ประสิทธิภาพของสารต้านอนุมูลอิสระจากธรรมชาติที่ใช้ในเนื้อไก่แยกกระดูกด้วยเครื่องและลูกชิ้นไก่

โดย

นางสาววารุณี เอี่ยมสอาด

สารนิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีอาหาร

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Potential natural antioxidants utilized in mechanically deboned chicken meat and chicken ball

By

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วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาประสิทธิภาพของสารต้านอนุมูลอิสระที่ได้จากธรรมชาติและจากการสังเคราะห์ในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นในเนื้อไก่แยกกระดูกด้วยเครื่องเปรียบเทียบกับเนื้อไก่แยกกระดูกด้วยเครื่องที่ไม่มีการใช้สารต้านอนุมูลอิสระที่เก็บในสภาวะแช่เย็นและแช่เยือกแข็ง โดยในการทดลองนี้ได้ใช้ค่าทีบีเอ (TBA value) เป็นดัชนีที่บ่งบอกถึงความสามารถในการต้านอนุมูลอิสระ การทดลองได้ใช้สารต้านอนุมูลอิสระ 5 ชนิด ได้แก่ บีเอชที, วิตามินอี, โรสแมร์รี่, แร่ธาตุจากนม และสารผสม (บีเอชที, บีเอชเอ, โพรพิลแกลเลตและกรดซิตริก) ที่ระดับความเข้มข้น 1000, 3000, 5000 ppm. และศึกษาผลกระทบที่เกิดจากการเก็บในสภาพแช่เย็นเป็นเวลา 3 วัน และแช่เยือกแข็งเป็นเวลา 28 วัน จากการทดลองพบว่าเมื่อเก็บรักษาเนื้อไก่แยกกระดูกที่นานขึ้นส่งผลให้ค่าทีบีเอสูงขึ้นและการเติมสารต้านอนุมูลอิสระส่งผลให้ค่าทีบีเอต่ำกว่าการที่ไม่เติมสารต้านอนุมูลอิสระอย่างมีนัยสำคัญทางสถิติ ($p\leq0.05$) ในสภาวะการเก็บในสภาพแช่เย็นเป็นระยะเวลา 3 วัน พบว่าโรสแมร์รี่มีประสิทธิภาพในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นได้ดีที่สุด โดยมีค่าทีบีเอลดลง 79.76 % ส่วนในสภาพการเก็บในสภาพแช่เยือกแข็ง พบว่าสารผสม (บีเอชเอ, บีเอชที, โพรพิลแกลเลตและกรดซิตริก) มีประสิทธิภาพในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นได้ดีที่สุดโดยมีค่าทีบีเอลดลง 54.06% เมื่อ ความเข้มข้นของสารต้านอนุมูลอิสระทุกชนิดสูงขึ้นส่งผลให้ค่าทีบีเอต่ำลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

เมื่อทำการศึกษาประสิทธิภาพของสารต้านอนุมูลอิสระที่ได้จากธรรมชาติในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นในลูกชิ้นไก่ การทดลองได้ใช้สารต้านอนุมูลอิสระ 4 ชนิด ได้แก่ บีเอชที, โรสแมร์รี่, วิตามินอีและแร่ธาตุจากนมที่ระดับความเข้มข้น 1000, 3000, 5000 ppm. ที่เก็บในสภาพแช่เย็น เป็นเวลา 35 วัน จากการทดลองพบว่าในการเก็บรักษาที่นานขึ้นส่งผลให้ค่าทีบีเอสูงขึ้นและการเติมสารต้านอนุมูลอิสระส่งผลให้ค่าทีบีเอต่ำกว่าการไม่เติมสารต้านอนุมูลอิสระอย่างมีนัยสำคัญทางสถิติ ($p\leq0.05$) สารบีเอชทีมีประสิทธิภาพในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นได้ดีที่สุด โดยมีค่าทีบีเอลดลง 45.00% และระดับความเข้มข้นของสารต้านอนุมูลอิสระที่เพิ่มขึ้นส่งเสริมประสิทธิภาพของสารต้านอนุมูลอิสระในการยับยั้งการเกิดปฏิกิริยาลิพิดออกซิเดชั่นได้ดีขึ้น

ภาควิชาเทคโนโลยีอาหาร บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา 2548

ลายมือชื่อนักศึกษา .....

ลายมือชื่ออาจารย์ผู้ควบคุมสารนิพนธ์ .....

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The objective of this research was to study potential natural antioxidant and synthetic antioxidant utilized in mechanically deboned chicken meat (MDCM) and then compared the result with MDCM without antioxidant under chilled and frozen storage. Thiobarbituric assay (TBA) was used to assess the effect of antioxidant on lipid oxidation of MDCM. Different antioxidants (BHT, vitamin E, rosemary, milk mineral and combination) at three levels of concentration (1000, 3000, and 5000 ppm) and two condition of storage (4 ^{o}C for 3 days and -20^{o} C for 28 days) were employed. The results shown that TBA values of both chilled and frozen MDCM increased with storage time. After 3 days of storage at 4 ^{o}C, rosemary was more effective antioxidant than BHT, vitamin E, milk mineral and combination, respectively. In contrast, after 28 days of storage at -20 ^{o}C, the combination of antioxidants was more effective antioxidant than BHT, rosemary, milk mineral and vitamin E, respectively. As increasing amount of antioxidant added in MDCM, lipid oxidation was significantly ($p \leq 0.05$) inhibited.

Final research was to study potential natural antioxidant utilized in chicken ball. Different antioxidants (BHT, rosemary, vitamin E, and milk mineral) at three levels of concentration (1000, 3000, and 5000 ppm) under chill storage (4^{o}C for 35 days) was examined. The result shown antioxidant activity, which assayed by TBA value of chicken ball with adding and without adding antioxidant was significantly different ($p<0.05$). BHT was the most powerful to retard lipid oxidation in this case.

Department of Food Technology Graduate School, Silpakorn University Academic Year 2005
Student's signature ……
Master's Report Advisors' signature ……

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TABLE OF CONTENTS

Page

Page

CHAPTER

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

Figure **Page**

CHAPTER 1

INTRODUCTION

Chicken meat and its products have experienced increasing popularity and become wide usage all over the world (Sallan et al., 2002). The consumer preference for chicken cuts instead of whole chicken and, later on, the demand for chicken fillets and convenience products, such as nuggets, hamburgers, and marinated cuts, requires the finding of ways to use backs, necks, and bones left over from manual deboning processes (Trindade et al., 2004). Mechanically deboned chicken meat (MDCM) is by product from poultry meat processing industry. In the processing of MDCM is removed from the skeletal bone tissues by grinding the starting material such as frames, backs, and necks by passing it through a sieve under high pressure (Froning and McKee, 2001). The remaining bony parts or carcass frame with attached meat are then ground and passed through a deboning machine which separates meat from bones. During the grinding and separating operations, a certain amount of bone marrow and bone flour gel into the meat (Essary, 1979). The muscle and other edible tissue pass through the opening; however, the bone particles, except for very small amounts, do not. The bone portion is shunted to one side and may be used for making broths or for producing bone meal used in animal feeds or as fertilizer. MDCM exhibits red color and its emerges from the machine as a finely ground paste like product (Institute of Food Technologists, 1979).

MDCM is a valuable co-product of chicken meat processing and provides a means of harvesting functional proteins. MDCM also is utilized in a wide range of emulsified and restructured meat products including frankfurters, bolongna, salami, breakfast sausage, hot dog, nuggets, and roasted, etc (Froning and McKee, 2001). Froning (1976) reported that the component affecting the composition of MDCM is the bone marrow. The mechanical deboning process incorporates heme and lipid components. The lipid contents from the bone marrow account for the large increase in fat content of MDCM, and this further dilutes noticeably the protein content. Heme/lipid interaction also is an important

factor affecting stability of MDCM. A major problem for products manufactured with MDCM is the rapid onset of oxidative rancidity, which results in off flavors and off odor (Mielnik et al., 2002). Oxidation of lipids in chicken meat and chicken meat products is responsible for the changes in its nutritional quality loss of vitamins and essential amino acids, color, flavor, odor, and texture (Aguirrezabal et al., 2000) and the reduced shelf life (Coronado et al., 2002). The problem of lipid oxidation has extensive economic importance for the meat industry as it leads to the development of rancidity and formation of potentially toxic reaction products and major cause of chemical spoilage in food system (McCarthy et al., 2001).

The rate of lipid oxidation can be effectively retarded by use of antioxidants (Coronado et al., 2002). Various synthetic antioxidants have been utilized to retard the development of rancidity in meat product, and extend their shelf life, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG). However, question regarding the safety of synthetic antioxidants together which consumer' preference have led to increased interest and research on natural antioxidants. It has been demonstrated many times that spices inhibit rancidity (Aguirrezabal et al., 2000). Natural antioxidants extracted from plants such as rosemary, sage, various vegetables, soybean, citrus peel, sesame seed, olives, carob pod, and grapes can be used as alternatives to the synthetic antioxidants because of their equivalent or greater effect on inhibition of lipid oxidation (Tang et al., 2001). Although these meat can be packaged appropriately using MAP or vacuum packaging, these technologies do not always completely removed oxygen. Permeation of oxygen may also occur through the packaging material resulting in deterioration of the food while these packaging techniques may extend the shelf life and keeping quality of food microbiological spoilage and lipid oxidation may occur (Smiddy et al., 2002).

The 2-thiobarbituric acid (TBA) method is the most widely used test for measuring the extent of lipid peroxidation in red meat and poultry, due to its speed and simplicity. The phospholipids fraction of mechanically deboned chicken meat is highly unsaturated, and thus very susceptible to oxidation, showing a great potential to produce 2-thiobarbituric acid reactive substances (Gomes et al., 2003).

The objective of this experiment was to select the most powerful natural antioxidant adding in MDCM and determine the optimum usage level of antioxidants to able retard

lipid oxidation in MDCM under chilled and frozen storage conditions. Subsequently, the last experiment was to determine the optimum usage level of antioxidant to able retard lipid oxidation in chicken ball under chilled storage condition.

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CHAPTER 2

LITERATURE REVIEW

MDCM is a valuable co-product of chicken meat processing (Hassan and Fan, 2004). In the process, the bony parts such as frame, backs, necks, or carcass pass through a deboning machine which separates meat from bones (Essary, 1979). MDCM involves grinding meat and bone together and forcing the mix through a fine screen or slotted surface to remove bone particles (Najdawi and Abdullah, 2002). MDCM emerges from the machine as a red color and finely ground paste. The bone portion is shunted to one side and many be used for making broths or for producing bone meal for use in animal feeds or as fertilizer (Institute of food Technologists, 1979). After mechanical deboning, the bone residue is often utilized in animal feed. That the bone residue some excellent potential as a feed ingredient or could be used to produce a protein isolate.

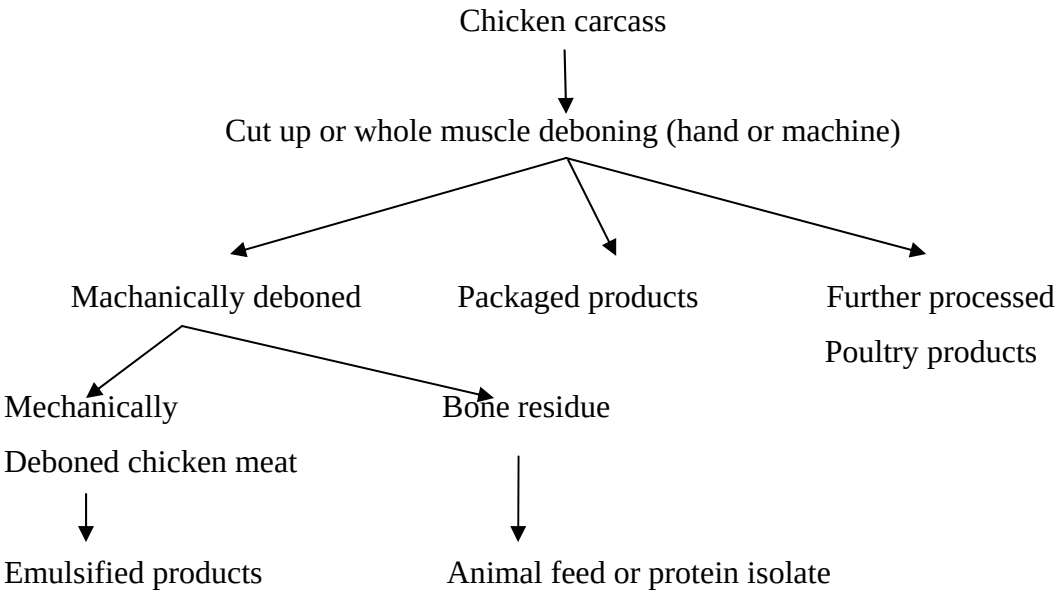

Figure 2.1 Mechanically deboned chicken meat (MDCM)
(Froning and McKee, 2001)

With increased environment concerns, utilization of the bone residue has become an important priority (Froning and McKee, 2001).

2.1 Mechanical deboner

Process for mechanical deboned of chicken consists of chopping the starting materials and then deboning the bone, sinew, and tendon from meat by passing it through a sieve under high pressure. There are two basic categories of mechanical deboner. One type forces meat from an outer chamber through slots of perforated drum, leaving the bone material in the outside of the drum. In a similar design, meat is forced outside through a perforated cylinder while the bone residue is maintained in the interior. Many factors related to the equipment can affect end product quality. For instance, yield is affected by the amount of pressure that is applied when pushing product though the sieve. However, when pressure increases, the deboned process can become slightly less efficient by allowing more bone, sinew, and other non meat residues in the final product. Processors determine the optimum machine settings to achieve high yield and product quality. Maintenance of the equipment is another factor that affects product quality. Maintaining sharp edges on cutting surfaces greatly influences the end product texture and consistency. Poor equipment maintained can cause product to smear and become pasty in texture. Texture can also be altered by changing screen or sieve sizes. Large pore sizes in sieves result in a course textured product. Product temperature is another factor that can alter end product quality. Most equipment can process meat that is chilled, but not frozen. One Midwest processor has modified the deboning equipment so that they are able to process meat that frozen. This is tremendous advantage because the product has a superior texture, long shelf life, and lower bacteria counts (Froning and McKee, 2001).

Figure 2.2 Mechanical deboner

2.2 Proximate composition of Mechanically deboned chicken meat

When poultry meat is mechanically deboned, considerable shearing action causes make cellular disruption. The muscle and other edible tissues through the opening. MDCM exhibits red color and its emerges from the machine as finely ground paste product (Institute of food Technologists, 1979).

Mechanical deboning of poultry affects the proximate composition of resulting meat. Considerable amount of lipids present in the raw material were incorporated in the MDCM, diluting protein and increasing the lipid contents of the deboned tissues. These lipids include those present in the bone marrow, the subcutaneous fat, the skin, and the abdominal fat, excluding the fat of the viscera removed during the slaughtering process (Trindade et al., 2004). Also, the bone marrow is released from broken bones during the deboning process, thereby contributing increased and heme components into in the deboned meat (Froning and McKee, 2001). As a result of inclusion of bone marrow in MDCM there is greater variation is the fatty acid content and high percentage of cholesterol and phospholipids (Najdawi and Abdullah, 2002). The phospholipids fraction of MDCM is highly unsaturated (Gomes et al., 2003). During mechanical deboning some bone marrow and flour enter the meat and as with hand deboned techniques, may leave small amount of powdered bone (Najdawi and Abdullah, 2002). Several authors have reported proximate composition of MDCM. Nagrao et al. (2005) reported that

proximate chemical composition, on dry basis, of MDCM and fresh chicken breast meat (FCBM) showed protein contents of 90.5% and 82.2%, lipid contents of 3.0% and 13.2%, and ash content of 6.1-4.2%, respectively. Hamm and Searcy (1981) reported proximate composition and mineral content of MDCM. Moisture content averaged 66.6-70.0%, fat content averaged 13.8-22.0%, protein content averaged 11.9-16.7%, and ash content averaged 0.9-1.5% wet weights. Mineral content from MDCM that potassium (K) 1360-2060 ppm, phosphorus (P) 1300-2420 ppm, calcium (Ca) 1200-2250 ppm, sodium (Na) 280-540 ppm, magnesium (Mg) 115-196 ppm, iron (Fe) 13-27 ppm, zinc (Zn) 11-18 ppm, copper (Cu) 0.35-0.63 ppm, manganese (Mn) 0.1-0.47 ppm, and lead (Pb) 0.02-0.17 ppm. Essary (1979) reported that moisture, fat, protein, and mineral content of MDCM from frame and necks without skin (1:1). Moisture content averaged 72.2%, protein content averaged 14.4%, and fat content averaged 13.4%. Mineral content of sample for potassium (K), sodium (Na), calcium (Ca), chlorine (Cl), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), rubidium (Rb), aluminium (Al), and bromine (Br).

Lipids are the food molecules most susceptible to oxidative free radical reactions. This instability is due to their content of polyunsaturated fatty acids (PUFA) and includes the esters of glycerol with fatty acids, triacylglycerols, and phospholipids. Pokorny et al. (2001) reported that in processing of a wide range of foods, fats may be added as part of the foods formulations. In animal tissues used as foods, the phospholipids present in all biological membranes may be an important substrate for oxidative deterioration.

