending on the components of the food (50).

Maillard reaction products can bind transition metals, such as iron and deactivate singlet oxygen. Because of their dark color and low solubility, they are unsuitable for use in fats and oils, but they may be used to improve the oxidative stability of many foods (51).

Other preventive antioxidants include several enzymes, such as catalase (47) and glutathione peroxidase (37), which reduce the generation of free radicals by decomposing hydrogen peroxide and hydroperoxides, respectively.

2.4.3 Type III- Control of Environment Factors

This category does not refer to compounds added to inhibit lipid oxidation. Rather, this category refers to factors, such as the reduction of the oxygen content, a decrease in temperature, altering the moisture content (46) and exclusion of light (47). As the temperature increases, oxygen becomes less soluble in lipids and water, slightly influencing the rate. The rate of oxidation increases proportionally to the surface of the lipid exposed to air. According to Labuza (46),
lipid oxidation can be reduced by altering the water activity which can alter the catalytic activity of metal catalysts. At an elevated water activity (aw > 0.55), the rate of oxidation can increase possibly due to the increased mobilization of metal catalysts.

Antioxidant synergism has been observed between primary antioxidants and between antioxidants with different modes of actions. This synergistic effect can substantially reduce the level of antioxidants needed in a food.

2.5 Keluak As A Natural Source of Antioxidant

2.5.1 Keluak – Introduction

_Pangium edule_ Reinw. (Figure 2.1) is a tall tropical tree that grows mainly in Micronesia, Melanesia, and Southeast Asia, including Indonesia. The taxonomy of the tree is uncertain and it may also be classified in the Flacourtiaceae or the Violaceae. According to Abdullah (55), the tree requires many years to mature and the seeds are therefore most frequently harvested from wild trees, as it is not economically feasible to cultivate the plant. The size of a Pangi tree is characterized as medium to large with large, glossy, heart-shaped leaves that are conspicuously veined and long-stemmed. The flowers are large and greenish and the sexes are separate. Flowering occurs from September to October with seed shed likely to occur in December to January. However, there are records of seed shed in April to May and July to September. This tropical tree is wild and not well cultivated (55).

The trees start fruiting when they are approximately 10 to 15 years old. The fruit is oval in shape and the size ranging from 12–30 cm (52) or about a size of a large husked coconut, brown and rough-suraced (55). The tree produces a large fruit that is poisonous because of the presence of cyanogenic glucosides (53). Almost all parts of the plant are poisonous including its leaves, barks and seed (52). Although poisonous to humans, the seeds of the tree are part of the natural diet of the babirusa (Babyroussa babyrussa) (53), a pig-like animal native to the region. However, the mature fruit is edible and the juice can be applied to sores and cuts (55).
In Indonesia, the seed kernel of this tree is edible after treatments following the removal of the cyanogenic glucosides. Keluak is fermented in a specific way; the seeds are harvested and placed in the field for 10 days. The seeds are then removed, washed, and boiled for 3 hours to remove the cyanide (54). After cooling, the seeds are buried (indoor) in ash, banana leaves, and earth for 40 days. The seed turn from a creamy white color to dark brown or black (51). The fermented seeds are cleaned and ready to be used as spices (54), image is shown in Figure 2.2. In some countries according to Ma (56) after harvesting the fruits are buried in the ground for ~100 days before the large seeds are removed; the rest of the plant is poisonous. The charcoal-colored nuts are braised for one hour so the soft flesh can be scooped out of the skin. The taste is bitter, buttery, and a bit like chocolate, while the texture is a familiar one - think mashed potatoes or roasted chestnuts (56).

**Taxonomy of Keluak (55)**

Kingdom : Plantae  
Division : Magnoliophyta  
Species : P.edule  
Family : Acariaceae  
Binomial name : *Pangium edule* Reinw, ex Blume  
Authority : Reinward  
Synonym names : football fruit, kluwek, Indonesia black nut, kepayang, kluwek / keluwek / kluwak, pangi, paying, picung, penarassan, seis.

According to Andarwulan et al., (54) the fermented seed product of this tree, depending upon the origin is called:

- **Indonesian:**
  - Keluak, kluwak, kluak, kluwek, keluwek or kloewak (Dutch spelling)  
  - Pucung or pucing (Sundanese)  
  - Rawan or rawon (adjective referring to food prepared with the seed of this tree) (Figure 2.3)

- **Malay:**
  - Kepayang
o Payang

- Philippines:
  o Samaun-referring to the oil
  o Pangi-referring to the tree

Keluak is commonly used as a spicy flavoring in soups in Java and South Sulawesi, several traditional dishes can be made with Keluak seeds, including “Rawon” (Figure 2.3) and “Black nut with golden snapper” (Figure 2.4). Keluak is also a raw material for another product, “kecap pangi” (“kecap” is a soy sauce-like product) and it has been used as a spice in Saparua, Maluku Island in Indonesia. Another of the edible products is called “Dage”, which is a boiled seed after the removal of kernels and water after soaking for 2-3 days. Dage is utilized in West java as a vegetable (54). In this document, I will use the term “Keluak” and “pangi to refer to the fermented seed that is commonly used as a spice.

2.5.2 Nutritional Profile of Keluak

The nutrition fact of the Keluak seed is shown in Table 2.2. Generally, phenolic compounds are found in the seeds and other parts of the plant. Most seeds contain large amounts of polyunsaturated oils and phenolic antioxidants are necessary to protect the polyunsaturated fatty acids in the oil against autoxidation (50).

The pangi seed contains some secondary metabolites (Table 2.3) of the cyanogen glycosides, which include the cyanogen glycoside, and various alkaloids, flavonoids, steroids, saponins, tannins, and quinine. These metabolites are present in both fresh and dried pangi seed (55).

2.5.3 Previous work on keluak

Previous research by Fardiaz and Romlah (57) on the fermented seed indicated that the methanol extract of keluak possessed antioxidant activity. Fardiaz and Romlah found that the addition of Keluak seed extract to cooking oil could lengthen the oxidation induction period of the oil. Their findings are summarized in the Table 2.4 and Figure 2.5
Recently, Chye and Sim (52) used several different extraction methods on Keluak seeds and examined the antioxidant activity of the extract. Extracts of *Pangium edule* Reinw. seed were investigated for their antioxidative activity using DPPH radical scavenging and β-carotene bleaching assays. For the extraction they used a mixture of acetone: acetic acid: water, which yielded the higher phenolic content of 22.22 ±0.05 mg GAE/g among the other methods of extraction and showed the most potent antioxidative activity in both DPPH radical scavenging and β-carotene (Figures 2.6- 2.7). They found that significant correlation was observed between the total phenolic content and its antioxidative activity (r=0.878) suggesting that the phenolics of the Keluak extract could be potential sources of natural antioxidants (52).
2.6 Figures

Figure 2.1. Pangium Plant

![Pangium Plant](http://www.pngplants.org/PNGtrees/TreeDescriptions/Pangium_edule_Reins.html)

Figure 2.2. Keluak Seed

Source: Wayang Brand Klwak Kupas, PT Wira Aksara Jakarta, Indonesia

Figure 2.3. A Dish Made With Keluak Seed– Black Beef Soup (Rawon)

![Black Beef Soup](http://www.lestariweb.com/resep-indonesia/English/Rawon.php)
Figure 2.4. A Dish made with Keluak Seed- Black Nut with Golden Snapper

http://delightfultastebuds.com/2011/05/01/rawon-indonesian-beef-black-soup/

Figure 2.5. The Effect of Kluwak Extract on Oxidation Induction Period of Palm Oil (57).
Figure 2.6. DPPH radical scavenging activity (%) of Keluak seed extracts at different concentrations (52).

