1999

Increased Nutritional Value and Oxidative Stability of Restructured Beef Roasts With Purified Extracts From Rice Bran.

Joo-shin Kim
Louisiana State University and Agricultural & Mechanical College

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

INCREASED NUTRITIONAL VALUE AND OXIDATIVE STABILITY OF RESTRUCTURED BEEF ROASTS WITH PURIFIED EXTRACTS FROM RICE BRAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
Joo-Shin Kim
B.S., Sang Myung Women's University, 1991
M.S., University of Tennessee, 1994
December 1999

ACKNOWLEDGMENTS

The author wishes to express her most sincere and deepest appreciation to her major professor, Dr. J. Samuel Godber, Professor, Department of Food Science, for his precious and patient guidance, inspiration, encouragement, advice, and support during the preparation of this dissertation.

Appreciation is expressed to Dr. Witoon Prinyawiwatkul, Assistant Professor of the Department of Food Science, for his valuable assistance and guidance with statistical analysis and sensory evaluation. Special thanks are also expressed to other committee members: Dr. Joan King, Assistant Professor of the Department of Food Science, Dr. Michael J. Keenan, Associate Professor of the School of Human Ecology, and Dr. Joseph Siebenaller, Professor of the Department of Biological Science for their advice and encouragement. Thanks are also extended to Dr. Douglas L. Park, Head of the Department of Food Science for his support during this research. The financial support provided by the Louisiana Beef Industry Council is very much appreciated. Also, all of her friends in the Department of Food Science are well appreciated for their encouragement and friendship.

The author would like to express her deepest indebtedness to her mother, Mrs. Mee-Kang Cho and her father, the deceased, Dr. Seung-Jin Kim for their endless love, sacrifice, and support and for her mother's endless prayers and special love during this endeavor. She wishes to acknowledge precious love and encouragement of her brother, Joo-Sang Kim, and his family. She would also like to thank her husband and sincere friend, Dr. Anthony H. Y. Chung, Assistant Professor of the Chinese University of

Hong Kong, for his endless love, priceless inspiration, understanding, sacrifice, and encouragement.

Most of all, the author deeply thanks the Lord, Jesus Christ. This dissertation is dedicated to the Lord.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The effects of semi-purified extracts of rice bran were studied in restructured beef roasts to increase nutritional value and oxidative stability. Crude rice bran oil at 0, 1, and 2% (w/w) was added to restructured beef roasts that were stored at 4 °C and analyzed at 0, 7, and 14 d. Saturated fatty acid to unsaturated fatty acid (SFA/UFA) ratio decreased, vitamin E vitamers increased ($p<0.05$), and cholesterol decreased in the beef roasts with rice bran oil. Thiobarbituric acid reactive substances (TBARs) numbers were lower ($p<0.05$) in the beef roasts with rice bran oil after 7 days of storage and the cholesterol oxidation product, 7-ketocholesterol, decreased in a similar manner. The addition of 2 % rice bran oil (RBO) was effective in increasing nutritional value and reducing the level of oxidative degradation.

Beef roasts containing either rice fiber (RF) or RF/RBO had higher oxidative stability ($p<0.05$) during storage (0, 4, and 8 d) compared to control roasts, with no additive. TBARs value and 7-ketocholesterol of beef roasts with RF and RF/RBO were lower ($p<0.05$) than those of the control during storage. SFA/UFA ratio was significantly reduced in beef roasts with additives, whereas vitamin E vitamers and UFA were higher ($p<0.05$) in the roasts with RF/RBO. Beef roasts containing RF and RBO were acceptable to consumers in sensory attributes.

Cholesterol autoxidation was examined in an aqueous meat model system with different levels of nonsaponifiable fraction from rice bran (0, 700, 1400, and 2100 ppm). The effect of nonsaponifiable fraction was determined at 4 h intervals for 16 h at pH 5.5 and 80 °C. Increased oxidation time reduced ($p<0.05$) total vitamin E vitamers

and cholesterol concentration. Oryzanol was not significantly reduced with the 2100 ppm treatment after 4 h cholesterol oxidation. An increase in the nonsaponifiable fraction resulted in a decrease ($p<0.05$) in the formation of 7-ketocholesterol. The highest level of additive (2100 ppm) was the most effective in inhibiting cholesterol autoxidation; the order of inhibition was 91.7% (2100 ppm)> 81.8% (1400 ppm)> 63.1% (700 ppm).

CHAPTER 1

INTRODUCTION

The beef industry is continually developing new products to improve health and nutrition. The products should meet the demand of today's consumer relative to nutritive value and taste. It is known that rancidity is a major deterrent to acceptable taste in precooked beef products (Arganosa et al., 1989; 1991; Tanchotikul et al., 1989). Thus, the use of antioxidants would be an approach to reduce rancidity in meat products. The values of certain red meat cuts might be enhanced through restructuring techniques from nutritional and functional points of view. Several chemical compounds in rice bran have functional potential as natural antioxidants, including tocopherols, tocotrienols, oryzanols and inositol phosphates.

Beef is an excellent source of high quality nutrients including most minerals, many water-soluble vitamins and all eight essential amino acids (Godber, 1993). However, beef is high in fat, particularly saturated fat and cholesterol, and is practically devoid of complex carbohydrate which is nutritionally important. Rice bran is high in fibers like cellulose and hemicellulose (Godber et al., 1991) and fat-soluble vitamin E (Shin and Godber, 1993) and possesses several plant sterol compounds that are thought to have a positive effect on cardiovascular diseases (Saunders, 1990). However, rice bran has been found to impair mineral bioavailability. Beef enhances mineral bioavailability when rice bran is consumed in the diet concurrently (Gordon and Godber, 1989; Godber, 1990; Qiao et al., 1992). Thus, combining these nutritional properties into a single product might constitute a nearly ideal product.