MDCM influences the color of the resultant meat. The process releases heme pigments from the bone marrow into the MDCM. This increased is primarily due to haemoglobin from bone marrow. Gopalakrishnan et al. (1999) reported that mechanically separated muscle would also contain components of blood marrow including lipids and heme ion. Haemoglobin is more subject to abnormal color problem since it is more easily oxidized and more susceptible to heat denaturation during processing and storage. Abnormal brown, green, and gray color defects have been reported in further-processed poultry meat products containing mechanically deboned poultry. During the deboning process the meat is exposed to considerable air, which may accelerate the oxidation of heme pigments. Composition and

processing variables have been show to affect the color characteristics of MDCM (Froning and McKee, 2001).

Proximate composition of various sources of MDCM as indicated, there is considerable variation in the composition. Factor influencing the composition included:

1. Bone to meat ratio: younger birds generally will have more heme and lipid components from bone marrow inflecting the proximate composition.
2. Skin content: skin content may greatly increase the fat content of the resulting deboned meat while the collagen from the skin is largely found in bone residue.
3. Deboner: deboner setting can affect the yields and the proximate composition substantially. If the setting is set for high yields, the fat and ash content in the resultant mechanical deboned chicken meat may be largely increased. High setting may also increase the temperature resulting in protein denaturation, which may ultimate affect functionality.
4. Cutting methods: protein quality of MDCM was comparable to that found from hand deboned sources. However, the fatty acid composition chicken bone marrow and MDCM was quite similar to that from hand deboned meat sources. Bone particles from hand deboned and MDCM have been characterized. Bone particles isolated from hand deboned sources were actually somewhat larger than that obtained from mechanically deboned meat. Any bone particles found in MDCM were indicated to be of a powdery from presenting no hazard to be consumer.
5. Species: species one concern has been the fatty acid and cholesterol content of MDCM. The bone marrow from chicken broilers contained a highly percentage of phospholipids and cholesterol than that found in other broiler meat (Froning and McKee, 2001).

2.3 Utilization of mechanically deboned chicken meat

Chicken meat and its products have experienced increasing popularity and become widely spread all over the world (Sallon et al., 2004). Meat recovered from

bones or carcass parts by mechanical procedure is generally considered to be of poor quality (Henckel et al., 2004) and has good nutritional, fine consistency, functional properties, and relatively low cost (Mielnik et al., 2002). MDCM was utilized in a wide range of emulsified and restructured meat products including frankfurters, bologna, chicken ball, and nuggets (Froning and McKee, 2001). This regulation allows the use of up to 60% of MDCM in substitution of the meat raw material in some types of emulsified sausages. The problem with the use of large ratios of MDCM in meat products was the low stability of this raw material, which is very prone to lipid and pigment oxidation as well as microbial growth (Trindade et al., 2004).

2.4 Lipid oxidation of MDCM

The mechanisms of oxidative reactions loading to decreased quality of processed foods are the same mechanisms described for lipid oxidation in general chemistry. Lipid oxidation is a multi-step, multi-factorial process, and in foods the variables encompassed include individual fatty acid susceptibility, molecular structure of lipids, physical state of lipids, initiation reactions, hydroperoxide (ROOH) decomposition catalysts (e.g., metals), presence of oxidized lipids, and the amounts and selectivity of antioxidants present (Branen et al., 2002).

MDCM represent a low value, under utilized source of muscle could be used to many products (Gopalakrishnan et al., 1999). A major problem for product manufactured with MDCM was the lipid on set of oxidative rancidity, which results in off-flavor and off odors (Mielnik et al., 2002). The mechanical deboner process produces considerable cellular disruption and release hemoglobin and lipids from bone marrow. Also, heat produced from the deboner process may accelerate lipid oxidation (Froning and McKee, 2001). The high oxidative potential of MDCM is due to extreme mechanical stress, extraction of considerable quantities of lipids and heme components from bone marrow, and aeration during the machine deboning process (Mielnik et al., 2002). Heme pigments are known to be strong catalysts for lipid oxidation in meat products. During mechanical deboning, oxygen incorporation, temperature increase, high pressure, and metal contact with the deboner may further

contribute to the lipid oxidation problem (Froning and McKee, 2001). Processing operations, such as particle size reduction, heating, and salting can alter the pro-oxidative (Gopalakrishnan et al., 1999).

2.5 Types of rancidity

2.5.1 Hydrolytic rancidity

Hydrolytic rancidity is only significant in meat and meat products in certain circumstances. The hydrolysis may be due either to direct chemical causes to enzyme activity. The commonest chemical cause in other fatty foods is the acidity of the food, either in its natural state or as developed in the course of other deteriorative changes. However, in meat the natural pH value is close to neutrality, 7.0 to 7.2 in the live animal, falling to 5.5 to 6.5 after death and only very exceptionally as low 5.0. Muscular tissue has a high buffering capacity and if acids are added to it, quite large quantities of strong acid are necessary to alter the pH value significantly. Furthermore, because of the structural factors outlined above, the possibility of chemical reaction between the lipids in the fatty tissue and acids in the muscular tissue is small, so hydrolytic rancidity in ordinary meat is not very common.

Hydrolytic changes initiated by enzymes may occur in meat or meat fats where there is microbiological growth. Lipolytic enzymes produced by microorganisms on the meat surface, especially by mould but sometimes by yeasts or bacteria, may well produce rancidity in the surface fat. In such circumstances microbial-induced proteolysis changes usually also occur. Proteolysis damages the fatty tissue cell walls and makes the fat more accessible to the lipolytic enzymes. However, the general level of microbiological activity usually leads to such obvious mouldiness, slime or smell that the meat is considered spoiled quite independently of any increase in rancidity. The problems can normally be avoided by minimizing microbial contamination, by good general hygiene and by storage at low temperature (Allen and Hamilton, 1994).

2.5.2 Oxidative rancidity

Oxidative rancidity (some time referred to as autooxidation) is much more common in meats than hydrolytic rancidity. Note again that the general chemical principles involved apply equally in meat systems as in other food, and although there may be modifications in their effects due to the nature of meat structure and other causes, some important simple predictions can be made from basic principles.

First, it is fundamental that the more unsaturated the fat, the more it is prone to oxidation: rancidity therefore develops faster and further in the relatively unsaturated pork fats than in the harder beef or mutton fats faster again (other factors being equal) in the very soft chicken fat and faster of all in fish oils.

As well as these well known species differences in fat hardness, in the animal body the lipids directly around the internal organ are harder (with higher melting point and lower degree of unsaturation) than those on the outside, and, likewise, the inner layers of body fat are harder than the outer layers. These differences may be related to a need in the live animal for lower melting points in the fats further away from the body centre, where the temperatures are slightly lower, so that the lipids in the tissue cells may always be in the liquid state.

There are differences in susceptibility to oxidation among different cuts of meat or individual muscles. These are probably related to differences in degree of unsaturation of the fats present but other factors may also be involved.

The second principle is that for oxidation to occur an oxidizing agent is required and this must be able to gain access to the fat. The commonest oxidizing agent, of course, is oxygen from the air. On the other hand, comminuting of the meat and the resultant increased exposure of the lipids will increase it.

In the case of the phospholipids in meat fat both these factors are at work. The phospholipids contain highly unsaturated fatty acids, very prone to oxidation, and there are located predominantly in or at the cell walls of the fatty tissue. They are therefore the first to be exposed to oxidation when the cells are damaged. Other phospholipids are more or less finely dispersed in the lean meat, where similar considerations apply (Allen and Hamilton, 1994).

Mechanism of autoxidation

-Initiation steps can occur by the action of external energy sources such as heat, light, or high energy radical or by chemical initiation involving metal ion or metalloproteins such as heme. The free radical $R^{\bullet}$ produced in the initiation steps can then react to form a lipid peroxy radical $ROO^{\bullet}$ which can react further to give the hydroperoxide ROO.

$$RH + O_2 \longrightarrow R^{\bullet} + {}^{\bullet}OOH$$

$$RH \longrightarrow R^{\bullet} + {}^{\bullet}H$$

-Propagation steps also provides a further free radical $R^{\bullet}$,making it a self-propagating chains process. In this way a small amout of catalyst, e.g. copper ions, can initiation the reaction, which then produces many hydroperoxide molecules, which ultimately break down to cause rancidity.

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$$

$$RO_2^{\bullet} + RH \longrightarrow RO_2H + R^{\bullet}$$

-Ternimation steps. The self propagation chain can be stopped by termination reactions, where two radicals combine to give products which do not feed the propagation reactions (Allen and Hamilton, 1994).

$$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$$

$$RO_2^{\bullet} + R^{\bullet} \longrightarrow RO_2R$$

2.6 Factor influencing rate of lipid oxidation in foods

2.6.1 Fatty acid composition

The number, position, and geometry of double bond affect the rate of oxidation. Relative rates of oxidation for arachidonic acid, linolenic, linoleic, and oleic acids are approximately 40:20:10:1, respectively. Cis acids oxidize more readily than their trans isomers, and conjugate double bonds are more reactive than nonconjugated. Autoxidation of saturated fatty acids is extremely slow: at room temperature, they remain practically unchanged when oxidative rancidity of unsaturated becomes detectable. At high temperature, however, saturated acids can

undergo oxidation at significant rates (Fennema, 1996). Gomes et al. (2003) reported that, the fat present in MDCM is rich in poly-unsaturated fatty acids due to the presence of phospholipids, originating in the fraction of bone and spinal marrow accompanying it. The heme iron from the same origin acts as an oxidation catalyst. In addition, the size reduction occurring during the extrusion process results in the inclusion process results in the inclusion of oxygen into the MDCM, which is the final factor involed in the development of off-favor and color change during storage (Fennema, 1996).

2.6.2 Free fatty acids versus the corresponding acylglycerols

Fatty acids oxidize at a slightly greater rate when free than when esterifies to glycerol. Randomizing the fatty acid distribution of natural fat reduces the rate of oxidation. The existence in a fat or oil of a small amount of free fatty acids does not have a marked effect on oxidative stability. In some commercial oils, however, the presence of relatively large amounts of free radicals can facilitate in corporation of catalytic trace metals from equipment or storage tanks and thereby increase the rate of lipid oxidation (Fennema, 1996).

2.6.3 Oxygen concentration

When oxygen is abundant, the rate of oxidation is independent of oxygen concentration, but at very low oxygen concentration the rate is approximately proportional to oxygen concentration. However, the effect of oxygen concentration on rate is also influenced by other factors, such as temperature and surface area (Fennema, 1996).

2.6.4 Temperature

In general, the rate of oxidation increase as the temperature is increased. Temperature also influences the relationship between rate and oxygen partial pressure. As temperature is increased changes in oxygen partial pressure have a smaller influence on rate because oxygen becomes less soluble in lipids and water as the temperature is raised (Fennema, 1996).

2.6.5 Surface area

The rate of oxidation increase in direct proportion to the surface area of the lipid exposed to air. Furthermore, as surface volume ratio is increased, a given reduction in oxygen partial pressure becomes less effective in decreasing the rate of oxidation. In oil in water emulsions the rate of oxidation is governed by the rate at which oxygen diffuses into the oil phase (Fennema, 1996).

2.6.6 Moisture

The rate of oxidation depends strongly on water activity. In dried foods with very low moisture contents (a_w values of less than about 0.1), oxidation proceeds very rapidly. Increasing the a_w to about 0.3 retards lipid oxidation and often produces a minimum rate. This protective effect of small amounts of water is believed to occur by reducing the catalytic activity of metal catalysts, by quenching free radicals, and/or by impeding access of oxygen to the lipid.

At somewhat higher water activities (a_w = 0.55-0.85), the rate of oxidation increases again, presumably as a result of increased mobilization of catalysts and oxygen (Fennema, 1996).

2.6.7 Molecular orientation

Molecular orientation of substrates has an important influence on the rate of lipid oxidation (Fennema, 1996).

2.6.8 Physical state

A recent study of cholesterol oxidation demonstrates the importance of physical state on rate of lipid oxidation. Fragments of microcrystalline cholesterol films, both solid and liquid, were suspended in aqueous media, and oxidation was studied under various conditions of temperature, time, pH, and buffer composition. The types and ratios of oxides differed depending on conditions, with physical state of the cholesterol having the most pronounced influence. Indeed, many of the factors considered in this section exert their effects on oxidation rates by influencing the physical state of both the substrate and/or the medium in which it exists (Fennema, 1996).

2.6.9 Emulsification

In oil in water emulsion, or in foods where oil droplets are dispersed into an aqueous matrix, oxygen must gain access to the lipid by diffusion into the aqueous phase and passage through the oil water interface. The rate of oxidation will depend on the interplay between a number of factors including type and concentration of emulsifier, size of oil droplets, surface area of interface, viscosity of the aqueous phase, composition, and porosity of the aqueous matrix, and pH (Fennema, 1996).

2.6.10 Molecular mobility and the glass transition

If the rate of lipid oxidation is diffusion limited, a low rate of oxidation would be expected below the glass transition and the rate would exhibit very large temperature dependence at temperature above the glass transition (Fennema, 1996).

2.6.11 Pro - oxidants

Transition metals, particularly those possessing two or more valence states and suitable oxidation-reduction potential between them (e.g., cobalt, copper, iron, manganese, and nickel), are effective pro-oxidants. If present, even at concentrations at low as 0.1 ppm, they can decrease the induction period and increase the rate of oxidation. Trace amounts of heavy metals are commonly encountered in edible oils, and they originate from the soil in which the oil-bearing plant was grown, from the animals, or from metallic equipment used in processing or storage. Trace metal are also naturally occurring components of all food tissues and of all fluid foods of biological origin (eggs, milk, and fruit juices), and they are present in both free and bound forms (Fennema, 1996).

2.6.12 Radiant energy

Visible, ultraviolet, and γ-radiation are effective promoters of oxidation (Fennema, 1996).

2.7 Method for measuring lipid oxidation

It is obvious from the preceding discussion that lipid oxidation is an exceedingly complete process involving numerous reactions that cause a variety of chemical and physical changes (Fennema, 1996).

2.7.1 Peroxide value

Peroxides are the main initial products of autoxidation. They can be measured by techniques based on their ability to liberate iodine from potassium (iodimetry):

$$ROOH + 2KI \longrightarrow ROH + I_2 + K_2O$$

Or to oxidize ferrous to ferric ions (thiocyanate method)

$$ROOH + Fe^{2+} \longrightarrow ROH + HO + Fe^{3+}$$

The peroxide value is usually expressed in terms of milliequivalents of oxygen per kilogram of fat. Various other colorimetric techniques are available. Although the peroxide value is applicable for follow peroxide formation at the early stages of oxidation, it is nevertheless highly empirical. Its accuracy is questionable, and the results vary with the specific procedure used, and with test temperature. During the course of oxidation, peroxide values reach a peak and then decline.

Various attempts have been made to correlate peroxide values with development of oxidative off flavors. Good correlations are sometimes obtained, but often not. It should be pointed out that the amount of oxygen that must be absorbed, or peroxides that must be formed, to produce noticeable oxidative rancidity varies with composition of the oil (those that are more saturated require less oxygen absorption to become rancid), the presence of antioxidants and trace metals, and the conditions of oxidation (Fennema, 1996).

2.7.2 Thiobarbituric acid test

This is one of the most widely used tests for evaluating the extent of lipid oxidation. Oxidation products of unsaturated systems produce a color reaction with thiobarbituric acid (TBA). It is believed that the chromogen results from condensation of two molecules of TBA with one molecule of malonaldehyde. However, malonaldehyde is not always present in all oxidized systems. Many

alkanals, alkenals, and 2,4-dienals produce a yellow pigment (at 450 nm) in conjunction with TBA, but only dienals produce a red pigment (at 530 nm). It has been suggested that measurement at both absorption maxima is desirable.

In general, TBA-reactive material is produced in significant amounts only from fatty acids containing three or more double bonds (Fennema, 1996).

Gomes et al. (2003) reported that the 2-thiobarbituric acid (TBA) method is the most widely used test for measuring the extent of lipid oxidation in red meat and poultry, due to its speed and simplicity.

2.7.3 Total and volatile carbonyl compounds

Methods for determining total carbonyl compounds are usually based on measurement of hydrazones that arise from reaction of aldehydes or ketone (oxidation products) with 2,4-dinitrophenylhydrazine. However, under the experimental conditions used for these tests, carbonyl compounds may be generate by decomposition of unstable intermediates, such as hydroperoxides, thus detracting from accuracy of the results. Attempts to minimize such interference have involved reduction of hydroperoxides to noncarbonyl compounds prior to determination of carbonyl, or conducting the reaction at a low temperature.

Because the carbonyl compounds in oxidized fats are of relatively high molecular weight they can be separated by a variety of techniques from lower molecular weight volatile carbonyl compounds. The lower molecular weight volatile carbonyl compounds are of interest because of their influence on flavor. The volatile carbonyl compounds are usually recovered by distillation at atmospheric or reduced pressure and then determined by reaction of the distillate with appropriate reagents or by chromatographic methods. A quantitative measurement of hexanal by headspace analysis is common techniques (Fennema, 1996).