Figure 2.7. Antioxidative Activity of Keluak Seed Extracts at 20 mg/mL measured by β-carotene bleaching method (52).

*Phenolic (Ace) = phenolic extracted by acetone; Phenolic (EtOAc) = Phenolic extracted by ethyl acetate; FPA = Free phenolic acid; GBPA = Glycoside-bound phenolic acid; Al = Alkaloid
2.7 Tables

Table 2.1 Fatty Acid Composition of Turkey Meat (50)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Wing (M ± SD)</th>
<th>Leg (M ± SD)</th>
<th>Breast (M ± SD)</th>
<th>Skin (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>1.5 ± 0.2 b</td>
<td>0.5 ± 0.1 c</td>
<td>6.9 ± 0.8 a</td>
<td></td>
</tr>
<tr>
<td>C16:1</td>
<td>6.5 ± 0.6 b</td>
<td>6.4 ± 0.1 b</td>
<td>15.5 ± 0.2 a</td>
<td>46.0 ± 2.0 a</td>
</tr>
<tr>
<td>C16:2</td>
<td>4.7 ± 0.4 a</td>
<td>4.5 ± 0.3 a</td>
<td>30.7 ± 0.6 a</td>
<td>209.3 ± 6.0 a</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.9 ± 0.9 b</td>
<td>10.4 ± 7.8 a</td>
<td>63.1 ± 6.9 a</td>
<td>395.5 ± 24.0 a</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.8 ± 0.1 a</td>
<td>0.5 ± 0.7 a</td>
<td>14.0 ± 3.8 a</td>
<td>150.5 ± 16.0 a</td>
</tr>
<tr>
<td>C22:0</td>
<td>1.3 ± 0.3 a</td>
<td>1.5 ± 0.6 a</td>
<td>20.0 ± 3.6 a</td>
<td>515.2 ± 16.1 a</td>
</tr>
</tbody>
</table>

Table 2.2 Nutrition Fact of Keluak Seed

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Per 100 grams (or 100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>2428 kj</td>
</tr>
<tr>
<td>Protein</td>
<td>15 g</td>
</tr>
<tr>
<td>Fat (Total)</td>
<td>50 g</td>
</tr>
<tr>
<td>Carbohydrate (Total)</td>
<td>17 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>4 g</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>10 g</td>
</tr>
<tr>
<td>Sodium</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
<td>680 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0</td>
</tr>
<tr>
<td>Iron</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: Wayang Brand Kluwak Kupas, PT Wira Aksara Jakarta, Indonesia
Table 2.3 Anti- Nutritional Factors of Pangium Seed (55)

<table>
<thead>
<tr>
<th>Anti- Nutritional Factors</th>
<th>Dry wt. Basis (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>N/A</td>
</tr>
<tr>
<td>Cyanide</td>
<td>1834</td>
</tr>
<tr>
<td>Lead</td>
<td>1.8</td>
</tr>
<tr>
<td>Phytic acid</td>
<td>N/A</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.46</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2.4 The Antioxidative Activity of Kluwak Expressed as Protective Factor (57)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protective factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fermentation time</td>
</tr>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Fermentation at the site of <em>Kluwak</em> producer</td>
<td></td>
</tr>
<tr>
<td>80 min. boiling time</td>
<td>1.36</td>
</tr>
<tr>
<td>40 min. boiling time</td>
<td>1.57</td>
</tr>
<tr>
<td>Fermentation at laboratory</td>
<td></td>
</tr>
<tr>
<td>80 min. boiling time</td>
<td>1.50</td>
</tr>
<tr>
<td>40 min. boiling time</td>
<td>1.75</td>
</tr>
</tbody>
</table>
2.8 References


35. Robinson, M. E. Haemoglobin and methaemoglobin as oxidative catalysts. Biochemical Journal. 1924, 18, 255-264


42. Coxon, D. Measurement of lipid oxidation. *Food Science and Technology Today*. 1987, 1164-6


CHAPTER 3. MATERIALS AND METHODS

3.1 Keluak Antioxidant and Chemicals

Keluak (Kupas Wayang Brand® Peeled Keluak Nuts, P.T. Wira Aksara, Jakarta, Indonesia, was manufactured for Empire International, East Chief Privado, Ontario, CA 91761). Butylated hydroxytoluene (BHT), gallic acid (ACS reagent), and 2-thiobarbituric acid were obtained from Sigma Chemical Company, St. Louis, MO, USA. Sodium chloride (crystal AR®, ACS) was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA).

The reagents used included acetone (Certified ACS) and chloroform (certified ACS) obtained from Fisher Scientific, Fair Lawn, NJ, USA. Acetic acid (Glacial, ACS reagent) was purchased from, J. T. Baker (Center Valley, PA 18034 USA) and Folin Ciocalteu’s phenol reagent (2 N) was purchased from Sigma-Aldrich (St. Louis, MO USA).

3.1.1 Phenolic Extraction

Keluak seeds were ground into fine pieces using a coffee grinder (Braun Inc., Lynnfield, MA, USA). The fatty material from the ground seed was removed first using a method modified from Wettasinghe and Shahidi (1). The ground seed was washed with n-hexane (1:6, g/g) and placed in a sonicator for 15 minutes with occasional stirring. The mixture was vortexed for 30 secs before it was centrifuged at 2688 x g for 10 minutes (Sorvall® Instrument RC-5B, Du Pont Company, Wilmington, DE, USA). The upper fat-soluble layer was discarded. The solid was washed 2 – 3 additional times. The solids remained at the bottom after washing. The defatted solids were then air-dried overnight under the hood to remove any solvent residue.

Forty grams of dried defatted Keluak seeds were extracted by stirring with 200 mL of solvent (acetone: water: acetic acid, 90: 9.5: 0.5, v/v/v) at 60°C and 600 rpm for 2 hrs. The mixture was then filtered through filter paper (Whatman #1, Buckinghamshire, UK). The filtrate was collected and dried at 45°C using a rotary evaporator until no further weight changes occurred. The concentrated extract was further dried using a vacuum oven at 45°C overnight. The extract was
collected, dissolved in 100% ethanol, flushed with Argon gas and stored at -20°C until further use.