2.7.4 Anisidine value

In the presence of acetic acid, *p*-anisidine reacts with aldehydes producing a yellowish color. The molar absorbance at 350 nm increases if the aldehyde contains a double bond. Thus, the anisidine value is mainly a measure of 2-alkenals. An expression termed the Totox or oxidation value (OV) which is equivalent to 2 x

peroxide value (PV) + anisidine value, has been suggested for the assessment of oxidation in oils (Fennema, 1996).

2.7.5 Kreis test

This was one of the first tests used commercially to evaluate oxidation of fats. The procedure involves measurement of a red color that is believed to result from reaction with phloroglucinol (Kreis reaction). Deficiencies of this test include: (a) fresh samples free of oxidized flavor frequently develop some color upon reaction with the Kreis reagent, and (b) consistent results among different laboratories are difficult to obtain (Fennema, 1996).

2.7.6 Ultraviolet spectrophotometry

Measurement of absorbance at 234 nm (conjugate dienes) and 268 nm (conjugated triens) is sometimes used to monitor oxidation. However, the magnitude of the absorbance does not correlate well with the degree of oxidation except in the early stages (Fennema, 1996).

2.7.7 Oxirane test

This method, which provides a measure of epoxide content, is based on the addition of hydrogen halides to the oxirane group. Epoxide content is determined by dissolving the sample in aqueous acetic acid in the presence of crystal violet, and titrating with HBr to a bluish green endpoint. The test, however, has poor sensitivity, lacks specificity, and does not provide an accurate quantitative result with some *trans*-epoxide. Hydrogen halides also react with β-unsaturated carbonyls and conjugated dienols. A colorimetric method for epoxides, based on the reaction of the oxirane group with picric acid, is reportedly more sensitive than the oxirane test and is not subject to the shortcomings mentioned (Fennema, 1996).

2.7.8 Iodine value

This test is measure of the unsaturated linkages in a fat and is determined by reacting the fat with a solution of iodine mono-chloride in a mixture of acetic acid and CCl_4 liberating excess iodine with KI, and titrating with $Na_2S_2O_3$ (Wijs method). The

results are expressed as grams of iodine absorbed by 100 g sample. In the Hanus method, an iodine bromide reagent is used instead of iodine mono-chloride. A decline in iodine value is sometimes used to measure the reduction of dienoic acid during the course of autoxidation (Fennema, 1996).

2.7.9 Fluorescence

Fluorescent compounds may develop from interaction of carbonyl compounds (produced by liquid oxidation) with constituents possessing free amino groups. Fluorescence methods provide a relatively sensitive measure of oxidation products in biological tissues (Fennema, 1996).

2.7.10 Chromatographic methods

Various chromatographic techniques, including liquid, thin layer, high-performance liquid, size exclusion, and gas, have been used to determine oxidation in oils or liquid-containing foods. This approach is based on the separation and quantitative measurement of specific fraction, such as volatile, polar, or polymeric compound or individual compounds, such as pentane or hexanal, that is known to be typically produced during autoxidation (Fennema, 1996).

2.7.11 Sensory evaluation

The ultimate test for oxidized flavor in foods is a sensory one. The value of any objective chemical or physical method is judged primarily on how well it correlates with results from sensory evaluation. The testing of flavor is usually conducted by trained or semitrained taste panels using highly specific procedure (Fennema, 1996).

2.7.12 Schaal oven test

The sample is stored at about 65^{o}C and periodically tested until oxidative rancidity is detected. Detection can be done organoleptically or by measuring the peroxide value (Fennema, 1996).

2.7.13 Active oxygen method (AOM)

This test is widely used. It involves maintenance of the sample at 98^{o}C while air is continuously bubbled through it at a constant rate. The time required to obtain a specific peroxide value is then determined (Fennema, 1996).

2.7.14 Rancimat method

Air bubbled through the oils as in the AOM test, and the increase in electrical conductivity due to generation of oxidation products is measured, usually at 100^{o}C, and expressed in terms of induction time (Fennema, 1996).

2.7.15 Oxygen absorption

The sample is placed in closed chamber and the amount of oxygen absorbed is determined and used as a measure of stability. This is done by measuring the time to produce a specific pressure decline, or by the time to absorb a pre-established quantity of oxygen under specific oxidizing conditions. This test has been particularly useful in studies of antioxidant activity (Fennema, 1996).

2.8 Control of rancidity

2.8.1 Use of antioxidants

With products made from or containing comminuted meat and fatty tissue antioxidants may be more useful. The rate of lipid oxidation can be effectively retarded by the use of antioxidants (Coronado et al., 2002). Antioxidants may react with highly reactive radical species to yield a second, less reactive free radical, or a non-radical species. This secondary radical is sufficiently stable to preferentially undergo chain termination rather than initiate further radical formation. Both synthetic and natural antioxidant typically include, as part of their molecular structure, an aromatic ring to delocalize the free electron of a radical and one or more hydroxyl porous to provide labile hydrogen atoms (Wei and Ho, 2006).

Autoxidation can be inhibited or retarded by adding a low concentration of an antioxidant (AH) which can interfere with either chain propagation or initiation

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

$$A^{\bullet} + ROO^{\bullet} \longrightarrow \text{non-radical product}$$

$$A^{\bullet} + A^{\bullet} \longrightarrow \text{non-radical product}$$

The free radical $A^{\bullet}$ derived from the antioxidant is stabilized by resonance and so does not participate in the propagation steps (Allen and Hamilton, 1994). According to reported, Mielnik et al. (2003) reported that effects of commercial rosemary extract on oxidative stability of mechanically deboned turkey meat (MDTM) compared with Trolox C (vitamin E), ascorbic acid (vitamin C) and control without antioxidant were investigated. Antioxidants were added to MDTM at three levels (low, medium, and high). Increased levels of TBA-reactive substances (TBARS) and volatile carbonyl compounds were noticed in all MDTM samples during storage, however most distinctly in MDTM without antioxidants. Retarding effect of antioxidants on the development of oxidation depended on the level and type antioxidants. Ascorbic acid was less efficient than Trolox C and Biolox HT-W (rosemary), but more potent than most rosemary extract in suppressing lipid oxidation especially in the long term frozen storage MDTM.

2.8.2 Vacuum or modified atmosphere packaging

The most obvious precaution to take against oxidative is to remove the source of oxygen. In most cases vacuum packing of meat or meat products, or modified atmosphere packaging in mixtures of carbon dioxide with nitrogen or oxygen, afford very satisfactory protection against color and rancidity problems.

Smiddy et al, (2002) introduction of vacuum and modified atmosphere packaging (MAP) techniques has solved some of the problems associated with the distribution of oxygen-sensitive foods. Packaging of precooked meats in an oxygen-free environment is effective in increasing the stability of the product oxidation.

2.8.3 Avoidance of pro oxidants

Similar to the removal of oxygen, it is an obvious yet sometimes overlooked precaution pre-caution, to eliminate or minimize pro-oxidants as far as possible. Substances and conditions which are well known to cause difficulty under practical conditions of use include (Allen and Hamilton, 1994).

- chlorine and chlorine-based cleaning agents, sanitizers etc.;

- ozone (e.g. from arc welding equipment);
- metal ions, especially iron and copper;
- free radicals-exposure to light, especially ultraviolet light, is highly destructive of the color of cooked cured meats and extreme cases may also be the cause of rancid flavors (Allen and Hamilton, 1994).

2.9 Types of antioxidants

2.9.1 Synthetic antioxidants

Some of the more popular synthetic antioxidants used are phenolic compounds. Synthetic phenolic antioxidants are always substituted by alkyls to improve their solubility in fat and oil. BHT, BHA, TBHQ, and ester of gallic acid e.g. propyl gallate (PG) in use are subjected to a good manufacturing practice limit of 0.02% of the fat or oil content of the food. Antioxidants that are heat stable have the properly referred to as carry through.

2.9.1.1 Butylated hydroxyanisole (BHA)

Butylated hydroxyl hydroxyanisole (MW, 180.24 melting point, 48-55^{o}C ; boiling point 264-270^{o}C) is mixture of 2-*tert*-butyl-4-methoxyphenol and 3-butyl-4-methoxyphenol, with the 3-isomer being 90% or more of the mixture. The molecule is a hindered phenol, and the *tert*-butyl groups *ortho* or meta to the hydroxyl group serves to suppress antioxidant activity. BHA has a strong phenolic odor that becomes particularly noticeable when and oils treated with these antioxidants are subjected to high temperatures. This water-insoluble, white, waxy solid is soluble in fats and oils. BHA is often produced in tablet form to prevent caking. BHA effectively controls the oxidation of animal fats, but is a relatively ineffective antioxidant in most vegetable oils. BHA provides good carry-through, which is the ability to be added to food, survive processing, and remain stable in food, especially in baked products. It is the most effective of all food-approved antioxidants for protecting the flavor and color of essential oils. BHA is also added to packaging materials, either being added directly to the wax used in making waxed inner liners or applied to the packaging as an emulsion. BHA exhibits antioxidant properties and

synergism with acids, BHT, propyl gallate, hydroquinone, methionine, lecithin, and thiodipropionic acids, etc (Branen et al., 2002).

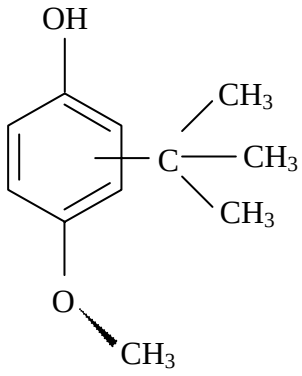

Figure 2.3 Butylated hydroxyanisole (BHA)

2.9.1.2 Butylated hydroxytoluene (BHT)

Butylated hydroxytoluene (2,6-di-tert-butyl-p-cresol; 2,6-bis(1,1-dimethylethyl)-4-methylphenol; MW, 220.34; melting point, 70°C; boiling point, 265°C) is a water insoluble, white, crystalline solid antioxidant that is more soluble in food oils and fats than is BHA. BHT is soluble in toluene, methanol, ethanol, isopropanol, methyl ethyl ketone, acetone, Cellosolve, petroleum ether, benzene, and most other hydrocarbon solvents. BHT is antioxidant for foods animal feed, petrol products, synthetic rubbers, plastics, animal, and vegetable oils, and soaps. While it is effective in animal fats, BHT is not effective in vegetable oils. BHT is frequently used in combination with BHA in foods because the two antioxidants are synergistic in their actions. BHT is noted for its high-temperature stability and its carry-through effect in fats or shortening in baked foods. However, BHT is less effective is less than BHA because of the greater stearic hindrance presented by two-tert-butyl groups surrounding the hydroxyl group in BHT. BHT is important as a food antioxidant it is readily soluble in glycerides, is insoluble in water and is susceptible to loss by volatilization and distillation under certain food processing conditions, such frying. BHT is often used in combination with other primary antioxidants (Branen et al., 2002).

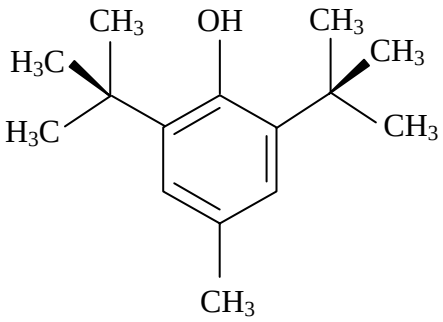

Figure 2.4 Butylated hydroxytoluene

2.9.1.3 Propyl gallate (PG)

Propyl gallate (3,4,5-tritrihydroxybenzoic acid propyl ester: MW, 212.20; melting point, 150°C; decompose above 148°C) is a white to light gray, crystalline antioxidant that is partially soluble in water, alcohol, ether, vegetable oils, and lard. Propyl gallate is used as an antioxidant in foods, fats, oils, ethers, emulsions, waxes, and transformer oils. This antioxidant is used to prevent rancidity in meat products. The low oil solubility of propyl gallate makes this antioxidant difficult to in corporate into fats and oils, and its solubility in water makes it more likely to complex with iron and iron salts, which causes dark dis coloration in some applications. Propyl gallate is usually used with citric acid to eliminate this unappealing discoloration. The low melting point of propyl gallate renders it ineffective at temperatures greater than 190°C for frying. As a result of heat labiality, propyl gallate provides little or no carry-through protection in many heat-processed foods. Propyl gallate is gallate is synergistic with BHA and BHT, and the combined effects provided storage stability and carry-through protection (Branen et al., 2002).

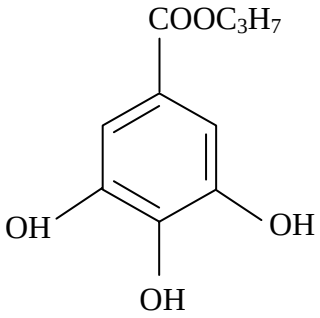

Figure 2.5 Propyl gallate

2.9.1.4 2-(1,1-Dimethylethyl)-1,4-Benzenediol

2-(1,1-Dimethyllethyl)-1,4-benzenediol (TBHQ; MW, 166.22: boiling point, 300^{o} C: melting point, 126.5-128.5^{o}C), also known as tertiary butylhydroquinone, is the most recently developed major phenolic antioxidant for food use. TBHQ is a white to light tan crystalline solid that effectively increases oxidative stability (shelf life) of polyunsaturated food fats and oils. Features that make this antioxidant favorable are its moderate solubility (5-10%) in fats and oils, its slight (1%) water solubility and its lack of discoloration with metals, such as iron. TBHQ is the best antioxidant for protecting frying oils against oxidation, and it provides good carry-through to the finished product. TBHQ also improves the color and stability of hydrogenated fats. TBHQ used in combination with citric acid further enhances its stabilizing properties, primarily in vegetable oils, shortenings, and animal fats. However, combination of TBHQ with propyl gallate is not permitted (Branen et al., 2002).

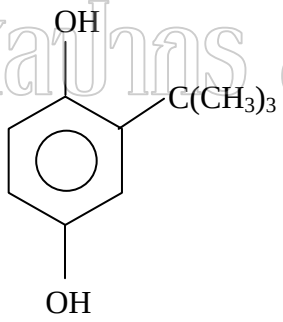

Figure 2.6 Tertiary butylhydroquinone

2.9.2 Natural antioxidants

Natural antioxidants are found in almost all plants, microorganisms, fungi, and even in animal tissues. The majority of natural antioxidants are phenolic compounds and the most important groups of natural antioxidants are tocopherols, flavonoids, and phenolic acids.

2.9.2.1 Citric acid

Citric acid, 2-hydroxy-1,2,3-propane-tricarboxylic acid (MW, 192.12), is highly soluble in water and primarily insoluble in fats. Citric acid was

found in almost all plants and animal species. It can chelate metal ions by forming bonds between the metal and carboxyl or hydroxyl groups of the citric acid molecule. Citric acid was very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorization (Hars et al., 2000). Although citric acid can counteract the pro-oxidant effect of iron, it can act as a synergist in the presence of phenolic synthetic antioxidant when no metallic accelerators are present. At least two free carboxylic groups are necessary for antioxidative potency. Although decomposed by heat, the thermal decomposition products are also good synergists (Branen et al., 2002).

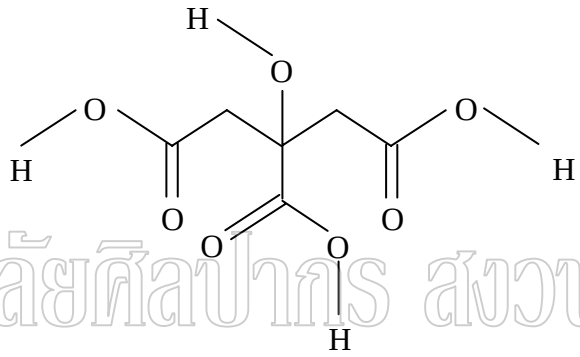

Figure 2.7 Citric acid

2.9.2.2 Tocopherol

Tocopherols are the natural antioxidant mixture of alpha, gamma, and delta. This yellow to amber, nearly odorless, clear, viscous oil that oxidizes and darkens in air and on exposure to light, insoluble in water, is freely soluble in alcohol, and is miscible with acetone, chloroform, ether, fats, and vegetable oils. Tocopherols are the most important natural antioxidants found in vegetable oil-derived food. Generally, they were lost during refining, deodorizing, and processing operations. At high concentrations and in presence of traces of iron and copper salts, tocopherols may act as pro-oxidants. The antioxidant activities range in the order: delta tocopherol (most effective) > gamma tocopherol > beta tocopherol > alpha tocopherol (least effective) (Fennema, 1996). α-tocopherol is an effective antioxidant in food and biological system. That is an extremely efficient inhibitor of free radical chain

reactions. α-tocopherol inhibits this free radical oxidation by reacting with the peroxyl radicals to stop chain propagation, and with the alkoxyl radicals to inhibit the composition of the hydroperoxides and decrease the formation of aldehydes. Thus α-tocopherols behave as a chain-breaking antioxidant by competing with the substrate for the chain-carrying peroxyl radicals, normally present in the highest in the system. The tocopherol radical can form non-radical products, including dimmers, stable peroxides, alkyl or unsaturated derivatives whereby the antioxidant is regenerated (Frankel, 1996). However, tocopherols may exert a prooxidant effect at high concentrations and their activity is temperature-dependent.

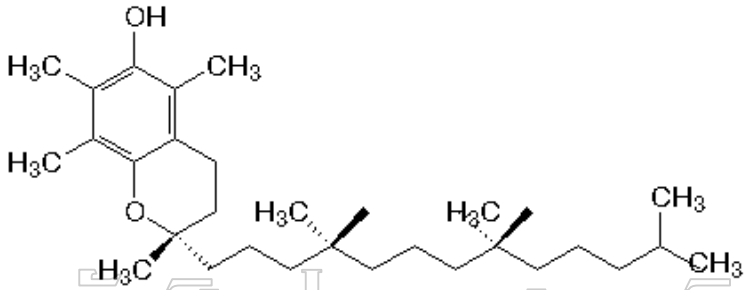

Figure 2.8 Vitamin E (α-tocopherols)

2.9.2.3 Rosemary extract

The antioxidant properties of rosemary, *Rosmarinus officinalis* L. (Labiatae), have been known for centuries (Richheimer et al., 1996). Several phenolic compounds with antioxidants activities have been isolated and identified from rosemary leaves. Carnosol, rosemanol, carnosic acid, and rosemaridiphenol have the same structural backbone of vicinal diphenol (Chen et al., 1992). There also are several reports that identify the compounds that are chiefly responsible for the antioxidant properties of rosemary extracts and that establish carnosic acid (CA) as the major phenolic diterpene present in fresh rosemary. It also is known that CA is converted to carnosol (CAR) upon heating and that CAR can degrade further to produce other compounds, such as rosmanol and 7-oxy derivatives of rosemanol. Because some commercial rosemary preparation contain significant amounts of these CA by product, it is important to know the relative antioxidant activity of all phenolic

diterpenes present in rosemary products, so that a total antioxidant index can be determined for products containing a mixture of several different antioxidant compounds (Richheimer et al., 1996). Carnosic acid and carnosol were effective as BHT and that their effectiveness was concentration dependent. Carnosic acid and carnosol showed the ability to chelate iron and were effective radical scavengers of peroxyl radicals. It has been established that the molecules of carnosol and the radicals formed from them participate in the reactions of chain inhibition and propagation to a much lower degree than is the case with most natural and synthetic antioxidants (Pokorny et al., 2001). Richheimer et al. (1996) reported that, the induction time of lard with rosemary extract compared other antioxidant. Longer induction time suggest stronger antioxidant activities both carnosol and carnosic acid had stronger antioxidants BHT and BHA.

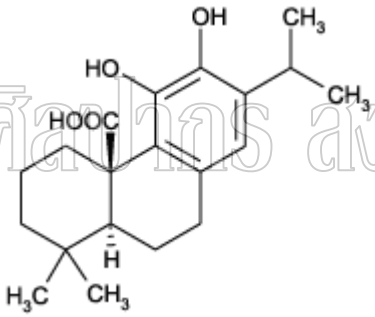

Figure 2.9 Carnosic acid

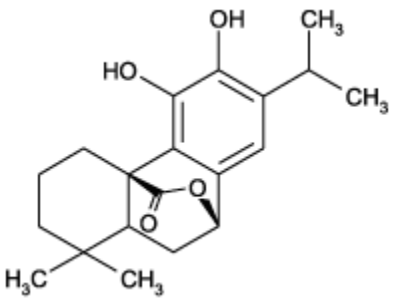

Figure 2.10 Carnosal

2.9.2.4 Milk mineral

Dried milk minerals (MM), the dried permeate of ultra-filtered whey, has antioxidant properties in cooked meats, apparently by iron-chelation to colloidal phosphate. The composition: 92% mineral, 1-5% protein, and 1-10% lactose. That contain about the 33-36% polyphosphate which inhibits oxidation by chelating soluble iron (Jayasingh and Cornforth, 2003).

2.9.2.5 Ascorbic acid

Ascorbic acid (3-oxo-L-guiofuranolactone; MW, 176.12) is a crystalline substance that decomposes near 160^{o}C. This natural antioxidant, which is extremely insoluble in fats, was first used as an antioxidant to improve the stability of mayonnaise. The synergists' antioxidant effect of ascorbic acid can be ascribed partly to the binding of metal ions. The free acid acts as a synergist with most phenolic antioxidant, but not with gallic acid (Branen et al., 2002). Ahn and Nam (2004) reported that, ascorbic acid is a reducing agent, which inhibited myoglobin oxidation and brown color development in non-irradiated beef.

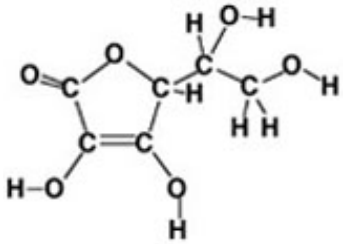

Figure 2.11 Ascorbic acid

2.9.2.6 Tea catechins

Tea catechins (TC), a predominant group of poly phenols present in green tea leaves (*Camellia sinensis* L.). Green tea has been acclaimed for its antioxidant properties, attributed to the presence of tea catechins (TC) including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). Tea catechins inhibit lipid oxidation in lard, canola oil,

rapeseed oils, marine oils, emulsified lipid systems, model food emulsion, and fish muscle model systems (Tang et al., 2001).

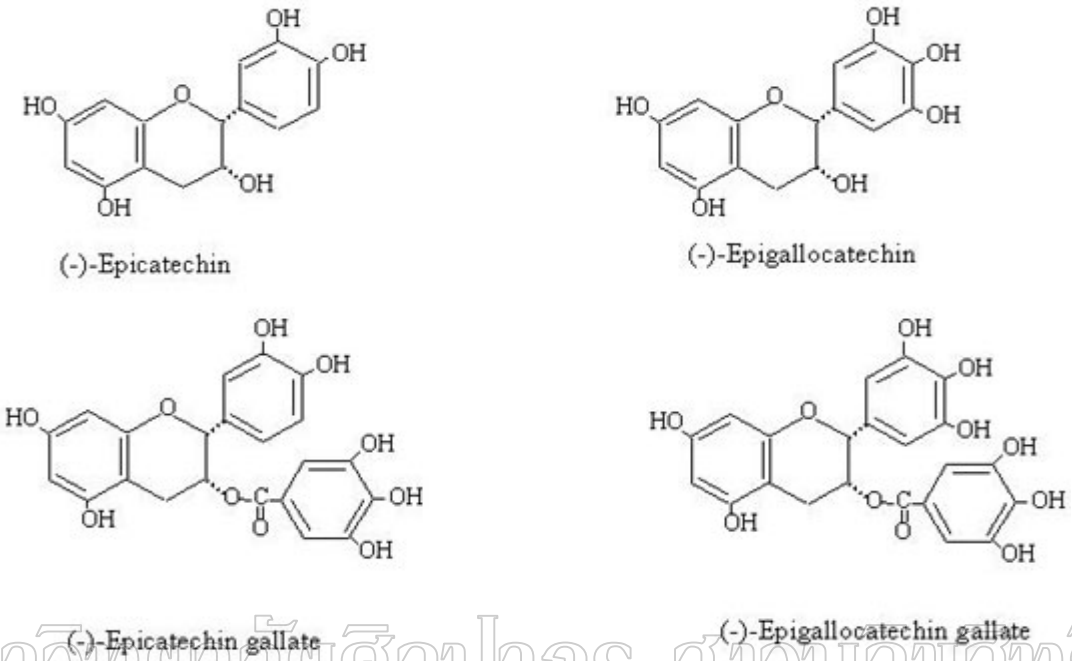

Figure 2.12 Tea catechins

Idea antioxidants meet the following demands: (Allen and Hamilton, 1994)

1. Safe in use,
2. Should impart no odor, flavor or color,
3. Effective at low concentration,
4. Should be easy to incorporate,
5. Should survive cooking processes such as baking and frying,
6. Should be available at a low cost in use.

2.10 Antioxidant effect

Antioxidants in food may be defined as any substance which is capable of delaying, retarding or preventing the development in food of rancidity or other flavor

deterioration due to oxidation. Antioxidants delay the development of off flavor by extending the induction period. Addition of antioxidants after the end of this period tends to be ineffective in retarding rancidity development.

Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, in which case the compound is described as a primary antioxidant, or by a mechanism that does not involve direct scavenging of free radicals in which case the compound is a secondary antioxidant. Primary antioxidants include phenolic compounds such as vitamin E (α-tocopherol). These components are consumed during the induction period. Secondary antioxidants operate by a variety of mechanisms including binding of metal ions scavenging oxygen, converting hydroperoxides to non radical species, absorbing UV radical or deactivating singlet oxygen. Normally, secondary antioxidants only show antioxidant activity when a second minor component is present (Pokorny et al., 2001).

Synergistic antioxidant, antioxidants are combined to take advantage of their different types of effectiveness. Specific combinations avoid or minimize solubility or color problems presented individual antioxidants; combinations permit better control and accuracy of application; combinations enable more complete distribution or solution of antioxidants and chelating agents in fats and oils; some combinations of antioxidants are more convenient to handle than individual antioxidant compound; and some provide synergistic effects offered by some antioxidant combinations.

The mechanisms of synergism vary, and while part of the activity of synergists is due to their inactivation of pro-oxidant metals, they may also function by inhibiting the decomposition of peroxides.

Many low molecular weight hydroxyl acids or amino acids exhibit synergistic activity. Among synergistic antioxidants are substituted mercaptopropionic acids, such as 3,3-thiodipropionic acid, phospholipids, citric acid, ascorbic acid, and phosphoric acids. As stated, mixtures of primary antioxidants, such as propyl gallate and BHA and mixtures of BHA and BHT are used synergistically in some food systems (Branen et al., 2002).

Carry-through, the ability of various antioxidants to survive cooking processes referred to as their carry-through property (Allen and Hamilton, 1994).

2.11 Changes during boling

For cooked meat, thermal processes can promote lipid oxidation by disrupting cell membranes and releasing pro-oxidants, thereby inducing warmed-over flavor during refrigerated storage (Pena-Ramos and Xiong, 2003). Boiling is very common procedure for food preparation. In this case boiling water transfer heat, it is useful to added food to hot water to shorten the time for enzyme deactivation, especially the deactivation of oxidoreductase. During boiling, the antioxidant activity of proteins is affected because of their denaturation. The effect on antioxidant is similar to that occurring during sterilization. The heat denaturation of heme pigments in foods of animal origin could increase the pro-oxidative effect of ion and thus reduce the activity of antioxidants. During boiling, antioxidants are partially extracted and remain in the boiling water. If the boiling water is not used but discarded these antioxidants are lost (Pokony et al., 2001).

2.12 Changes during storage

However during storage, quality attributes of the product deteriorate due to lipid oxidation. Lipid oxidation is responsible for reduction in nutritional quality as well as change in flavor (Sallam et al., 2004). Storage of meat can lead to the development of off-flavor in raw meat and warmed-over flavor in cooked meat (Sarraga and Regueiro, 1999). During frozen storage of MDCM extensive lipid oxidation occurs, resulting in decreased functionality of the meat. However, the storage life of MDCM can be extending through the use of antioxidant (Mielnik et al., 2003).

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CHAPTER 3

POTENTIAL NATURAL ANTIOXIDANTS UTILIZED IN MECHANICALLY DEBONED CHICKEN MEAT (MDCM)

ABSTRACT

Effects of types, concentrations of antioxidants, and storage duration to retarded lipid oxidation in MDCM were investigated. BHT, rosemary, vitamin E, milk mineral, and combination were individually added in MDCM at three different levels (1000, 3000, and 5000 ppm) to figure out the optimum usage in this raw material. Thiobarbituric acid (TBA) assay was used to assess the effects of antioxidant on lipid oxidation of MDCM during 3 days for chill storage and 28 days for frozen storage. An increase in TBA value was noticed in all samples during storage, however most distinctly in sample without antioxidants. Retarding effect of antioxidants on the development of lipid oxidation depended on the concentration and type of antioxidants. Rosemary at 5000 ppm exhibited the most effectiveness to delay ($p<0.05$) lipid oxidation in MDCM at 3 days of chill storage by 79.76%, about 77.82% when used mix synthetic antioxidants. In contrast, mix synthetic antioxidant (combination) at 5000 ppm. Would be the most potential compounds utilized in MDCM when kept in frozen state. It could decrease the lipid oxidation about 54.06% as compared to the control.

Key words: mechanically deboned chicken meat, antioxidants, lipid oxidation, TBA value

INTRODUCTION

Mechanically deboned chicken meat (MDCM) is the raw material removed from the skeleton bone tissues by grinding the starting materials such as frame, backs, necks by passing it through a sieve under high pressure (Froning and McKee, 2001). During the grinding and separating operations, a certain amount of bone marrow and flour simultaneously contaminate and gel into the meat (Essary, 1979). MDCM normally exhibits red in color and appears as a finely ground paste like product (Instituted of Food Technologists, 1979). Yield and composition of MDCM varies from 55 to 80% depending on the origin of raw material, i.e. skin tissues increase the lipid fraction and conversely the protein fraction decreases, bone to meat ratio, age, and species of bird, cutting method, and deboner setting (Froning and McKee, 2001; Mielnik et al., 2002).

One of the most important components affecting the chemical and physical attributes of MDCM is bone marrow (Froning, 1976). In general, the mechanical deboner process produces considerable cellular disruption and releases hemoglobin and lipids from the bone marrow (Froning and McKee, 2001). These lipid components from the bone marrow account for an increase in fat content of MDCM, dilute noticeably the protein content, and pursue the lipid oxidation in the meat. Thus, heme/lipid interaction also plays an important role affecting oxidative stability of MDCM (Froning, 1976) since the iron in heme can be a potential catalyst in an initial period of lipid oxidation. However, some researches proposed that MDCM contains good nutritional and functional properties and is suitable for the formulation of many types of meat products such as various frankfurters, loaf products, fermented sausages, and restructured chicken products (Mielnik et al., 2002).

Lipid oxidation occurs easily in poultry because its fat contain highly unsaturated. It is known that heat treatment enhances lipid oxidation (Beltran et al., 2003). The polyunsaturated fatty acid located primarily in the phospholipids derived from the bone marrow is the major factor promoting the autoxidation (Mielnik et al., 2002). Thus, MDCM is highly susceptible to oxidative deterioration due to the extensive stress and aeration during the machine deboning process and the compositional nature such as bone marrow, heme, and lipids.

During frozen storage, lipid oxidation and microbial growth in MDCM extensively occurs and ultimately results in a decrease functionality of the meat, reduction in nutritional quality as well as changes in flavor and color (Mielnik et al., 2003; Sallan et al., 2004).

However, the storage life of MDCM can be extending with the use of antioxidants (Hassan and Fan, 2004). Various synthetic antioxidants such as BHT, BHA and propyl gallate have been utilized to retard the development of rancidity, and thus extend their shelf life (Aguirrezabal et al., 2000). However, because of potential health hazards, the use of synthetic antioxidants in food products in many countries is either forbidden or under strict regulation. Therefore, there is an increased demand for safe natural ingredients that can extend the shelf life of both processed and unprocessed meat products (Lanari et al., 2004). Natural antioxidants extracted from plants such as rosemary, sage, sesame seed, various vegetable, and grapes can be used as alternatives to the synthetic antioxidants because of their equivalent or greater effect on inhibition of lipid oxidation (Aguirrezabal et al., 2000).

The objectives of this experiment was to select an appropriate natural antioxidant used in MDCM and determine the optimum usage level of antioxidants to able retard lipid oxidation in MDCM under chilled and frozen storage conditions.

MATERIALS AND METHODS

Preparation of sample

MDCM was produced in sausage plant of BETTER FOODS CO., Ltd. Chicken carcasses were stored overnight at 0-4^{o}C before deboning process. Chicken carcasses with back and neck bones were passed through a mechanical deboner (model POSS-PDE 1500, Holland). Each 20 kg of cold MDCM was individually packed in plastic bags for further usage. Four different antioxidants (BHT, rosemary extract, Milk mineral, and α-tocopherol) and a mixture (BHT, BHA, propyl gallate, and citric acid at ratio 2:2:1:1) were individually added into the chilled MDCM using a kitchen aid. Three different usage levels (1000, 3000, and 5000 ppm.) were applied in this experiment. Each batch of 200 g-MDCM was blended at high speed for 5 min. To solve the speadability of small amounts of antioxidant, 3 ml of triacetin was use to

dilute the antioxidant before mixing with MDCM. The control treatment would contain only 3 ml of triacetin without antioxidant added. Each meat treatment was then divided into small portions (approximately 30 g). The meat samples were individually packed in plastic bag and then stored at two different conditions; chilled storage at 4 $\pm$ 1^{o}C for 3 days, and frozen condition at -18^{o}C for 28 days. The meat samples were randomly selected to measure the TBA test daily for chilled samples and weekly for frozen samples.