3.1.2 Total Phenolics content

The total phenolic content was measured using the Folin-Ciocalteu assay as modified by Waterhouse (13). The phenolic extract was diluted with 100% ethanol to make several dilutions (1:100, 2:100 and 10:100). One milliliter of each dilution was added to a test tube containing 5 mL of deionized water. Then 0.5 mL of 50% Folin-Ciocalteu reagent was added to the test tube. The solution was mixed and allowed to stand for 8 mins at 22°C before adding 1 mL of 5% sodium carbonate (w/v). The mixture was vortexed using a touch mixer (model 231, Fisher Scientific, Pittsburgh, PA, USA) and incubated in the dark for 1 hr. The solution was vortexed a second time, before reading the absorption with a UV-spectrophotometer (Spectronic Genesys 5 spectrophotometer, Thermo Electron Corporation, Madison, WI, USA) at 725 nm against DI water. A standard curve was prepared at the same time with gallic acid at concentrations ranging from 0 to 100 mg/L. The quantity of total phenolic content in the sample was calculated as gallic acid equivalents (GAE) from the standard curve using the formula:

\[ C = c \times \left( \frac{V}{m} \right) \]

where:
- \( C \) is total phenolic content (mg GAE/g extract)
- \( c \) is the concentration of gallic acid obtained from the calibration curve (mg/mL)
- \( V \) is volume of sample (mL)
- \( m \) is mass of the sample extract (g)

3.2 Sample Preparation

Ground turkey meat (15% fat, Jennie-O Turkey Store, Wilmar, MN, USA) purchased from a local grocery store at day 0 and was immediately divided into 4 treatments according to the formulation (Table 3.1) (2, 3):

1. Control- No antioxidant
2. Commercial antioxidant- 0.02% BHT (w/w of lipid)
3. Natural antioxidant- 0.02% Keluak extract (w/w of lipid)
(4) Natural antioxidant – 0.04% Keluak extract (w/w of lipid)

The level of Keluak extract antioxidant used was based on the amount of fat in each formula. Salt was dissolved in water prior to addition to the meat. The water addition was 5% of the ground meat (w/w). The antioxidant and salt were mixed in the liquid first before adding it to the meat by sprinkling while mixing. After the antioxidant, salt and liquid addition, mixing by hand (wearing gloves) was continued for 5 mins. The meat mixture was prepared into patties of uniform size (using a cookie cutter, diameter = 5.5 cm, thickness = 1.5 cm). The patties were cooked in an electrical skillet and temperature was monitored by DigiSense® Scanning Thermometer (Model 92000-00, Cole Parmer Instrument Co., Barrington, IL) using copper-constantin thermocouple wires (Type T, Omega Engineering Inc., Stamford, CT) to an endpoint temperature of 74°C. The cooked patties were cooled and placed in a single layer on a tray and then overwrapped in commercial PVC (water vapor transmission rate = 15 g/100 m2/24 h; oxygen transmission rate = 880 cm³/m2/24 h) and aluminum foil over the wrap. The patties were stored at 2°C in the dark and were assayed for the extent of oxidation by odor sensory, TBARS, conjugated dienes, and peroxide value analysis at days 0, 1, 3, 5, 7 for the first study (2).

In a second, separate study, a longer storage time study was completed to examine the effect of antioxidants on the cooked ground turkey over a longer time period. The same procedure for antioxidant addition and meat sample preparation was used. However, in the second study, the samples were stored for a total of 15 days (5; 6). Samples were collected at days 0, 1, 3, 5, 7, 10, 13, 15 for TBARS, conjugated dienes, peroxide values, and pH determinations.

3.3 Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Tarladgis and others (4) and Yu et al. (5). Briefly, 10 g of meat was homogenized with 50 mL of deionized water using a homogenizer (IKA® T25 digital Ultra-Turrax, Wilmington, NC, USA). The homogenate was filtered through #1 Whatman paper. Five mL of the clear filtrate was mixed with 5 mL TBA reagent,
containing 0.02 M TBA in 90% glacial acetic acid. The mixture was capped, vortexed for 30 secs and incubated in the dark for 15-18 hrs at room temperature. After the color developed, the mixture was measured for absorbance at 532 nm against a blank (deionized water). The absorbance was converted to TBAR values using 1,1,3,3- tetraethoxypropane to prepare a standard curve. Triplicates of 10 g samples were prepared for each treatment. TBA value is expressed as mg MDA/kg meat.

3.4 Conjugated Dienes

Conjugated dienes (CD) were determined by the method described by Juntachote and others (6) with slight modifications. A cooked meat sample (0.5 g) was suspended in 5 mL of deionized water and homogenized using a digital Ultra-Turrax T25 (IKA®, Wilmington, NC, USA) to obtain a smooth slurry. A 0.5 mL aliquot of this suspension was mixed with 5 mL of extracting solution containing hexane and isopropanol (3:1, v/v) and mixed for 1 min using a vortex mixer. After centrifugation at 2000 g for 5 mins, the supernatant was collected and the absorbance of the supernatant was determined at 233 nm. The concentration of conjugated dienes was calculated using a molar extinction coefficient of 25,200 M⁻¹cm⁻¹ and the result were expressed as nmole of conjugated dienes per mg meat sample.

3.5 Peroxide Value

The peroxide value was determined according to the AOAC method 965.33 (Association of Official Analytical Chemists) (13) with a few modifications. The lipids from meat samples were extracted by the method of Kim et al. (7). First, 5 g of meat sample was broken into small pieces and placed in a water bath at 60°C to melt the fat for 30 mins. The meat was extracted with a solution containing acetic acid: chloroform (3:2) and then filtered with Whatman #1 filter paper. A saturated solution of potassium iodide (KI, 0.5 mL) was added to the filtrate. The solution color changed to yellow and then 30 mL of deionized water was added. The mixture was allowed to let stand at room temperature for at least 5 mins, and the color would turn to pale to bright yellow. Before titrating with 0.01 N of
sodium thiosulfate (Na$_2$SO$_3$), 0.5 mL of 1% starch solution was added (a color changed occurred - the color turned purple to blue-black). The PV is expressed as meq O$_2$/kg lipid.

3.6 pH value

To determine the pH of the meat samples, cooked meat samples (10 g) were blended with 50 mL deionized water for 15 seconds in a homogenizer (Ultra-Turrax T25, IKA®, Wilmington, NC, USA). The pH was measured with a pH meter (Accumet Basic model AB 15, Fisher Scientific Company). The pH meter was calibrated daily with standard buffers of pH 4.0 and 7.0 at 25°C.