Proximate analysis

The MDCM samples were analyzed to determine the chemical components in triplicate. Moisture content was measured by Infrared moisture analyzer. Crude protein content was determined by Kjeldahl nitrogen determination. Crude fat content was determined by Soxhlet Etraction. Ash content was determined by Dry Ashing. Total saturated fatty acid analyses were done according to the method of National Food Institute T 974 based on AOAC (2000), 963.22, 969.33 by GC. Iron (Fe) analyses were done according to the method of National Food Institute T 965 based on AOAC (2000), 985.35 by Atomic Absorption.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Jayasingh and Cornforth (2003). Duplicate samples of mechanically deboned chicken meat (2.0 $\pm$ 0.1 g) were mixed with 10 ml of stock solution containing 0.375 % TBA, 15% TCA, and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (100^{o}C) to develop a pink color, cooled in tap water and then centrifuged at 8000 rpm for 20 min (Centrifuge, Hettich type Universal 16). The absorbance of the supernatant was measured spectrophotometrically at 532 nm (UV-Visible Spectrophotometer, Genesys type 10 uv) against a blank that contained all the reagents minus the meat and stock solution to blanks. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56 x 10 ^{5} M^{-1} cm^{-1}. The MDA concentration was converted to TBA number (mg MDA/kg meat sample) as follow;

TBA NO. (ppm) = Sample A_{532} $\times$ 2.77

Statistical Analysis

Statistic analysis were performed using General Linear Model Program (GLM) to test the effects of various antioxidants, concentration of antioxidants, condition, and time of stored as compared with a control sample (without antioxidant). Augmented Factorial was used to estimate the significant differences among of each treatment at 5% the probability level using SAS program (Ver. 8.1, SAS Inst., Cary, NC, USA).

RESULTS AND DISCUSSIONS

Proximate composition

MDCM known as a value added by-products was normally used in sausage and meat ball productions. It absolutely retained from the meat with bone and cut parts with lower commercial value. In deboning process, after the removal of the usual meat cut, there always was meat which was firmly attached to the bones (Trindade et al., 2004). Regarding to this point, this raw material usually contained a great variety of chemical components released from bone fragments, bone marrow, and blood. This made the MDCM exhibiting high amounts of lipid, meat mineral, such as copper, calcium and iron, when compared to the regular ground meat. In this experiment, MDCM from the deboner was a byproduct primarily received from the back and neck without skin of whole chicken carcasses.

Froning and McKee (2001) had examined on proximate MDCM compositions from chicken backs and necks and found that MDCM samples comprised 9.3-14.5% of protein, 63.4-66.6% of moisture, 14.4-27.2% of lipid (by wet basis). The nearly same results were reported by Macneil et al. (1978). In addition, some important selected nutrient contents of MDCM (net weight basis) were also investigated to explain the chemical and physical changes of MDCM; for instance, the MDCM considerably contained 0.95% of ash, 1.76 mg/100g of iron, 1.77 mg/100g of zinc, and 126.7 mg/100g of calcium. This showed that MDCM normally contained high meat mineral and was greatly susceptible to lipid oxidation during its storage.

The proximate compositions of our MDCM were presented in Table 3.1. The percentages of MDCM fat and protein was 27.17% and 14.66%, respectively. It

potentially increased up to 61.13%, and 35.25%, respectively as compared to the MDCM from Froning and McKee (2001). According to Najdawi and Abdullah (2002), MDCM with skin and without skin in contained high lipid content when compared to the regular meat since the major fat was directly extracted from bone marrow. Normally, the lipid content of chicken bone marrow was approximately 46.5%. Release of fat from skin and bone marrow elevated overall fat content in and this raw material simultaneously decreased the amount of protein. As expected, ash content of MDCM also increased 97.72%. The high ash resulted by bone particles incorporated into the meat (Najdawi and Abdullah, 2002). It was noticeable that iron content in MDCM increased 10.20%. This larger iron observed could be attributed to the presence of heme released from bone marrow (Crosland et al., 1995). Thus, it was not curious that MDCM was so pinky paste in characteristic.

Table 3.1 Proximate composition of mechanically deboned chicken meat

Proximate composition	**% Wet basis**
Moisture content	56.10 %
Total saturated fatty acid	4.49 mg/100 g
Iron (Fe)	19.62 mg/kg
Crude Fat	27.17 %
Crude Protein	14.66 %
Ash	94.81 ppm

The moisture of our MDCM was 56.10%. Regarding to the preliminary study, it found that A_w and pH of MDCM was about 0.981-0.985 and 6.64-6.66, respectively. The rate of oxidation depended strongly on water activity. In general, dried foods with very low moisture (a_w values of less than about 01), oxidation proceeded very rapidly. Increasing the a_w to about 0.3 retarded lipid oxidation and other produced a minimum rate. This protective effect of small amounts of water was believed to occur by reducing the catalytic activity of metal catalysts, by quenching free radicals, and/or by impeding access of oxygen to the lipid. At somewhat higher water activities (a_w about 0.55-0.85), the rate of oxidation increased again,

presumably as a result of increased mobilization of catalyst and oxygen (Fennema, 1996). It caused MDCM so sensitive to be off odor and off color. The total saturated fatty acid content was 4.49 mg/100 g. Jantawa and Dawsons (1979) studied lipid oxidation in MDCM, they reported that the predominant fatty acid of the triglyceride fraction were palmitic, stearic, oleic and linoleic acid. The phospholipid fraction contained higher levels of 18 carbon saturated and 20:3 to 22:6 carbon polyunsatulated fatty acid than did the triglyceride fraction.

The color of MDCM was L^* 48.60, a^* 8.84, and b^* 14.44. Mechanical deboning of chicken was influenced the color of resultant meat. The process released heme pigments from the bone marrow into the MDCM and resulted in. Increasing the heme protein. This increase was primarily due to hemoglobin from the bone marrow. Hemoglobin was more subject to abnormal color problems since it was more easily oxidized and more susceptible to heat denaturation during processing and storage. Abnormal brown, green, and gray color defects had been reported in further-processed chicken meat products containing MDCM. During the deboning process the meat was exposed to considerable air, which might accelerate the oxidation of heme pigments (Froning and McKee, 2001).

Najdawi and Abdullah (2002) reported that MDCM was highly susceptible to oxidative deterioration. This was related to the release of heme, oxidative enzymes and incorporation of oxygen into the product during mechanical deboning, promoting auto-oxidation of polyunsaturated fatty acids (PUSFA) in the phospholipids content of poultry tissue. Factor effecting MDCM lipid oxidation included: fat content, PUFSA, metals e.g. iron (Fe) and copper (Cu), heme catalysts and enzymes such as lipoxygenase or cycloxygenase.

MDCM had pH value between 6.64-6.66. Trinded et al. (2004) reported that MDCM presented higher pH than manually deboned meats, in general as a result of the incorporation of red marrow, in which pH ranges from 6.8 to 7.4. The pH of manually deboned meat lied between 5.8 and 5.9 for the breast and 6.2 and 6.3 for thigh, whereas MDCM had values between 6.5 and 7.0. These high pH values favored the water holding capacity, but on the other hand contributed to increase in the bacterial load, speeding up the spoilage process. Ostovar et al. (1971) also reported that psychrophilic microorganisms did account for a high percentage of total

microflora of raw chicken and contribute to low temperature. The average microbial counted for MDCM increased after each step of processing, increased temperature, surface area of the product and improper cleaning of the equipment after usage.

Lipid oxidation of MDCM was more importance than spoilage of MDCM from microbial growth because several of these products were mixture of meat from various spices and preservatives. Parts to be deboned should be fresh and held at near freezing temperature (0-4^{o}C). After deboning, MDCM needed be incorporate into a formulated product within a one day period unless frozen and should not be held longer than 30 days in frozen storage. Its use required good quality assurance guidelines to avoid speeding up the spoilage of MDCM from microbial growth.

Froning and McKee (2001) reported that factors influencing the composition included bone to meat ratio, age of the chicken, skin content, cutting methods, deboner setting, and species. In general, younger chickens generally would have more heme and lipid component from the bone marrow influencing the proximate composition. Skin content might greatly increase the fat content. Deboner setting could affect the yields and the proximate composition. If the setting was set for high yields, the fat and ash content in MDCM might be largely increased. In addition, composition of MDCM varied depending on the origin of raw material, i.e. skin tissues increase the lipid fraction and conversely the protein fraction decrease (Nagrao et al., 2005). Henckel et al. (2004) reported that the chemical composition of MRM meat was subjected to an extremely large variability and relied on spices, breed, and age of the animal as well as carcass parts used, degree of trimming, machine type, and machine setting. The fat present in MDCM was rich in poly-unsaturated fatty acids due to the presence of phospholipids, originating in the fraction of the bone and spinal marrow accompanying its (Gomes et al., 2003). Najdawi and Abdullab (2002) reported ash content of MDCM was higher than HDCM. The high ash values were likely to be result of bone particles incorporated into meat. Another aspects, Trindade et al. (2004) evaluated total saturated fatty acid and total unsaturated fatty acid were 31.8% and 68.2%, respectively.

Chilled storage condition

The effect of different antioxidant, concentration of antioxidant and storage condition on MDCM shown in Table 3.2. The interaction between different antioxidant and various concentration at chilled storage condition was shown in Figure 3.4 (a, b, and c). In this present study, therefore, chilled storage condition of MDCM for a long time increased lipid oxidation. At chilled storage TBA value increased both in control and treatment added some antioxidants. The sample with antioxidant could be successfully impeded the formation of malanaldehyde in MDCM in chilled storage condition. However, TBA value of MDCM in control group was higher than MDCM treated with antioxidant group. The differences in TBA value between the control and the combination, rosemary, vitamin E, milk mineral, and BHT at day 1, 2, and 3 were significantly ($p<0.05$) (Table 3.3). From the study, rosemary had shown the most effective in decrease of TBA value in MDCM at 3 days by decrease up to 79.76%, 78.34% for combination, 73.92% for BHT, 67.32% for vitamin E, and 59.27% for milk mineral. Rosemary exhibited good quality to inhibit lipid oxidation in MDCM. The antioxidative substances of rosemary were phenolic compounds that neutralized free radicals by donating a hydrogen atom, but there was evidence that rosemary also acted as a metal chelator (Beltran et al., 2003). The other study, the rosemary spices contained a number of compounds including rosemanol, carnosol, rosmaridiphenol, and rosemariquinone, which imparted antioxidant activity by acting as free radical scavengers to protect fat from oxidation by absorbing or binding oxygen in a manner similar to that of synthetic antioxidant (e.g. BHA and BHT) (Lee et al., 1996).

Considering other antioxidants, BHT was a synthetic antioxidant for foods, animal feed, animal and vegetable oils and soap. While it was effective in animal fat, BHT was not as effective in vegetable oils. BHT was frequently used in combination with BHA in foods because the two antioxidants were synergistic in their action (Fennema, 1996).

Vitamin E (α-Tocopherols) was highly potent antioxidant that was widely used as a natural alternative to BHA and BHT and reported that addition of vitamin E at a level of 300 mg/kg significantly inhibited lipid oxidation in cooked beef and chicken compared with control sample (Mielnik et al., 2003).

Dried milk mineral had also antioxidant properties in cooked meat, apparently by iron-chelation to colloidal phosphate. That contained about the 33-36% polyphosphate which inhibited oxidation by chelating soluble iron (Jayasingh and Cornforth, 2003). In this study, major mineral in MDCM was iron. In general iron was the pro-oxidant affecting of transition metal ion in MDCM. Thus, polyphosphate in milk mineral was found to deactivate metal ions by chelation. However, MDCM adding milk mineral obtained higher TBA value than other treatment.

The study shown the concentration of antioxidant affected to the rate of lipid oxidation. Samples with higher concentration of antioxidant revealed significantly ($p<0.05$) lower TBA value than sample with low concentration of antioxidant. There were tendencies of decreasing lipid oxidation in the MDCM containing the highest concentration of antioxidants (Table 3.4).

Frozen storage condition

The effects of different antioxidant, concentration and frozen storage condition were shown in Table (3.5). The interaction between different antioxidant, concentration of antioxidant at frozen storage condition was shown in Figure 3.4 (a, b, and c). Considering of storage time of MDCM at frozen storage condition, TBA values for all samples increased ($p<0.05$) during storage. In this study, after 1 week of frozen storage, TBA value of samples with antioxidant added was lower than control. After 4 weeks, the significantly highest TBA value of control sample was found. Also, samples with various antioxidants revealed significantly higher TBA value than 3 week and 2 week, respectively.

In this present study, the effect of antioxidant type on the TBA value of MDCM during frozen storage condition at different time was shown in Table (3.6). The study shown that the use of antioxidant in frozen MDCM could decrease ($p<0.05$) TBA value. From this study, the combination was the most effective antioxidant to decrease lipid oxidation for 28 days by decrease up to 54.06%, 47.97% for rosemary, 44.46% for BHT, 42.07% for vitamin E, and 18.45% for milk mineral. The effective of combination might be largely attributed to the synergistic effect of the antioxidants BHA, BHT, propyl gallate, and citric acid (Lee and et al., 1996). Chen et al. (1992) studied antioxidant activity of rosemary by the rancimat method.

They presented induction time of lard with rosemary, both carnosol and carnosic acid had stronger antioxidant activities than the commonly known antioxidants BHT and BHA. Vitamin E supplementation resulted in chicken meat being protected against oxidation during frozen storage and cooking. This was in agreement in with this study which had lower TBARS values in vitamin E supplemented meat than control meat in all samples (Sullivan et al., 2004).

The effect of antioxidant concentration on lipid oxidation by measured TBA value was reported. The study showed that the higher concentration of antioxidant. The more decrease on TBA value of frozen MDCM for 28 days ($p<0.05$) (Table 3.7). There were tendencies of decreasing lipid oxidation in the samples containing the highest concentration of antioxidant.

CONCLUSIONS

The effects of different antioxidants, concentrations, storage duration on the rate of lipid oxidation were investigated. The TBA value of MDCM added antioxidants and control increased for a longer storage time. The lipid oxidation rate tremendously decreased with higher concentration of antioxidant applied. The induction time for lipid oxidation in MDCM with antioxidant added would depend on type and concentration of antioxidant applied prior to chilled and frozen storage. Rosemary extract, a natural phenolic antioxidant, was the most effective to inhibit lipid oxidation in chilled MDCM. In contrast, the combination is a mixture of antioxidant (BHA, BHT, propyl gallate, and citric acid) appeared most effective to inhibited lipid oxidation in MDCM revealed by TBA value under frozen storage condition. The TBA value of MDCM depended on concentration of antioxidant. The highest concentration of antioxidant decrease TBA value in all sample compared control sample. The storage condition and time of storage have effect to TBA value of sample. For along time of storage was increase TBA value and frozen storage condition was decrease TBA value because during frozen storage of MDCM extensive lipid oxidation occurs, resulting in decreased functionality of the meat. TBA value was not significantly altered by freezing, possibly freezing protected MDCM from lipid oxidation.

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Table 3.2 The effect of antioxidant on the TBA value of MDCM during chilled storage condition.

Antioxidants	Concentration (ppm)	Time (day)			
		0	1	2	3
BHT	1000	0.152 (0.004)	0.212 (0.010) ^{a}	0.214 (0.004) ^{gh}	0.218 (0.002) ^{f}
	3000	0.152 (0.004)	0.183 (0.004) ^{b}	0.194 (0.004) ^{i}	0.208 (0.004) ^{g}
	5000	0.152 (0.004)	0.146 (0.009) ^{e}	0.174 (0.002) ^{k}	0.179 (0.002) ^{j}
Milk mineral	1000	0.152 (0.004)	0.176 (0.001) ^{bc}	0.434 (0.002) ^{b}	0.344 (0.004) ^{b}
	3000	0.152 (0.004)	0.169 (0.004) ^{c}	0.391 (0.004) ^{c}	0.310 (0.004) ^{c}
	5000	0.152 (0.004)	0.126 (0.001) ^{f}	0.384 (0.002) ^{c}	0.290 (0.002) ^{d}
Rosemary	1000	0.152 (0.004)	0.101 (0.005) ^{g}	0.221 (0.002) ^{g}	0.198 (0.006) ^{h}
	3000	0.152 (0.004)	0.061 (0.000) ^{hi}	0.211 (0.004) ^{h}	0.158 (0.000) ^{k}
	5000	0.152 (0.004)	0.057 (0.002) ^{ij}	0.189 (0.008) ^{ij}	0.111 (0.004) ^{l}
Vitamin E	1000	0.152 (0.004)	0.097 (0.007) ^{g}	0.323 (0.005) ^{d}	0.315 (0.002) ^{c}
	3000	0.152 (0.004)	0.104 (0.002) ^{g}	0.252 (0.000) ^{e}	0.270 (0.001) ^{e}
	5000	0.152 (0.004)	0.068 (0.002) ^{h}	0.235 (0.003) ^{f}	0.172 (0.000) ^{j}
Combination	1000	0.152 (0.004)	0.069 (0.004) ^{h}	0.193 (0.002) ^{i}	0.190 (0.002) ^{i}
	3000	0.152 (0.004)	0.069 (0.000) ^{h}	0.185 (0.002) ^{j}	0.158 (0.000) ^{k}
	5000	0.152 (0.004)	0.049 (0.002) ^{j}	0.171 (0.002) ^{k}	0.153 (0.004) ^{k}
Control	-	0.152 (0.004)	0.157 (0.005) ^{d}	0.495 (0.002) ^{a}	0.771 (0.004) ^{a}

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdefghijk} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 3.3 The effect of antioxidant types on the TBA value of MDCM during chilled storage condition.

Antioxidants	Time (day)			
	0	1	2	3
BHT	0.153 (0.003)	0.180 (0.030) a	0.194 (0.018) e	0.201 (0.018) d
Milk mineral	0.153 (0.003)	0.157 (0.024) b	0.403 (0.024) b	0.314 (0.025) b
Rosemary	0.153 (0.003)	0.073 (0.022) d	0.207 (0.015) d	0.156 (0.039) f
Vitamin E	0.153 (0.003)	0.089 (0.018) c	0.270 (0.042) c	0.252 (0.065) c
Combination	0.153 (0.003)	0.062 (0.011) e	0.183 (0.010) f	0.167 (0.018) e
Control	0.153 (0.003)	0.157 (0.006) b	0.495 (0.002) a	0.771 (0.004) a

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdef} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 3.4 The effect of antioxidant concentrations on the TBA value of MDCM during chilled storage condition.

Concentrations	Time (day)			
	0	1	2	3
0	0.153 (0.003)	0.157 (0.057) [a]	0.495 (0.095) [a]	0.771 (0.067) [a]
1000	0.153 (0.003)	0.131 (0.053) [b]	0.277 (0.080) [b]	0.253 (0.064) [b]
3000	0.153 (0.003)	0.117 (0.042) [c]	0.246 (0.084) [c]	0.221 (0.063) [c]
5000	0.153 (0.003)	0.089 (0.006) [d]	0.230 (0.002) [d]	0.181 (0.004) [d]

numerical number in the table presented $\bar{x}$ (SD)

n = 0

[abcd] means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 3.5 The effect of antioxidant on the TBA value of MDCM during frozen storage condition.

Antioxidants	Concentration (ppm)	Time (day)				
		0	7	14	21	28
BHT	1000	0.233 (0.003)	0.252 (0.004) ^{b}	0.259 (0.001) ^{c}	0.257 (0.002) ^{f}	0.319 (0.004) ^{f}
	3000	0.233 (0.003)	0.241 (0.000) ^{c}	0.246 (0.002) ^{ef}	0.246 (0.002) ^{g}	0.306 (0.001) ^{g}
	5000	0.233 (0.003)	0.226 (0.002) ^{d}	0.239 (0.002) ^{f}	0.199 (0.002) ^{h}	0.277 (0.008) ^{jk}
Milk mineral	1000	0.233 (0.003)	0.240 (0.002) ^{c}	0.257 (0.002) ^{cd}	0.308 (0.004) ^{b}	0.516 (0.008) ^{b}
	3000	0.233 (0.003)	0.211 (0.004) ^{ef}	0.251 (0.002) ^{de}	0.287 (0.006) ^{c}	0.432 (0.004) ^{c}
	5000	0.233 (0.003)	0.204 (0.002) ^{f}	0.244 (0.004) ^{f}	0.273 (0.002) ^{de}	0.378 (0.001) ^{d}
Rosemary	1000	0.233 (0.003)	0.227 (0.000) ^{d}	0.271 (0.000) ^{b}	0.279 (0.002) ^{d}	0.293 (0.002) ^{hi}
	3000	0.233 (0.003)	0.208 (0.004) ^{f}	0.211 (0.004) ^{h}	0.255 (0.004) ^{f}	0.283 (0.004) ^{ij}
	5000	0.233 (0.003)	0.196 (0.002) ^{g}	0.171 (0.002) ^{j}	0.244 (0.004) ^{g}	0.270 (0.006) ^{k}
Vitamin E	1000	0.233 (0.003)	0.227 (0.004) ^{d}	0.259 (0.006) ^{c}	0.269 (0.004) ^{e}	0.333 (0.004) ^{e}
	3000	0.233 (0.003)	0.218 (0.002) e	0.225 (0.004) ^{g}	0.291 (0.004) ^{c}	0.328 (0.006) ^{ef}
	5000	0.233 (0.003)	0.194 (0.000) ^{g}	0.216 (0.004) ^{h}	0.261 (0.004) ^{f}	0.284 (0.001) ^{hij}
Combination	1000	0.233 (0.003)	0.185 (0.002) ^{h}	0.223 (0.001) ^{g}	0.190 (0.000) ^{i}	0.294 (0.004) ^{h}
	3000	0.233 (0.003)	0.168 (0.002) ^{i}	0.183 (0.004) ^{i}	0.257 (0.002) ^{f}	0.230 (0.004) ^{l}
	5000	0.233 (0.003)	0.150 (0.004) ^{j}	0.171 (0.002) ^{k}	0.247 (0.004) ^{g}	0.223 (0.006) ^{l}
Control	-	0.233 (0.003)	0.261 (0.008) ^{a}	0.315 (0.002) ^{a}	0.337 (0.006) ^{a}	0.543 (0.008) ^{a}

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdefghijk} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 3.6 The effect of antioxidant types on the TBA value of MDCM during frozen storage condition.

Antioxidants	Time (day)				
	0	7	14	21	28
BHT	0.233 (0.003)	0.240 (0.012) ^{b}	0.248 (0.009) ^{b}	0.234 (0.027) ^{d}	0.301 (0.019) ^{d}
Milk mineral	0.233 (0.003)	0.218 (0.017) ^{c}	0.250 (0.006) ^{b}	0.289 (0.016) ^{b}	0.442 (0.062) ^{b}
Rosemary	0.233 (0.003)	0.210 (0.014) ^{d}	0.217 (0.045) ^{d}	0.259 (0.016) ^{c}	0.282 (0.011) ^{e}
Vitamin E	0.233 (0.003)	0.213 (0.015) ^{d}	0.233 (0.002) ^{c}	0.286 (0.021) ^{b}	0.314 (0.024) ^{c}
Combination	0.233 (0.003)	0.167 (0.016) ^{e}	0.193 (0.002) ^{e}	0.257 (0.010) ^{c}	0.249 (0.035) ^{f}
Control	0.233 (0.003)	0.261 (0.002) ^{a}	0.315 (0.002) ^{a}	0.337 (0.006) ^{a}	0.542 (0.008) ^{a}

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdef} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 3.7 The effect of antioxidant concentrations on the TBA value of mechanical debonded chicken meat (MDCM) during frozen.

Concentrations	Time (day)				
	0	7	14	21	28
0	0.233 (0.003)	0.261 (0.027) [a]	0.315 (0.017) [a]	0.337 (0.025) [a]	0.543 (0.094) [a]
1000	0.233 (0.003)	0.226 (0.028) [b]	0.254 (0.029) [b]	0.284 (0.021) [b]	0.351 (0.077) [b]
3000	0.233 (0.003)	0.209 (0.030) [c]	0.223 (0.030) [c]	0.267 (0.030) [c]	0.316 (0.060) [c]
5000	0.233 (0.003)	0.194 (0.008) [d]	0.209 (0.002) [d]	0.245 (0.006) [d]	0.286 (0.008) [d]

numerical number in the table presented $\bar{x}$ (SD)

n = 0

[abcd] means within a column with unlike superscript letters are significantly different ($p<0.05$).

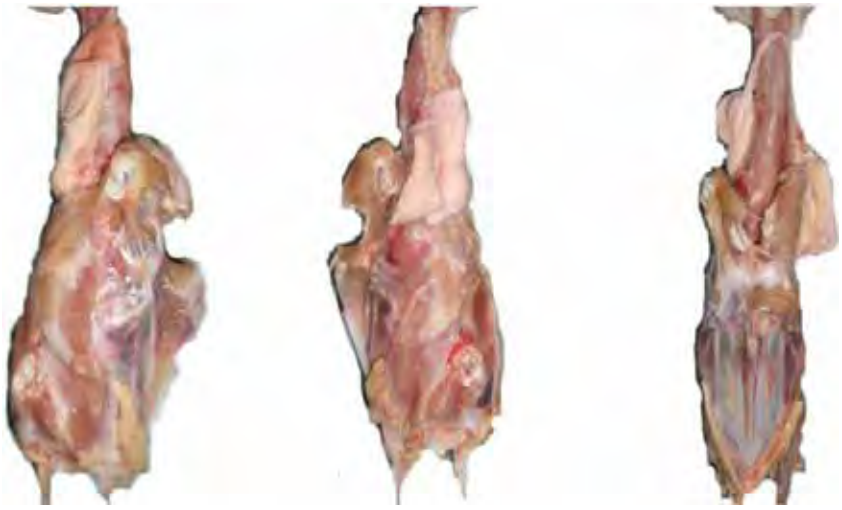

Figure 3.1 Chicken carcass with backs and necks

Figure 3.2 Mechanically deboned chicken meat.

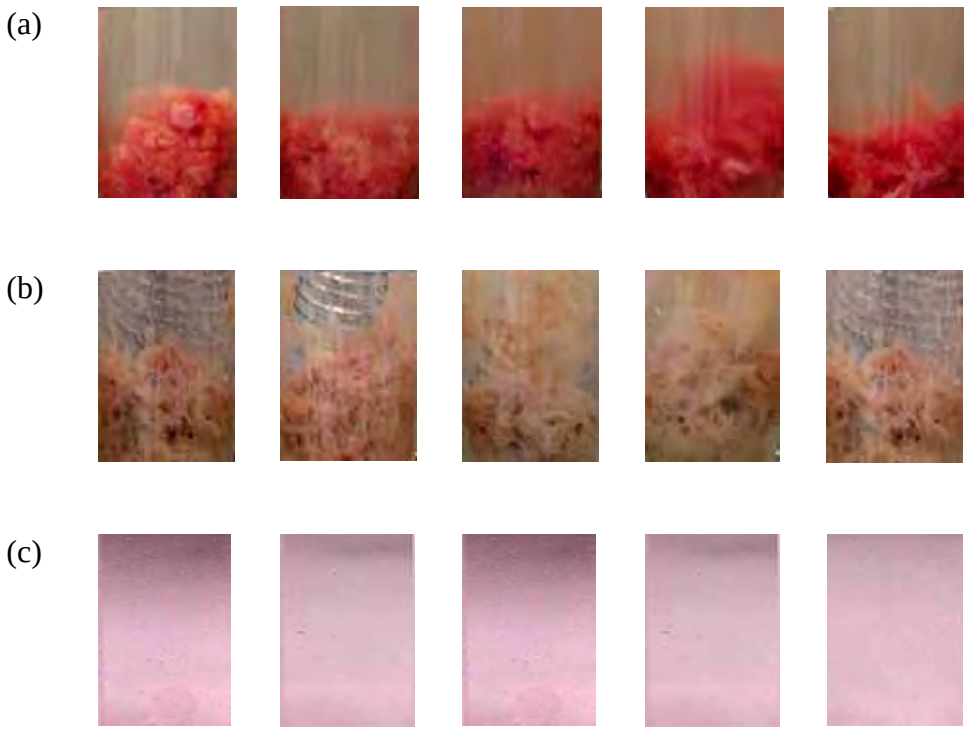

Figure 3.3 TBA analysis of mechanically deboned chicken meat; (a) MDCM in stock solution before boiling; (b) MDCM in stock solution after boiling; and (c) the absorbance of the supernatant was measured spectrophotometrically at 532 nm.