3.7 Sensory Evaluation

Trained panelists (n=15) were recruited from among University of Illinois graduate students and staff. The panelists were prepared using an extensive training program. During each training session, panel members practiced and discussed the techniques developed for sample presentation, score sheet terms, and odor sensory evaluation method. A panel leader led the panelists to develop terms to describe the aroma characteristics of fresh cooked turkey meat including the aroma characteristics of cooked poultry with or without the added Keluak extract. Panel members were trained regularly for 7 sessions for total of two weeks to identify and discriminate aroma differences. Some of the terms were derived from reference standards including fresh boiled ground turkey meat, lard, oxidized vegetable oil, hard-boiled egg, and wet cardboard (Table 3.2). The panelists agreed on the following aroma descriptors for warm-over-flavor: meaty, fatty, rancid, eggy, and cardboardy. These descriptors were judged for the presence and overall intensity using a 15 cm-structured line scale (0 = none, 15 = extreme) along with an anchor value for each respective standard. Table 2 showed the form used to evaluate the aroma descriptors described above.

Cooked patties were uniformly cut into four pie-shaped wedges and placed in a sniff bottle (Nalgene Teflon PTFE wash bottle, Nalge Company, Rochester, NY, USA) covered with foil to hide the identity of the sample inside. Each sample represented a treatment labeled with three-digit random numbers and were
served at random order during each test session. The samples were held in an incubator (45°C ± 1°C) for 30 mins prior to presentation to the panelists. The panelists were asked to clean their palate by chewing a piece of unsalted saltine crackers and rinse with water prior to evaluating the samples. Samples were sniffed. Four samples were evaluated for each test session. Roasted coffee beans were presented for panelists to clear the palate between samples. Standard references were prepared daily. The second session, replicates of the first session, was conducted one week after the first session.

3.8 Statistical Analysis

Statistical assessment was carried out with the program SAS. Data were analyzed as 4 (treatments) by 5 (days of storage). The results from the TBARs analysis, peroxide value analysis, conjugated dienes analysis and the odor/sensory analysis were analyzed using one-way analysis of variance (ANOVA). Differences were considered significant at the $P < 0.05$ level. Comparison of treatment means was based on Fisher’s Least Significant Difference (LSD).
### 3.9 Tables

#### Table 3.1 Formulation of Cooked Ground Turkey Meat for Each Treatment

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>Control</th>
<th>BHT</th>
<th>AO @ 200ppm</th>
<th>AO @400ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenny-O 15% fat Ground Turkey (g)</td>
<td>650</td>
<td>650</td>
<td>650</td>
<td>650</td>
</tr>
<tr>
<td>Salt (2% w/w) (g)</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>BHT (0.02% of total fat) (g)</td>
<td></td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keluak extract (mL)</td>
<td></td>
<td></td>
<td>24.87 (0.02 g phenolics)</td>
<td>49.74 (0.04 g phenolics)</td>
</tr>
<tr>
<td>Cold water (mL)*5% by sample wt (w/w)</td>
<td>32.5</td>
<td>32.5</td>
<td>32.5</td>
<td>32.5</td>
</tr>
</tbody>
</table>

#### Table 3.2 Descriptive Odor Standards for Refrigerated Ground Turkey Meat

<table>
<thead>
<tr>
<th>Descriptive Odor term</th>
<th>Standard</th>
<th>Location on the 15-cm line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rancid</td>
<td>Oxidized vegetable oil in water (1:100, v,v)</td>
<td>7</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1 cm² cardboard in 15 mL water</td>
<td>8.5</td>
</tr>
<tr>
<td>Eggy</td>
<td>Fresh hard-boiled egg white (0.1 g)</td>
<td>9</td>
</tr>
<tr>
<td>Meaty</td>
<td>Fresh boiled ground turkey meat in water (1:5, g/v)</td>
<td>8</td>
</tr>
<tr>
<td>Fatty</td>
<td>Lard (0.2 g)</td>
<td>9</td>
</tr>
</tbody>
</table>
3.10 References


Chapter 4. RESULTS AND DISCUSSION

4.1 Phenolic Extraction Yield

The phenolic extract yield using acetone: water: acetic acid (90: 9.5: 0.5) extraction was 5.03 ± 1.40 g of extract per 70 g of defatted ground Keluak seed or 0.07 g phenolic extract per gram of defatted Keluak. The samples contained approximately 7.19% phenolic compounds by weight; the values are equal to the average ± standard deviation of the three replicate analyses. This value was moderately greater than that reported by Chye and Sim (1) which reported a 5.48% yield. The total phenolic content, as determined using Folin-Ciocalteau reagent, was 19.73 ± 2.03 mg GAE/g extract, which was also moderately greater than the result reported by Chye and Sim, which was 16.39± 0.04 mg GAE/g extract (1). It is possible that the difference is due to the Keluak source. Chye and Sim obtained their seeds material from local indigenous markets in Kota Kinabalu, Sabah, Malaysia (1), while the seeds used in this study were grown in from Indonesia. The geographical and horticultural differences could affect the phenolic content and concentration of the seed.

According to Kahkonen et al. (2), different phenolic compounds can have different responses in the Folin Ciocalteau method depending on their chemical structure. Therefore, the antioxidant activity of an extract cannot be predicted solely on its total phenolic content (3; 4).

4.2 Effect of Keluak Extract and storage time on TBARS values during refrigerated storage of cooked ground turkey.

The TBARS values represent the content of secondary lipid oxidation products, particularly malonaldehyde. The carbonyls are primarily responsible for the production of off-flavors in oxidized meat and meat products. Aldehydes are formed by the oxidation of unsaturated fatty acids. They react with the TBA reagent to form a pink complex (TBA chromogen) with a maximum absorbance at 532 nm. The intensity of this pink color is directly related to the concentration of the TBA-reactive substances in the original sample. The results of the TBARs analysis for the preliminary study (7 days) and the extended storage study (15 days) are shown in Tables 4.1a, b and Figures 4.1a, b, respectively. Overall, lipid
oxidation increased substantially as the refrigerated storage progressed, due to the effects of heating and in combination with storage under aerobic conditions.

On the first day of analysis for both the 7 and 15 days storage study, all the antioxidant treated samples (BHT and both concentrations of the Keluak extracts) the TBARs values were significantly lower than the control, an indication that the antioxidants had a positive effect in suppressing oxidation. Similar findings were observed by Juntachote et al. and Teets et al. (5; 6). TBARS values for both 7 and 15 days storage study ranges from 0.35- 4.75 mg MDA/ kg meat which was comparable to other study by Juntachote (5) for 14 days of storage study of pork meat. There were examining the effect other natural sources of antioxidants on oxidation during refrigerated storage of cooked meat.

During the preliminary study (Table 4.1a), the control and BHT treatments did not show significant differences ($P>0.05$) from day 1-7. However, both of the Keluak extract treatments showed a significant ($P<0.05$) effect on the suppression of the progression of lipid oxidation as compared to the control (Figure 4.1a).

On the other hand, during the 15 day study, all the samples with added antioxidants had significantly lower TBAR values, compared to the control, throughout the entire storage period. This indicates that both the commercial treatment and the treatment with Keluak extracts provided substantial protection to the meat with respect to lipid oxidation. The efficacy of the Keluak extracts to inhibit lipid oxidation is probably related to their phenolic content. The results of this study showed agreement with those obtained by Fardiaz and Romlah (14) which stated that the addition of Keluak extract to palm oil lengthened the oxidation induction period from 22 hours to 50 hours.

Lipid oxidation was considerably greater with longer storage times due to the exposure to oxygen. TBARs measure the formation of secondary lipid oxidation products which contribute to WOF in oxidized meat. Heat increases the release of non-heme iron from the heme pigments, a major catalyst for lipid oxidation in cooked meats (7). Cooking also alters cellular compartmentalization and exposes membrane phospholipids to molecular oxygen and metal ions (8)
resulting in greater amounts of lipid oxidation (9). The addition of antioxidants during processing, cooking or packaging can be done to increase the oxidative stability of cooked meat (10).

4.3 Effect of Keluak Extract and Storage Time on Conjugated Diene Content during Refrigerated Storage of Cooked Ground Turkey Meat.

The development of conjugated dienes (CD) is related to the formation of hydroperoxides found in the extracted lipids of the cooked ground turkey. Unsaturated fatty acid double bonds occur naturally in a methylene interrupted structure. As meat begins to oxidize, the double bonds of these fatty acids are altered creating conjugated systems, which can be measured spectrophotometrically (6). During lipid peroxidation, non-conjugated double bonds (C=C–C=C=C), which are present naturally in unsaturated lipids, are converted to conjugated double bonds (C=C–C=C) with a large increased in absorption at 232–234 nm (11).

In the preliminary study there were no significant differences among the antioxidant treated sample and the control ($P>0.05$) except on day 5 (Figure 4.2a and Table 4.2a). The CD value for all the treatments peaked at day 5 and the samples treated with antioxidants were significantly different that the control ($P<0.05$).

Meanwhile, during the second storage study (Table 4.2b and Figure 4.2b) when the CD values were compared to the control, on day 0 and day 1 the 0.02% BHT and both of the Keluak extract treated samples were not significantly different ($P>0.05$). However, from day 3 on, all the antioxidant-treated samples had less CD formation than the control ($P<0.05$). The CD content of the control generally increased especially during the last 5 days (Day 10, 13, and 15) of the study. Additionally, cooked ground turkey treated with Keluak at 400 ppm had slightly lower concentrations of conjugated dienes compared to cooked ground turkey containing Keluak extract at 200 ppm and the commercial antioxidant (BHT). The trend of CD formation was similar to several other studies (5; 6; 12; 13). They generally observed that the CD concentration for most samples peaked
on Day 3 and decreased thereafter. This phenomenon of decreased CD is likely due to the decomposition of the conjugated diene hydroperoxides into secondary oxidation products over time. Conjugated dienes hydroperoxides are expected to decompose into secondary products, as was observed in this study. According to Frankel (15), the formation of conjugated dienes, which is related to the production of hydroperoxides, occurs in the early stages of lipid oxidation.

According to Estevez et al. (17), measurement of conjugated dienes (CD) in meat products containing elevated levels of PUFA is limited depending on the fatty acid composition of the sample as well as the presence of other non-hydroperoxides CDs. In agreement with Grau et al (18), the TBA test showed a higher sensitivity with respect to lipid oxidation than did the CD determination.

### 4.4 Effect of Keluak extract and storage time on peroxide value during refrigerated storage of cooked ground turkey

Peroxide values (PV) were also used to assess the level of lipid oxidation in cooked ground turkey during storage at 2°C (Table 4.3 and Figure 4.3). However, while there were differences between the control and samples treated with AOs on a few days, the result of this study showed that treatments had no significant effect ($P > 0.05$) on the peroxide values compared to the control. Generally, based on the results in Table 4 and Figure 4, the PVs increased during storage and peaked on day 10. At the time, it is possible that much of the polyunsaturated membrane lipids may have been oxidized at that point, so that further storage and oxidation resulted in a decrease in the concentration of peroxides and thus the PV.

The PV differences among the samples did not provide proof that any of the AOs were effective. It is not clear why the PV analysis did not provide any evidence of AO efficacy. According to Estevez (17), the sensitivity of this method is about 0.5 miliEquivalent/kg of lipid. Lower peroxide values cannot be measured satisfactorily by the AOCS method because of the uncertainty with the iodometric titration endpoint, unless the iodine-starch end is determined potentiometrically at levels as low as 0.06 Eq/kg lipid. The iodometric method is highly empirical and very small procedural differences can cause variation in the
results (19). Two major sources of error in this test could be the liberation of iodine by air oxidation of the KI and absorption of iodine by fatty acid double bonds (17).

4.5 Effect of Keluak Extract and storage time on Odor of Refrigerated Cooked Ground Turkey

No significant interaction ($P > 0.05$) occurred between antioxidant and storage time for most of the odor characteristics (Tables and Figures 4.4-4.8). For meaty odor characteristic (Figure and Table 4.4), the score of all the treatments over the 7 days of storage did not show significant differences ($P > 0.05$). The scores ranged from 5.02 – 7.83 which was slightly below the freshly boiled turkey meat standard for meaty (which was ranked 8 in the 15-cm line scale). Therefore, the panelists were unable to score and detect the cooked meat aroma differences in the treated samples relative to the standard reference. The meaty aroma in this study was used to determine if there were possible masking effects from the AO treatments.

For the rancid odor (Figure and Table 4.5), both of the Keluak treated samples were perceived as more rancid than both control and BHT after day 1. Overall, both of the Keluak treated samples were ranked higher than the control and BHT which disagrees with the results of TBA test. However, there was no significant difference among the treatments between day 3-7. According to Ruth (20), rancidity, as a measure of lipid oxidation, is usually best measured by the concentration of TBARS.

The minimum TBA values that corresponds to dark chicken meat samples that were significantly different in rancid aroma was 1,933 μg MDA/kg of meat (21). For consumer acceptability the TBA values must be less than 785 μg of MDA/kg (21). The results from Bou et al. (21) were comparable to the results reported by Gray and Pearson (22) showing that rancid flavor in meats is initially detected between 500 and 2,000 μg of MDA/kg of meat. In addition, O’Neill et al. (23) found that the threshold for WOF detection in cooked, dark chicken meat was at TBA values ≥800 μg of MDA/kg of meat. Since chicken and turkey meat
have a comparable PUFA content, those values were considered useful in this experiment.

On the other hand, the odor score for Eggy, cardboard and fatty were statistically not different ($P > 0.05$). Panelists were unable to detect differences which could be to different reasons such as that Keluak extract has an aroma masking effect that confuses the panelists with the “off-odor”. The Keluak did have a slight aroma and it is likely that it masked the effects of storage on lipid oxidation. The aroma of Keluak has been described as bitter, dirt-like and chocolate-like odor (16). This aroma masking could be perceived as undesirable by the panelists and it might have been mistaken as an off-odor or as an oxidized sample, even though the TBARs and CD data showed otherwise.