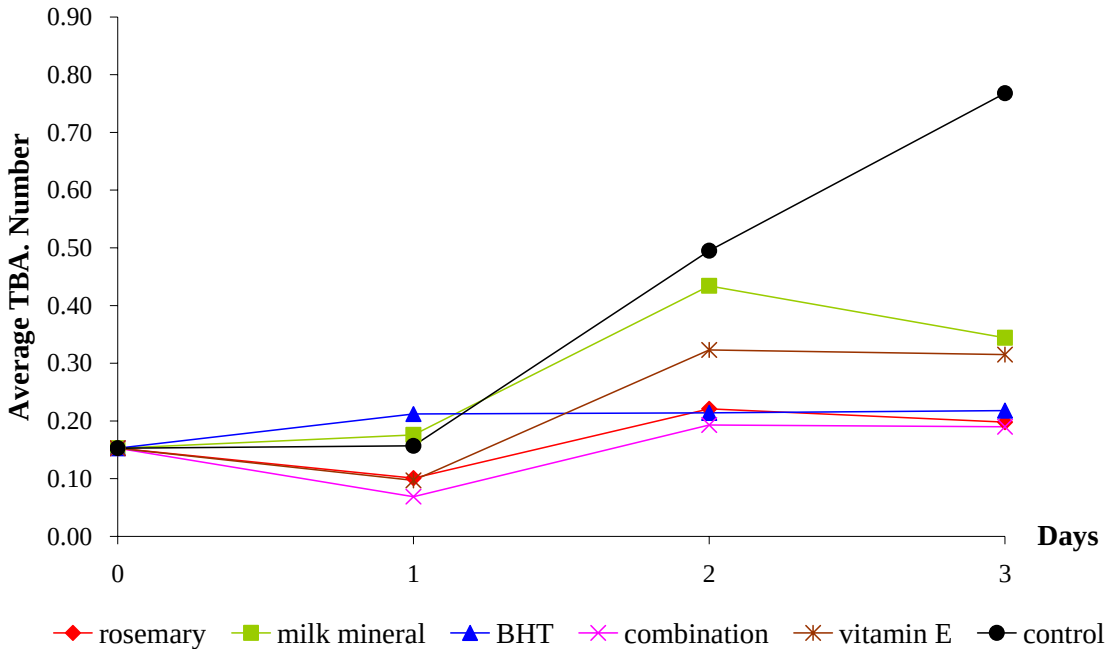

Figure 3.4 (a) TBA value of MDCM at 1000 ppm under chilled storage condition

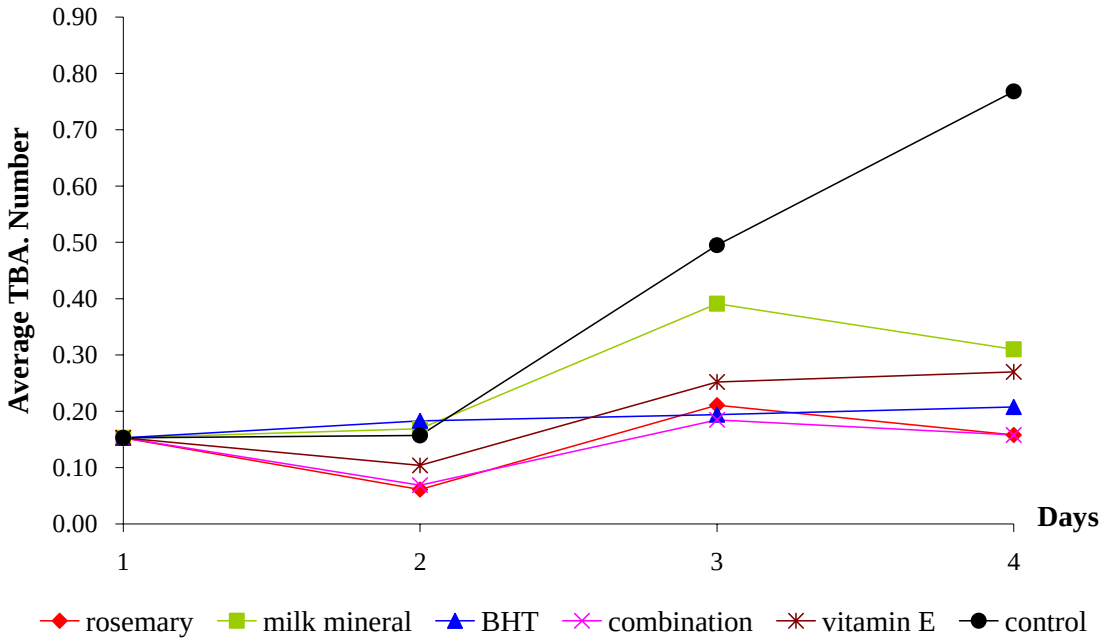

Figure 3.4 (b) TBA value of MDCM at 3000 ppm under chilled storage condition

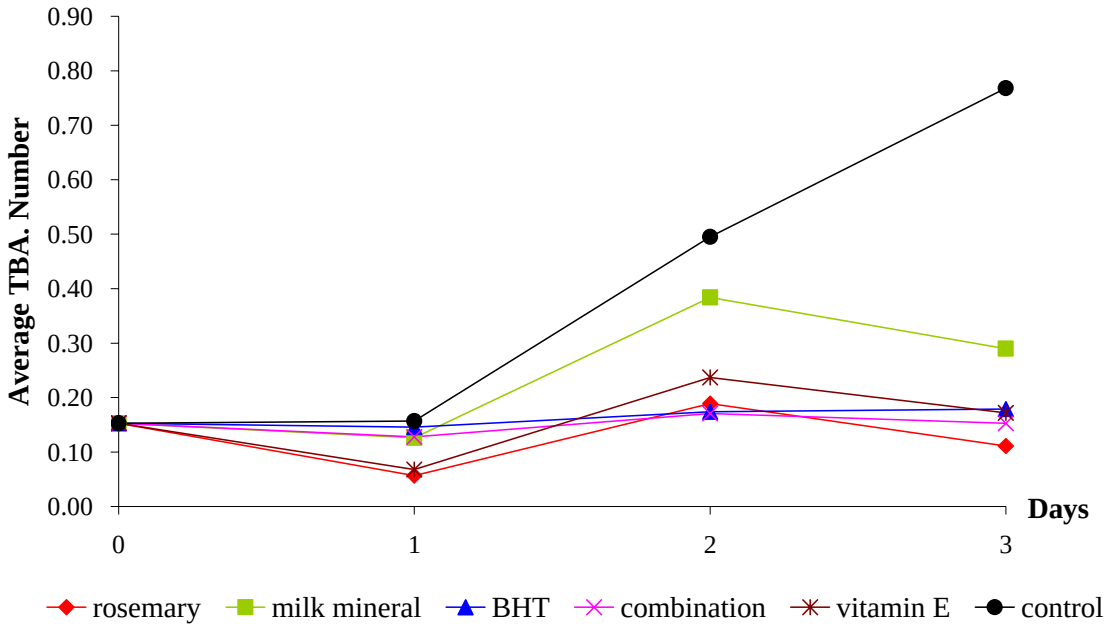

Figure 3.4 (c) TBA value of MDCM at 5000 ppm under chilled storage condition

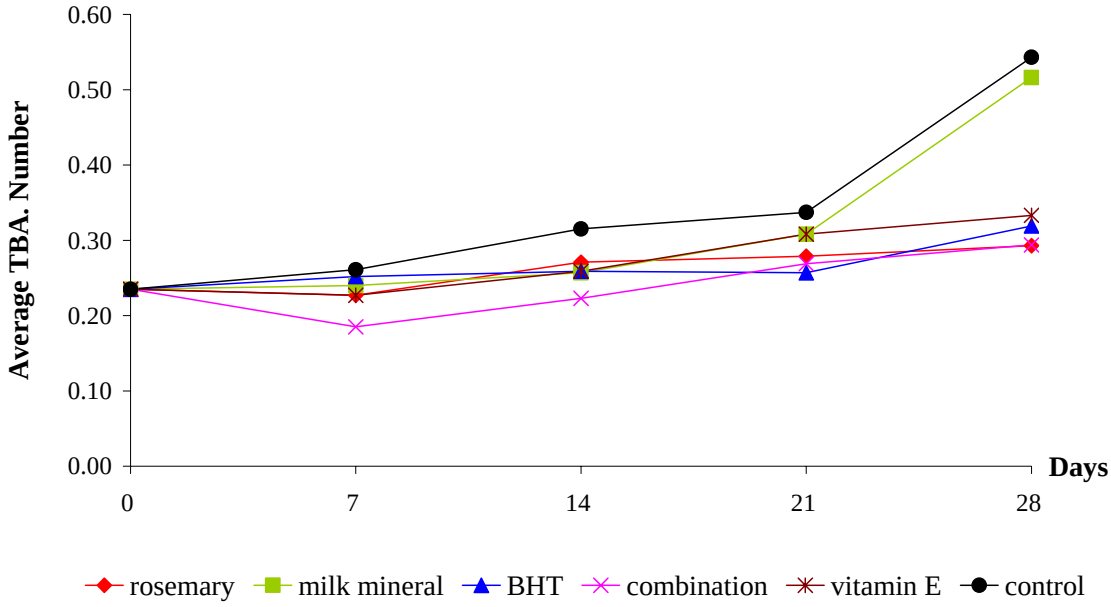

Figure 3.5 (a) TBA value of MDCM at 1000 ppm under frozen storage condition

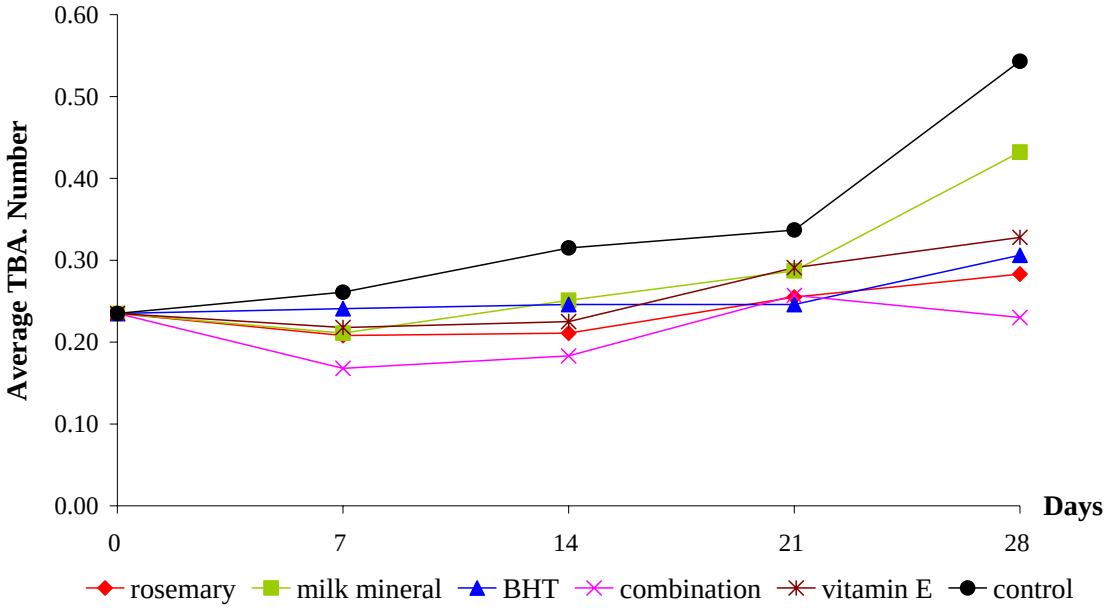

Figure 3.5 (b) TBA value of MDCM at 3000 ppm under frozen storage condition

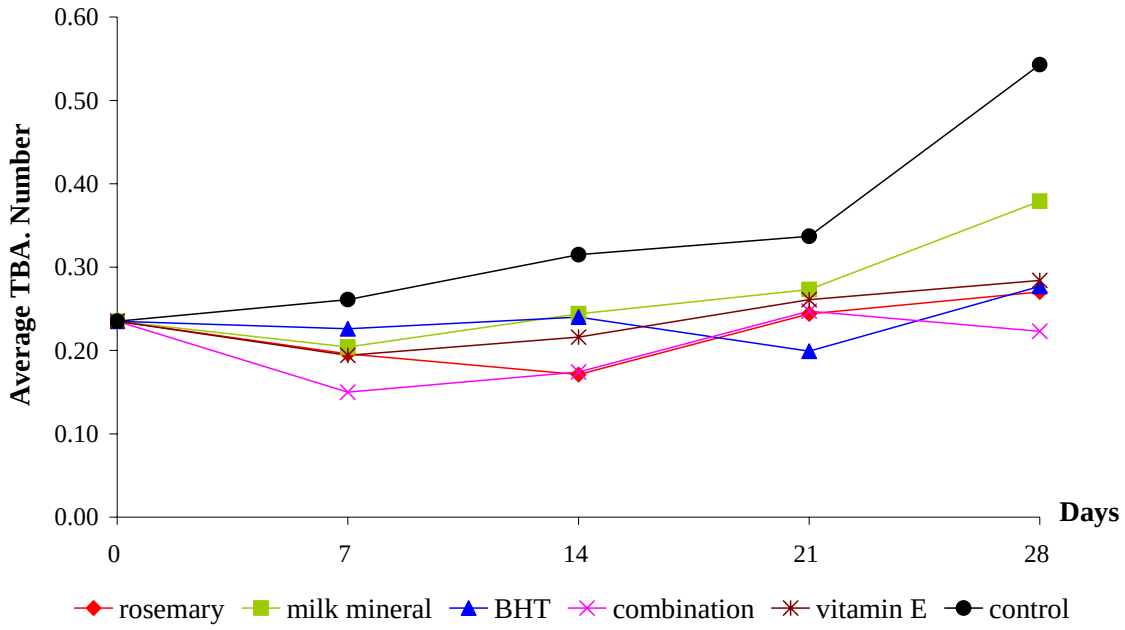

Figure 3.5 (c) TBA value of MDCM at 5000 ppm under frozen storage condition

CHAPTER 4

POTENTIAL NATURAL ANTIOXIDANTS UTILIZED IN CHICKEN BALL

ABSTRACT

Chicken ball formulated with mechanically deboned chicken meat (MDCM) treated with four different antioxidants (rosemary, vitamin E, milk mineral, and BHT) and three different concentrations (1000, 3000, and 5000 ppm) during mixing step and then stored at chill for 35 days have been investigated. The treatment sample was finally compared to the control sample (without antioxidant). Lipid oxidation in the samples was measured by means of 2-thiobarbituric acid (TBA value). After 35 days of chilled storage, the significant difference ($p<0.05$) was found in 2-thiobarbituric acid (TBA value) between the treatment and control sample. The higher amount of antioxidants added, the lower lipid rancidity occurred. As expected, retarding effect of antioxidants on the development of lipid oxidation depended on the concentration and type of antioxidants. From this study BHT at 5000 ppm was the most effective to inhibit lipid oxidation among samples. The chicken ball with BHT extremely decreases the rate of lipid oxidation indicated by TBA value up to 45.0%.

Keyword : chicken ball, antioxidant, TBA value, lipid oxidation

INTRODUCTION

MDCM continues to have usage and provides an economical meat source. MDCM provides a means of harvesting functional proteins which can be used in the preparation of a variety of further processed meat products. MDCM has been widely utilized in further processed chicken meat products such as bologna, salami, frankfurters, restructured meat products, and soup mixes (Froning and McKee, 2001). The quality of further processed products containing MDCM was effected by the particular carcass part used. A major problem for products manufactured with MDCM was the rapid onset of oxidative rancidity, when resulted in off-flavors and off odors (Mielnik et al., 2002). Oxidation of lipids not only produced rancid odors and flavors, but a decreased the nutritional quality and safety by the formation of secondary products in food after cooking and processing (Frankel, 1996). Oxidation of lipids, which occurred during raw material storage, processing, heat treatment and further storage of final products, was one of the basic processes causing rancidity in food products, leading to their deterioration (Tomino et al., 2005).

Cooked meat was more susceptible to lipid oxidation than raw meat during refrigerated and frozen storage (Tang et al., 2001). For cooked meat, thermal processes could promote lipid oxidation by disrupting cell membranes and releasing pro-oxidants, thereby inducing warmed over flavor (WOF) during refrigerated storage and subsequent reheating (Ramose and Xiong, 2003). The rate and degree of auto-oxidation degradation had been directly to the degree of unsaturation of the lipids present and degree of oxygen exposure (Jayasingh and Cornforth, 2003). The degree of unsaturation of muscle lipids and susceptibility of muscle to lipid oxidation varies with meat species and this could be influenced by diet of the animal (Tang et al., 2001). Lipids hydroperoxides formed during propagation phase of the peroxidation process are unstable and produced various chemicals which adversely affected quality and safety (Biswas et al., 2004).

The stability of foods against lipid oxidation was dependant on the concentrations, composition and activity of reactive substrates, pro-oxidants and anti-oxidants present. These might be balanced in order to produce oxidatively stable foods. Strategies to preserve or enhance the endogenous oxidation controlling

systems were utilized to minimize the use of food additives (Smiddy et al., 2002). For many years the most industry has used synthetic antioxidants like butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) to prevent or reduce flavor deterioration. However, because of potential health hazards, the use of synthetic antioxidants in food products in many countries is either for bidden for salt natural ingredients that can extends the shelf life of both processed and unprocessed meat products (Lanari et al., 2004). Particular emphasis is given to herbs of the *Labiatae* family, particulally rosemary, sage, and oregano, which have been reported to posses substantial antioxidant activity (Botsoglou et al., 2003). Food technologists to preserve or enhance have been concerned with removal of oxygen from the headspace of food packages. Introduction of vacuum and modified atmosphere packaging (MAP) techniques has solved some of the problems associated with the distribution of oxygen-sensitive foods packaging of pre-cooked meats in an oxygen-free environment is effective in increasing the stability of the product oxidation (Smiddy et al., 2002).

The objective of this experiment was to determine the optimum usage level of antioxidants to able retard lipid oxidation in chicken ball under chilled storage conditions.

MATERIALS AND METHODS

Preparation of chicken ball

Raw materials used in chicken ball production were MDCM, the remaining meat cut from skinless boneless breast meat (SBB), the scrapped meat from SBB, and the chicken tendon. According to, MDCM production chicken carcasses with backs and necks without skin passed through a machine deboner (model POSS-PDE 1500, Holland). The other meats were ground through a 4.0 mm grinder plate but chicken tendon was passed through a 10 mm grinder plate. The mincing operation was composed of four steps. First step, SBB (23.50%), scrapped (4.5%), MDCM (6.0%) were mixed with sodium chloride (1.82%), garlic (1.82%), STPP (0.3%), and iced (4.45%) into the chopper for 120 s. Second step, skin (8.0%), modified starch (4.0%), native starch (4.0%), sugar (25%), monosodium glutamate (0.8%), white

pepper (0.64%), dexylose (0.1%), sorbate (0.1%), and thitanium dioxide (0.05%) were added into minced meat and chopped for 60 s. Third step, modified starch (4.0%), native starch (4.0%), and iced (4.45%) were then added to the patty for 90 s. Four steps, chicken tendon was finally added in chopper and minced for 10 s.

Four different antioxidants (BHT, rosemary, vitamin E, and milk mineral) were individually added into patties using a kitchen aid. Three different usage levels (1000, 3000, and 5000 ppm.) were applied in this experiment. Each batch of 200 g patties was blended at high speed for 5 min. To enhance the speadability antioxidant, 3 ml of triacetin was used as a solvent to dissolve the antioxidant before mixing with patties. The control treatment would contain only 3 ml of triacetin. Each patty treatment was divided into small portion (approximately 27-30 g.) and then boiling in water bath at 80-85^{o}C for 23 min. The chicken ball samples were individually packed in plastic bag and then stored under chilled at 4.0 $\pm$ 1.0^{o}C for 35 days. The chicken ball sample was randomly selected to measure the TBA measurements every five days interval.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Jayasingh and Cornforth (2003). Duplicate samples of chicken ball (2.0 $\pm$ 0.1 g) were mixed with 10 ml of stock solution containing 0.375 % TBA, 15% TCA, and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (100 ^{o}C) to develop a pink color, cooled in tap water, and then centrifuged at 8000 rpm for 20 min (Centrifuge, Hettich type Universal 16). The absorbance of the supernatant was measured spectrophotometrically at 532 nm (UV-Visible Spectrophotometer, Genesys type 10 uv) against a blank that contained all the reagents minus the meat and stock solution to blanks. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1}. The MDA concentration was converted to TBA number (mg MDA/kg meat sample) as follow;

TBA NO. (ppm) = Sample $A_{532} \times 2.77$

Statistical Analysis

Statistic analysis were performed using General Linear Model Program (GLM) to test the effects of various antioxidants, concentration of antioxidants, condition and time of stored compare with control sample (without antioxidant). Augmented Factorial was used to estimate the significant differences among of each treatment at 5% the probability level using SAS program (Ver. 8.1, SAS Inst., Cary, NC, USA).

RESULTS AND DISCUSSIONS

The effect of antioxidant on the TBA value of chicken ball during chilled storage condition was showed in (Table 4.1). The interaction between type, concentration of antioxidant, and time of storage was reported in Figure 4.1 (a, b, and c). In this study, the TBA values of chilled chicken ball increased ($p<0.05$) with longer storage of time. The effects of antioxidant type on TBA value of chicken ball stored under chilled storage condition were presented in Table 4.2. Estevez et al. (2005) reported that oxidative reactions in foodstuffs were enhanced after cooking and chilled storage through the increase of their oxidative instability due to the degradation of natural antioxidants and the release of free fatty acid and iron from heme molecule. Particularly remarkable rancid developed in cooked meats during chilled storage as results of the generation of some volatile compounds from polyunsaturated degradation, known as warmed-over flavor. From this study, BHT was the most effective antioxidant to be able to decrease TBA value in chicken ball for 35 days by 45.00%, 36.17% for rosemary, 25.01% for vitamin E, and 10.16% for milk mineral.

BHT was a synthetic antioxidant and very effective in animal fats. BHT was widely used in the meat industry. It was able to survive at high temperatures encountered during food processing operations (Allen and Hamilton, 1994).

Rosemary were mainly related to their content of phenolic compounds; suggesting that their antioxidant actions were similar to those of synthetic phenolic antioxidants. Rosemary oleoresin was as efficient as a mixture of BHA and BHT in suppressing lipid oxidation in breakfast sausage containing 25% MDCM stored

refrigerated for 2 weeks. Addition of rosemary to deboned poultry meat had been shown to provide protection from oxidation during cooking, and to prolong the incubation period required for oxidation in chicken fat (Mielnik et al., 2003).

Vitamin E supplementation resulted in chicken meat being protected against oxidation during frozen storage and cooking. Sage, rosemary, and tea catechins, had antioxidant activity comparable to that dietary vitamin E and BHA/BHT (combined) for fresh and previously frozen chicken meat, while only tea catechins was as effective as vitamin E and BHA/BHT for cooked meat (Sullivan et al., 2004).

Dried milk mineral had antioxidant properties in cooked meats, apparently by iron-chelation to colloidal phosphate (Jayasingh and Cornforth, 2003).

For cooked meat, thermal processes could promote lipid oxidation by disrupting cell membrane and releasing pro-oxidants, thereby inducing warmed over flavor during refrigerated storage (Pena-Ramos and Xiong, 2003). The heat denaturation of heme pigments in foods of animal origin could increase the pro-oxidant effect of iron and thus reduce the activity of antioxidants. During boiling, the antioxidant activity of proteins was affected because of their denaturation and antioxidants were partially extracted and remained in the boiling water. If the boiling water was not used but discarded these antioxidants were lost (Pokony et al., 2001).

The effect of antioxidant concentration in chicken ball on the TBA value was presented in Table 4.3. The study showed that higher concentration of antioxidant in chicken ball were decreased the TBA value compared with control sample were significantly ($p<0.05$).