4.6 Effect of Keluak Extract and storage time on pH of Refrigerated Cooked Ground Turkey

Table 4.9 shows the effect of antioxidant treatments in pork muscle on pH values during storage at 2°C. The values of the pH ranged between 6.33-6.68 Generally, there are no correlations of pH value among the treatments during storage. The trend for the pH value is inconclusive.
4.7 Figures

Figure 4.1a. The profile of TBARS in cooked ground turkey treated with various antioxidants during storage at 2°C - A Preliminary Study

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively**
Figure 4.1b The profile of TBARS in Cooked Ground Turkey Treated with Various Antioxidants During Storage at 2°C - Extended Storage Time.

* K200 and K400 represent Keluak extract at concentration of 200 and 400 ppm, respectively.

Figure 4.2a. Conjugated dienes values of different antioxidant treatments over storage at 2°C - A Preliminary Study

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively
Figure 4.2b. Conjugated dienes values of different antioxidant treatments over storage at 2°C- Extended Storage Time

![Graph showing conjugated dienes values](image)

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively.**

Figure 4.3. Peroxide Value (meq/ kg meat) of Different Antioxidant treatments over Storage at 2°C.

![Graph showing peroxide values](image)

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively.**
Figure 4.4: Intensity of Meaty Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

**K200 and K400 represent Treatment with Keluak extract at concentration of 200 and 400 ppm, respectively.**

Figure 4.5: Intensity of Rancid Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.**
Figure 4.6 Intensity of Eggy Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.**

Figure 4.7 Intensity of Cardboard Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.**
Figure 4.8 Intensity of Fatty Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.**
### 4.8 Tables

**Table 4.1a.** TBARS Value of Cooked Ground Turkey Meat Treated with Various Antioxidants during Storage at 2° C- A Preliminary Study

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>Keluak 200ppm</th>
<th>Keluak 400ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.31±0.09(^{A,a})</td>
<td>0.94±0.21(^{B,a})</td>
<td>0.50±0.04(^{C,a})</td>
<td>0.37±0.10(^{C,a})</td>
</tr>
<tr>
<td>1</td>
<td>1.89±0.03(^{A,ab})</td>
<td>1.61±0.25(^{A,ab})</td>
<td>0.80±0.06(^{B,a})</td>
<td>0.57±0.26(^{B,ab})</td>
</tr>
<tr>
<td>3</td>
<td>2.43±0.10(^{A,c})</td>
<td>2.36±0.25(^{A,bc})</td>
<td>1.11±0.22(^{B,ab})</td>
<td>0.55±0.08(^{C,ab})</td>
</tr>
<tr>
<td>5</td>
<td>3.97±0.16(^{A,d})</td>
<td>3.19±0.64(^{A,cd})</td>
<td>1.70±0.25(^{B,bc})</td>
<td>1.08±0.23(^{B,b})</td>
</tr>
<tr>
<td>7</td>
<td>4.73±0.76(^{A,d})</td>
<td>3.73±0.74(^{AB,d})</td>
<td>2.17±0.40(^{AC,c})</td>
<td>1.01±0.44(^{C,ab})</td>
</tr>
</tbody>
</table>

*Different letters (A-D) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-d) within the same treatment are significantly different (P<0.05)*

**Table 4.1b.** TBARS Value of Cooked Ground Turkey Meat Treated with Various Antioxidants during Storage at 2° C- Extended Storage Time

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K(_{200})</th>
<th>K(_{400})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.29±0.08(^{A,e})</td>
<td>0.91±0.04(^{B,cd})</td>
<td>0.65±0.15(^{B,e})</td>
<td>1.01±0.03(^{C,f})</td>
</tr>
<tr>
<td>1</td>
<td>1.59±0.08(^{A,de})</td>
<td>0.76±0.05(^{C,d})</td>
<td>0.83±0.12(^{C,e})</td>
<td>1.27±0.06(^{B,f})</td>
</tr>
<tr>
<td>3</td>
<td>2.20±0.11(^{A,cd})</td>
<td>0.74±0.04(^{D,d})</td>
<td>1.19±0.03(^{C,d})</td>
<td>1.60±0.04(^{B,e})</td>
</tr>
<tr>
<td>5</td>
<td>2.58±0.14(^{A,e})</td>
<td>0.82±0.06(^{C,d})</td>
<td>0.76±0.14(^{C,e})</td>
<td>1.50±0.04(^{B,d})</td>
</tr>
<tr>
<td>7</td>
<td>3.77±0.01(^{A,b})</td>
<td>1.09±0.02(^{C,ab})</td>
<td>1.58±0.06(^{B,c})</td>
<td>1.60±0.06(^{B,d})</td>
</tr>
<tr>
<td>10</td>
<td>4.05±0.61(^{A,ab})</td>
<td>1.21±0.09(^{B,b})</td>
<td>1.97±0.12(^{B,b})</td>
<td>1.95±0.09(^{B,e})</td>
</tr>
<tr>
<td>13</td>
<td>4.65±0.14(^{A,a})</td>
<td>1.51±0.04(^{C,a})</td>
<td>2.24±0.03(^{B,ab})</td>
<td>2.18±0.03(^{B,b})</td>
</tr>
<tr>
<td>15</td>
<td>4.59±0.24(^{A,a})</td>
<td>1.54±0.13(^{D,a})</td>
<td>2.31±0.14(^{C,a})</td>
<td>2.84±0.10(^{B,a})</td>
</tr>
</tbody>
</table>

*Different letters (A-D) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-f) within the same treatment are significantly different (P<0.05)*

**K\(_{200}\) and K\(_{400}\) represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively.**
Table 4.2a. Conjugated Dienes Concentrations with Antioxidant Treatments and Storage At 2°C - A Preliminary Study

Conjugated Dienes (nmole/mg meat)

<table>
<thead>
<tr>
<th>Storage (Days)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.15±0.03&lt;sup&gt;A,ac&lt;/sup&gt;</td>
<td>0.22±0.01&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.20±0.08&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.12±0.01&lt;sup&gt;A,ac&lt;/sup&gt;</td>
<td>0.12±0.03&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.11±0.01&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;B,ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.06±0.00&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>0.03±0.02&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>0.02±0.02&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.04±0.02&lt;sup&gt;A,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.38±0.00&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.24±0.01&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>0.27±0.07&lt;sup&gt;AB,a&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;B,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>0.23±0.08&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.26±0.01&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.29±0.03&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.16±0.01&lt;sup&gt;A,ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-d) within the same treatment are significantly different (P<0.05)

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively.

Table 4.2b. Conjugated Dienes Concentrations After Antioxidant Treatments and Storage At 2°C - Extended Storage Time

Conjugated Dienes (nM/ mg meat)

<table>
<thead>
<tr>
<th>Storage (Days)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.13± 0.01&lt;sup&gt;A,ab&lt;/sup&gt;</td>
<td>0.09±0.00&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.10± 0.00&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.10± 0.01&lt;sup&gt;b,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.08± 0.01&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.08± 0.00&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>0.07± 0.01&lt;sup&gt;B,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.11± 0.00&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.08± 0.00&lt;sup&gt;b,bc&lt;/sup&gt;</td>
<td>0.06± 0.00&lt;sup&gt;C,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.12±0.01&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;b,b&lt;/sup&gt;</td>
<td>0.06± 0.00&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.06± 0.00&lt;sup&gt;b,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>0.11±0.01&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>0.08± 0.00&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.06± 0.01&lt;sup&gt;b,cd&lt;/sup&gt;</td>
<td>0.07± 0.00&lt;sup&gt;b,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>0.14± 0.00&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.09±0.00&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.09± 0.01&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.07± 0.00&lt;sup&gt;C,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>0.14± 0.01&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.09±0.00&lt;sup&gt;b,ab&lt;/sup&gt;</td>
<td>0.08± 0.00&lt;sup&gt;b,bc&lt;/sup&gt;</td>
<td>0.06± 0.00&lt;sup&gt;C,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>0.14± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08± 0.01&lt;sup&gt;b,c,cd&lt;/sup&gt;</td>
<td>0.07± 0.00&lt;sup&gt;C,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-d) within the same treatment are significantly different (P<0.05)

**K200 and K400 represent treatments with Keluak extract at concentration of 200 and 400 ppm, respectively.
Table 4.3. Peroxide Value (meq/ kg meat) of Different Antioxidant treatments during Storage of cooked ground turkey meat at 2°C.

<table>
<thead>
<tr>
<th>Storage (Days)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.10 ± 0.42&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>0.70 ± 0.14&lt;sup&gt;A,e&lt;/sup&gt;</td>
<td>0.80 ± 0.28&lt;sup&gt;A,d&lt;/sup&gt;</td>
<td>1.30 ± 0.14&lt;sup&gt;A,cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>2.30 ± 0.14&lt;sup&gt;A,ab&lt;/sup&gt;</td>
<td>1.30 ± 0.14&lt;sup&gt;B,d&lt;/sup&gt;</td>
<td>0.90 ± 0.14&lt;sup&gt;BC,d&lt;/sup&gt;</td>
<td>0.70 ± 0.14&lt;sup&gt;C,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.80 ± 0.00&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>0.70 ± 0.14&lt;sup&gt;A,e&lt;/sup&gt;</td>
<td>0.70 ± 0.14&lt;sup&gt;A,d&lt;/sup&gt;</td>
<td>0.70 ± 0.14&lt;sup&gt;A,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>2.80 ± 0.28&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>1.40 ± 0.00&lt;sup&gt;B,cd&lt;/sup&gt;</td>
<td>1.10 ± 0.14&lt;sup&gt;B,cd&lt;/sup&gt;</td>
<td>0.80 ± 0.28&lt;sup&gt;B,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>1.70 ± 0.42&lt;sup&gt;A,abc&lt;/sup&gt;</td>
<td>1.90 ± 0.14&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>1.95 ± 0.21&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>2.25 ± 0.49&lt;sup&gt;A,bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>3.10 ± 0.42&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>3.30 ± 0.14&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>2.90 ± 0.42&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>3.40 ± 0.28&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>2.55 ± 0.07&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>2.65 ± 0.21&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>2.40 ± 0.28&lt;sup&gt;A,ab&lt;/sup&gt;</td>
<td>2.60 ± 0.28&lt;sup&gt;A,ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>2.55 ± 0.64&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>1.50 ± 0.14&lt;sup&gt;AB,cd&lt;/sup&gt;</td>
<td>0.86 ± 0.06&lt;sup&gt;B,cd&lt;/sup&gt;</td>
<td>1.00 ± 0.28&lt;sup&gt;B,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-e) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.

Table 4.4. Intensity of Meaty Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.95±2.50&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>7.27±1.44&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>6.32±2.02&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>6.06±1.89&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>7.83±2.08&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>7.31±1.74&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>5.43±2.20&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>5.42±2.94&lt;sup&gt;B,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>6.13±2.01&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>6.93±1.43&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>5.02±2.66&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>5.52±2.28&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>7.34±2.77&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>7.10±2.90&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>4.74±2.53&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>4.87±2.63&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>6.94±2.96&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>7.51±2.65&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>5.32±2.59&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>5.43±2.36&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-b) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.
Table 4.5 Intensity of Rancid Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.28±1.94\textsuperscript{A,a}</td>
<td>1.93±1.75\textsuperscript{A,a}</td>
<td>3.34±2.31\textsuperscript{A,a}</td>
<td>3.50±2.74\textsuperscript{Aa}</td>
</tr>
<tr>
<td>1</td>
<td>1.77±1.94\textsuperscript{A,a}</td>
<td>1.72±1.63\textsuperscript{A,a}</td>
<td>3.92±2.74\textsuperscript{AB,a}</td>
<td>4.26±2.65\textsuperscript{B,a}</td>
</tr>
<tr>
<td>3</td>
<td>2.60±2.10\textsuperscript{A,a}</td>
<td>2.72±2.65\textsuperscript{A,a}</td>
<td>4.19±3.06\textsuperscript{A,a}</td>
<td>3.37±2.47\textsuperscript{A,a}</td>
</tr>
<tr>
<td>5</td>
<td>3.44±2.55\textsuperscript{A,a}</td>
<td>3.29±2.15\textsuperscript{A,a}</td>
<td>4.27±2.48\textsuperscript{A,a}</td>
<td>3.95±2.53\textsuperscript{A,a}</td>
</tr>
<tr>
<td>7</td>
<td>4.08±3.00\textsuperscript{A,a}</td>
<td>2.89±2.44\textsuperscript{A,a}</td>
<td>4.48±2.41\textsuperscript{A,a}</td>
<td>3.85±2.44\textsuperscript{A,a}</td>
</tr>
</tbody>
</table>

\*Different letters (A-B) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-b) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.

Table 4.6 Intensity of Eggy Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.43±2.42\textsuperscript{A,a}</td>
<td>5.13±2.44\textsuperscript{A,a}</td>
<td>5.02±3.24\textsuperscript{A,a}</td>
<td>5.65±2.87\textsuperscript{A,a}</td>
</tr>
<tr>
<td>1</td>
<td>5.97±2.48\textsuperscript{A,a}</td>
<td>4.76±3.21\textsuperscript{A,a}</td>
<td>5.99±3.32\textsuperscript{A,a}</td>
<td>5.84±3.60\textsuperscript{A,a}</td>
</tr>
<tr>
<td>3</td>
<td>3.45±2.58\textsuperscript{A,b}</td>
<td>4.00±2.70\textsuperscript{A,a}</td>
<td>5.15±3.35\textsuperscript{A,a}</td>
<td>5.09±4.25\textsuperscript{A,a}</td>
</tr>
<tr>
<td>5</td>
<td>3.64±3.03\textsuperscript{A,ab}</td>
<td>4.24±3.20\textsuperscript{A,a}</td>
<td>4.75±2.69\textsuperscript{A,a}</td>
<td>4.69±1.80\textsuperscript{A,a}</td>
</tr>
<tr>
<td>7</td>
<td>4.73±3.06\textsuperscript{A,ab}</td>
<td>3.64±2.10\textsuperscript{A,a}</td>
<td>4.85±2.68\textsuperscript{A,a}</td>
<td>4.31±3.05\textsuperscript{A,a}</td>
</tr>
</tbody>
</table>