CONCLUSIONS

Oxidation is the main cause of MDCM deterioration, occurred spontaneously in lipids or lipid containing foods. This study concluded that BHT, rosemary, vitamin E, and milk mineral provided antioxidant benefits to chicken ball during chilled storage (4^oC) and effectiveness of each antioxidant relied on the concentration usage. The more addition of antioxidants, the fewer lipids rancid reacted. Therefore, chilled chicken ball could be more rancid with longer storage time. In this study, BHT at 5000 ppm was the most antioxidant effective to inhibited lipid oxidation in chicken

ball. BHT was able to survive the high temperature during cooking. BHT, rosemary, vitamin E, and milk mineral were tended to decrease lipid oxidation in the chicken ball at every concentrations usage. It was also suggested that rosemary as a natural antioxidant, could be used to extend the shelf life of chicken ball and other chicken products providing the consumer with food containing natural antioxidant which might be seen more healthful than those of synthetic origin.

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Table 4.1 The effect of antioxidant on the TBA value of chicken ball during chilled storage condition.

Antioxidants	Concentration (ppm)	Time (day)						
		0	5	10	15	20	25	35
BHT	1000	1.593 (0.015)	2.021 (0.142) ^{f}	2.198 (0.061) ^{fg}	2.297 (0.004) ^{gh}	2.232 (0.037) ^{gh}	2.205 (0.173) ^{f}	2.329 (0.139) ^{gh}
	3000	1.593 (0.015)	1.939 (0.141) ^{f}	2.093 (0.104) ^{g}	2.201 (0.072) ^{h}	2.090 (0.107) ^{h}	2.007 (0.127) ^{g}	2.163 (0.086) ^{h}
	5000	1.593 (0.015)	1.914 (0.126) ^{f}	1.971 (0.010) ^{g}	1.985 (0.186) ^{i}	1.892 (0.106) ^{i}	1.884 (0.102) ^{g}	1.972 (0.047) ^{i}
Milk mineral	1000	1.593 (0.015)	3.259 (0.061) ^{ab}	3.439 (0.069) ^{ab}	3.547 (0.010) ^{ab}	3.655 (0.034) ^{a}	3.947 (0.184) ^{a}	3.389 (0.153) ^{a}
	3000	1.593 (0.015)	3.185 (0.135) ^{bc}	3.238 (0.097) ^{bc}	3.345 (0.194) ^{bc}	3.481 (0.093) ^{b}	3.700 (0.182) ^{b}	3.410 (0.008) ^{b}
	5000	1.593 (0.015)	3.021 (0.072) ^{c}	3.184 (0.100) ^{c}	3.205 (0.059) ^{c}	3.352 (0.016) ^{b}	3.602 (0.076) ^{b}	3.312 (0.057) ^{bc}
Rosemary	1000	1.593 (0.015)	2.326 (0.018) ^{e}	2.452 (0.004) ^{e}	2.598 (0.051) ^{ef}	2.679 (0.066) ^{d}	2.760 (0.163) ^{d}	2.680 (0.065) ^{e}
	3000	1.593 (0.015)	2.296 (0.020) ^{e}	2.395 (0.013) ^{ef}	2.427 (0.004) ^{fg}	2.409 (0.033) ^{ef}	2.514 (0.096) ^{e}	2.452 (0.016) ^{fg}
	5000	1.593 (0.015)	2.258 (0.004) ^{e}	2.349 (0.047) ^{ef}	2.345 (0.014) ^{gh}	2.296 (0.020) ^{fg}	2.492 (0.100) ^{e}	2.371 (0.011) ^{g}
Vitamin E	1000	1.593 (0.015)	2.669 (0.081) ^{d}	2.927 (0.010) ^{d}	2.863 (0.112) ^{d}	3.126 (0.057) ^{c}	3.154 (0.069) ^{c}	3.134 (0.041) ^{cd}
	3000	1.593 (0.015)	2.339 (0.030) ^{e}	2.734 (0.137) ^{d}	2.673 (0.035) ^{de}	2.684 (0.062) ^{d}	2.980 (0.004) ^{c}	3.076 (0.218) ^{d}
	5000	1.593 (0.015)	2.218 (0.002) ^{e}	2.442 (0.269) ^{e}	2.435 (0.113) ^{fg}	2.474 (0.168) ^{e}	2.783 (0.009) ^{d}	2.603 (0.206) ^{ef}
Control	-	1.593 (0.015)	3.386 (0.006) ^{a}	3.514 (0.002) ^{a}	3.625 (0.002) ^{a}	3.664 (0.375) ^{a}	3.765 (0.004) ^{b}	3.918 (0.006) ^{a}

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdefghijk} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 4.2 The effect of antioxidant types on the TBA value of chicken ball during chilled storage condition.

Antioxidants	**Time (day)**						
	0	5	10	15	20	25	35
BHT	1.593 (0.015)	1.958 (0.117) ^{e}	2.087 (0.115) ^{e}	2.161 (0.169) ^{e}	2.071 (0.168) ^{e}	2.032 (0.180) ^{d}	2.155 (0.177) ^{e}
Milk mineral	1.593 (0.015)	3.154 (0.131) ^{b}	3.287 (0.139) ^{b}	3.366 (0.179) ^{b}	3.496 (0.143) ^{b}	3.750 (0.200) ^{a}	3.520 (0.261) ^{b}
Rosemary	1.593 (0.015)	2.293 (0.033) ^{d}	2.398 (0.051) ^{d}	2.457 (0.118) ^{d}	2.461 (0.179) ^{d}	2.589 (0.164) ^{c}	2.501 (0.147) ^{d}
Vitamin E	1.593 (0.015)	2.409 (0.212) ^{c}	2.701 (0.260) ^{c}	2.657 (0.205) ^{c}	2.761 (0.309) ^{c}	2.972 (0.169) ^{c}	2.938 (0.294) ^{c}
Control	1.593 (0.015)	3.386 (0.004) ^{a}	3.514 (0.002) ^{a}	3.625 (0.002) ^{a}	3.664 (1.611) ^{a}	3.765 (0.003) ^{a}	3.918 (0.004) ^{a}

numerical number in the table presented $\bar{x}$ (SD)

n = 0

^{abcdef} means within a column with unlike superscript letters are significantly different ($p<0.05$).

Table 4.3 The effect of antioxidant concentrations on the TBA value of chicken ball during chilled storage condition.

Concentrations	Time (day)						
	0	5	10	15	20	25	35
0	1.593 (0.015)	3.386 (0.496) [a]	3.514 (0.509) [a]	3.625 (0.496) [a]	3.664 (0.566) [a]	3.764 (0.688) [a]	3.918 (0.609) [a]
1000	1.593 (0.015)	2.569 (0.495) [b]	2.754 (0.461) [b]	2.826 (0.464) [b]	2.923 (0.554) [b]	3.016 (0.672) [b]	2.995 (0.535) [b]
3000	1.593 (0.015)	2.440 (0.440) [c]	2.615 (0.483) [c]	2.662 (0.483) [c]	2.666 (0.575) [c]	2.800 (0.664) [c]	2.775 (0.527) [c]
5000	1.593 (0.015)	2.353 (0.004) [d]	2.487 (0.002) [d]	2.492 (0.002) [d]	2.504 (1.444) [d]	2.690 (0.003) [d]	2.564 (0.004) [d]

numerical number in the table presented $\bar{x}$ (SD)

n = 0

[abcd] means within a column with unlike superscript letters are significantly different ($p<0.05$).

Figure 4.1 Chicken ball

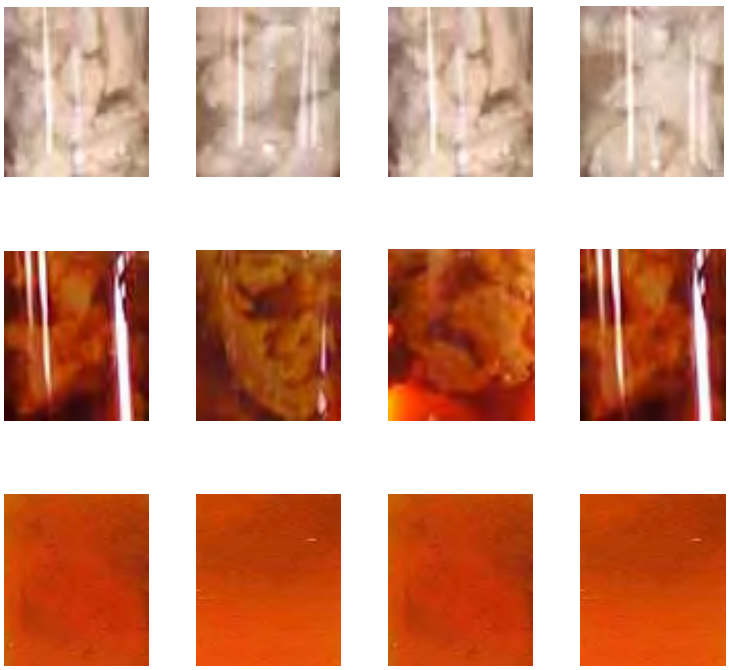

Figure 4.2 TBA analysis of chicken ball; (a) chicken ball in stock solution before boiling; (b) chicken ball in stock solution after boiling; (c) the absorbance of the supernatant was measured spectrophotometrically at 532 nm.

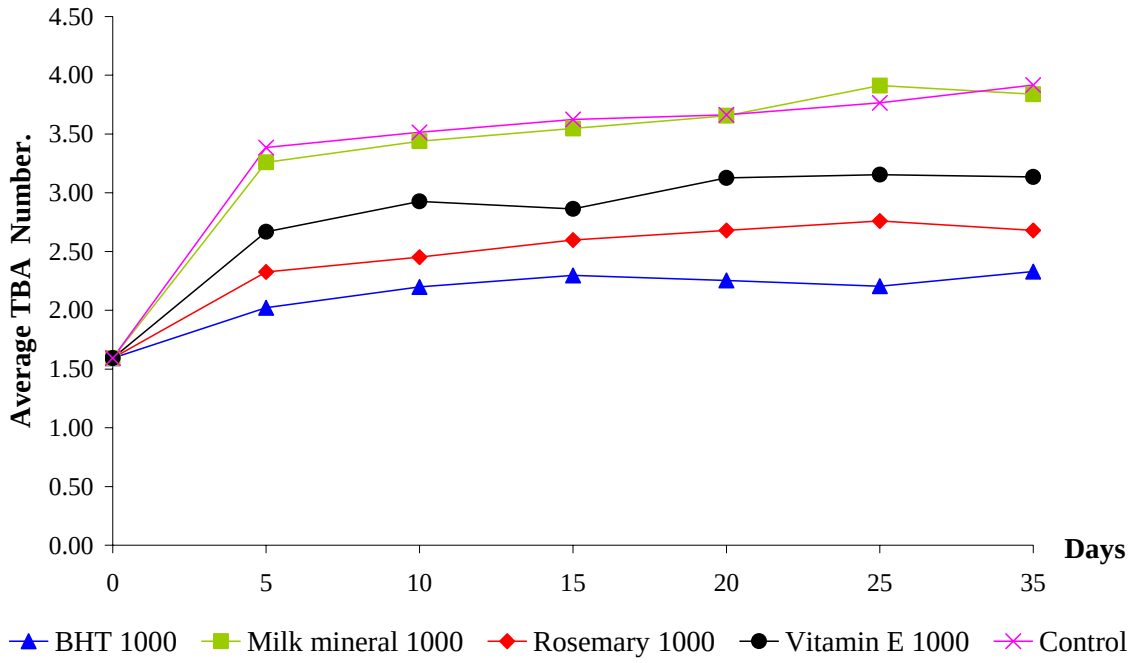

Figure 4.3 (a) TBA value of chicken ball at 1000 ppm under chilled storage condition

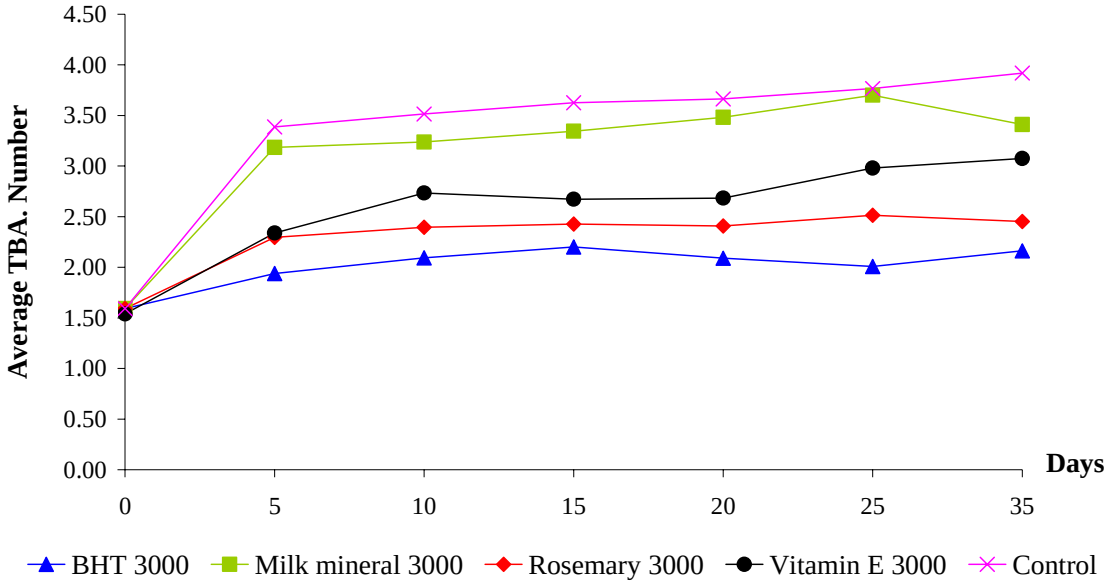

Figure 4.3 (b) TBA value of chicken ball at 3000 ppm under chilled storage condition

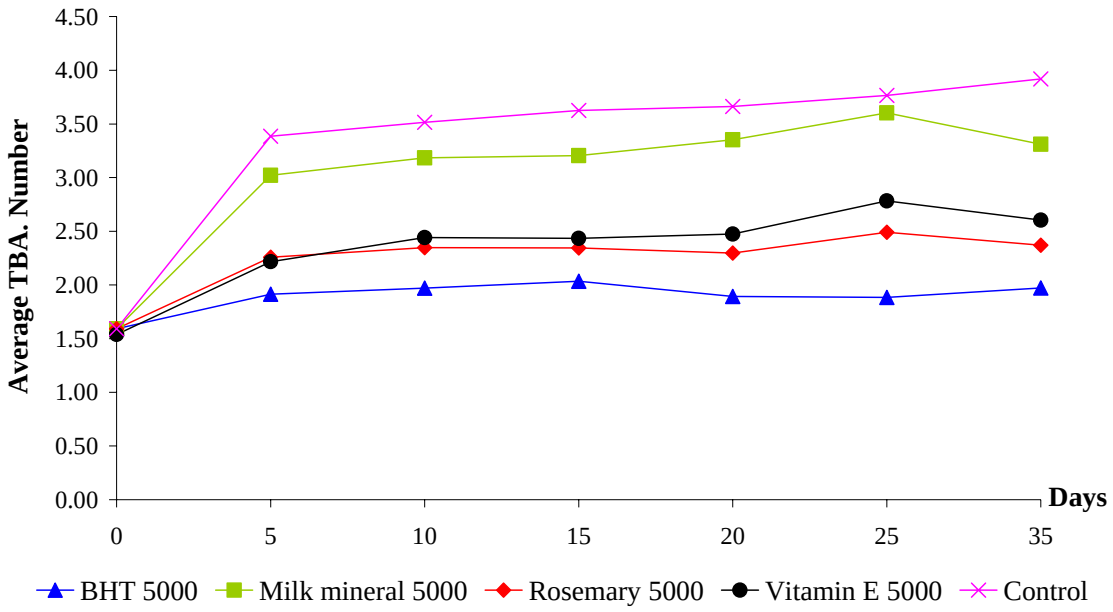

Figure 4.3 (c) TBA value of chicken ball at 5000 ppm under chilled storage condition

CHAPTER 5

SUMMARY

The objective of this research was to evaluate the natural potential and synthetic antioxidant added in chilled and frozen MDCM and compared to the control. Thiobarbituric assay (TBA) was used to assess antioxidant activity to retard lipid oxidation of MDCM. The TBA value of MDCM added antioxidants and control increased with longer storage time. The lipid oxidation rate tremendously decreased with higher concentration of antioxidants applied. Rosemary at 5000 ppm exhibited the most effectiveness to delay lipid oxidation in chilled MDCM. In contrast, combination (mix synthetic antioxidant) at 5000 ppm would be the most potential compound utilized in frozen MDCM. It could decrease the lipid oxidation about 54.06% when compared to the control.

Further research was to determine the natural antioxidant utilized in chicken ball. This study concluded that, BHT, rosemary, vitamin E, and milk mineral provided antioxidant benefits to chicken ball during chilled storage. The effectiveness of each antioxidant relied on the concentration usage. The more addition of antioxidants, the less lipid oxidation obtained. The chicken ball with and without antioxidant added could be more rancid with longer storage time. BHT at 5000 ppm was the most effective to impede lipid oxidation among samples. The chicken ball with BHT extremely decreases the rate of lipid oxidation indicated by TBA value up to 45.0%.

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