\*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-e) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.
Table 4.7 Intensity of Cardboard Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.99±2.22^A,a</td>
<td>2.09±1.82^A,a</td>
<td>2.61±1.98^A,a</td>
<td>3.12±1.57^A,a</td>
</tr>
<tr>
<td>1</td>
<td>2.62±2.49^A,a</td>
<td>1.80±1.92^A,a</td>
<td>3.39±2.38^A,a</td>
<td>3.42±2.29^A,a</td>
</tr>
<tr>
<td>3</td>
<td>2.99±2.03^A,a</td>
<td>2.84±2.54^A,a</td>
<td>3.88±2.35^A,a</td>
<td>2.87±1.85^A,a</td>
</tr>
<tr>
<td>5</td>
<td>2.52±2.43^A,a</td>
<td>2.90±1.90^A,a</td>
<td>3.09±1.93^A,a</td>
<td>3.20±2.04^A,a</td>
</tr>
<tr>
<td>7</td>
<td>3.01±2.10^A,a</td>
<td>2.73±2.53^A,a</td>
<td>3.37±1.95^A,a</td>
<td>2.84±2.05^A,a</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-e) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.

Table 4.8 Intensity of Fatty Odor Descriptor in Cooked Ground Turkey Meat Treated with Different Antioxidants Rated in a 15-cm Line Scale

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.44±2.26^aA</td>
<td>3.50±2.56^aA</td>
<td>3.95±3.09^aA</td>
<td>3.95±2.77^aA</td>
</tr>
<tr>
<td>1</td>
<td>4.17±2.85^aA</td>
<td>3.89±2.30^aA</td>
<td>4.01±3.45^aA</td>
<td>4.38±2.87^aA</td>
</tr>
<tr>
<td>3</td>
<td>3.88±2.20^aA</td>
<td>3.78±2.29^aA</td>
<td>3.96±3.28^aA</td>
<td>4.03±2.85^aA</td>
</tr>
<tr>
<td>5</td>
<td>4.79±2.52^aA</td>
<td>4.61±2.93^aA</td>
<td>3.89±2.55^aA</td>
<td>4.28±3.20^aA</td>
</tr>
<tr>
<td>7</td>
<td>4.60±2.88^aA</td>
<td>4.92±2.86^aA</td>
<td>4.26±2.81^aA</td>
<td>4.28±3.40^aA</td>
</tr>
</tbody>
</table>

*Different letters (A-C) within a row indicates that mean values are significantly different (P< 0.05)
Different letters within a column (a-e) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent Treatment with Keluak extract at concentrations of 200 and 400 ppm, respectively.
Table 4.9 pH Values of Cooked Ground Turkey Treated with Different Antioxidants Stored at 2°C

<table>
<thead>
<tr>
<th>Storage (Day)</th>
<th>Control</th>
<th>BHT 0.02%</th>
<th>K200</th>
<th>K400</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.65± 0.02⁴,⁵</td>
<td>6.44± 0.03⁶,⁷</td>
<td>6.46± 0.04⁸,⁹</td>
<td>6.58± 0.03⁴,⁵</td>
</tr>
<tr>
<td>1</td>
<td>6.59± 0.03⁴,⁶</td>
<td>6.43± 0.03⁸,⁹</td>
<td>6.51± 0.01⁶,⁷</td>
<td>6.62± 0.01⁴,⁵</td>
</tr>
<tr>
<td>3</td>
<td>6.45± 0.03⁶,⁷</td>
<td>6.34± 0.02⁸,⁹</td>
<td>6.43± 0.04⁸,⁹</td>
<td>6.54± 0.00⁴,⁵</td>
</tr>
<tr>
<td>5</td>
<td>6.63± 0.02⁴,⁵</td>
<td>6.40± 0.02⁸,⁹</td>
<td>6.45± 0.04⁶,⁷</td>
<td>6.54± 0.02⁴,⁵</td>
</tr>
<tr>
<td>7</td>
<td>6.47± 0.01⁴,⁵</td>
<td>6.33± 0.03⁸,⁹</td>
<td>6.48± 0.04⁸,⁹</td>
<td>6.46± 0.06⁴,⁵</td>
</tr>
<tr>
<td>10</td>
<td>6.65± 0.01⁴,⁵</td>
<td>6.46± 0.02⁶,⁷</td>
<td>6.54± 0.01⁶,⁷</td>
<td>6.60± 0.03⁴,⁵</td>
</tr>
<tr>
<td>13</td>
<td>6.65± 0.02⁴,⁵</td>
<td>6.48± 0.02⁸,⁹</td>
<td>6.55± 0.02⁶,⁷</td>
<td>6.64± 0.02⁴,⁵</td>
</tr>
<tr>
<td>15</td>
<td>6.68± 0.01⁴,⁵</td>
<td>6.51± 0.01⁷,⁸</td>
<td>6.55± 0.01⁷,⁸</td>
<td>6.63± 0.01⁴,⁵</td>
</tr>
</tbody>
</table>

*Different letters (A-D) within a row indicate that mean values are significantly different (P< 0.05)
Different letters within a column (a-c) within the same treatment are significantly different (P<0.05)
**K200 and K400 represent treatments with Keluak extract at concentrations of 200 and 400 ppm, respectively.
4.9 References


Chapter 5. CONCLUSION AND FUTURE WORK

The results of the present study indicated that the Keluak extract incorporated into cooked ground turkey can reduce lipid oxidation. The addition of Keluak extracts at 200 and 400 ppm had a positive effect on reducing the TBAR values and the conjugated dienes content compared to the control without added AOs during storage at 2°C for 15 days. The Keluak extract used in this study was comparable to BHT (0.02%) in reducing lipid oxidation in meat. Peroxide value analysis did not differentiate between the control and the treatments on the extent of lipid oxidation. Due to confusion of the odor components in the Keluak extracts by the panelists, there were no significant differences between the treatments in the odor sensory evaluation.

Keluak seed is a culinary spice with no known toxic effect. Its moderate pungent aroma makes it valued as a traditional ethnic food flavorant in parts of South East Asia. With its substantial phenolic content and antioxidant properties, it could be an effective source of natural antioxidants. However, the application of this natural antioxidant might be limited in Western cultures due to its effect on the color and odor of meat products. Further investigations are needed to identify selective extraction and clean-up techniques that could be used to eliminate the color and odor problem. Other chemical analysis methods that might provide better information on the extent of oxidation in meat products include the p-anisidine value, Food Oil Sensor (FOS) and Gas chromatography techniques (GC analysis) of selected volatiles.