This combination could also provide complimentary functional attributes. This is especially true with regard to lipid and cholesterol oxidation in that rice bran possesses several compounds that can act as antioxidants. The antioxidant compounds,

which can influence the nutritional properties and oxidative stability in the meat product, mainly come from the nonsaponifiable fraction of rice bran oil. Furthermore, the nonsaponifiable fraction present in rice bran oil might be responsible for lowering cholesterol. The hypercholesterolemic property is believed to be due to its high content of tocotrienols, oryzanol, β-sitosterols and other nonsaponifiables (Seetharanaiah and Chandrasekhara, 1988, 1989; Sharama and Rukimini, 1986). Those specific compounds also have other important beneficial effects on human health, which is that they act as an antioxidant to protect the oxidative stability of lipids *in vivo* and *in vitro*. By including rice bran in beef products, it may be possible to achieve a high degree of lipid stability; lipid instability is the primary reason for deterioration in precooked beef products. However, previous research has discovered that whole rice bran increased an undesirable cereal flavor and caused a decrease in overall acceptability of ground patties with rice bran (Liu, 1995). Thus, access to a semi-purified fiber component from rice bran may be necessary to serve as a water binding agent and source of complex carbohydrates (Pierce, 1996).

Smith (1980) indicated that cholesterol autoxidation proceeds by a free radical mechanism, similar to autoxidation of unsaturated fatty acids (Nawar, 1985). Factors affecting rate and onset of lipid oxidation might be the same as those of cholesterol oxidation. As a result, several reactive components like cholesterol oxide products can be generated (Nawar, 1985). Because there appears to be no practical method to lower cholesterol in certain foods like muscle tissue, research directed to protect against cholesterol oxidation in a meat product might be more valuable. Techniques are being developed to lower cholesterol oxide content of the product.

This study focused on developing a beef product that included purified components of rice bran, which would maximize sensory and nutritional properties as well as antioxidative properties and result in a functional food from the point of view that it could possess therapeutic value.

1.1. Restructured Meat Products

Restructured beef roast products are becoming more important products of the meat industry due to their benefits such as convenience of preparation and economic advantages in production. Restructuring is a process in which various muscle and trimmed parts are manipulated and structured into new forms (Akamittath et al., 1990). The primary rationale is to transform undervalued meat into products having an increased market value (Secrist, 1987). In restructured meat, raw meat components consist of lean and fatty tissues. Fresh lean tissues are the most important ingredient because they contain both myosin and actomyosin, the only naturally occurring binders in meat (Trout and Schmidt, 1987). These proteins were shown to bind meat pieces strongly upon heating (MacFarlane et al., 1977). Water and salt are considered as additives that exert their effects on the functional properties of restructured meat products (Trout and Schmidt, 1987).

Water is added primarily as a processing aid to help mixing and dissolution of salt. The amount of added water is a critical factor from the point of view of binding and cooking yield. If too little water is added, the product will be dry resulting from evaporation and other processing losses, whereas excessive water decreases the concentration of the meat proteins resulting in decreased binding. Salts are incorporated to increase functional properties of protein, improve flavor, and

minimize microbial growth (Trout and Schmidt, 1987). Salt extracts the structural proteins from muscle cells at the surface of meat during mixing and a three-dimensional protein network is formed when salts interact with the muscle proteins during cooking (Trout and Shimidt, 1987).

In addition to those non-protein additives, rice bran oil and rice fiber could also be used as ingredients in restructured beef roasts. Use of non-meat ingredients in processed beef products is not new. A variety of vegetable protein products have been used to extend beef products (Schmidt, 1986). The use of soy proteins as a vegetable extender has been very common and it has been shown to have functional properties such as increased water-binding and cooking yield (Ray et al., 1981) and as antioxidants (Pratt et al., 1981; Arganosa et al., 1991). A more recent trend has been the use of polysaccharides such as carageenan and alginate as functional ingredients (Egbert et al., 1991). The traditional view of the use of non-meat additives as lower-cost extenders and functional additives might need to be modified to meet the demand of health-conscious consumers. These non-meat additives could be considered nutritional modifiers in that they impart nutritional properties as well as potential functionality. The focus for nutritionally modified beef products would be for use of non-meat ingredients that have a high proportion of calories as complex carbohydrate and a high amount of fiber, with higher proportions of mono- and polyunsaturated fat.

1.2. Nutritional Attributes of Meat

Meat is not only an excellent source of energy, protein, vitamins, and minerals but also a nutritionally complete protein food containing all eight essential amino acids needed by human beings for good health. Meat protein is often considered nutritionally

superior to plant protein. However, the difference in protein quality is not very great (Bender, 1974). Individual plant proteins may have lower nutritional values than those of individual animal proteins. The biological value (BV) of meat is for instance 75, for soya it is 70, for wheat gluten 44, and for gelatin 0. Because people eat a mixed diet, not single foods, the difference between plant and animal protein is even less marked. Amino acids from different vegetables proteins in the diet complement each other and the BV of protein in a mixture of plant foods can be as high as that of animal foods (Bender, 1974).

Consumption of meat is associated with consumption of fat. Red meats tend to have higher levels of fat in the range between 10 and 40 % (Godber, 1993). The fatty acid components of meat fats contain in the range of 50-60 percent unsaturated fatty acids, primarily oleic acid (C18:1), with the polyunsaturated fatty acids-linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) acids-present in the aggregate at levels ranging from 3-10 percent of the total fatty acids. Because meat contains virtually no carbohydrates, the food energy of meat is only provided by fat and protein (Food and Nutrition Board, 1980).

Cholesterol, while not a nutrient in the classic sense, is a constituent of meat that continues to receive a great deal of attention due to its relationship with the incidence of atherosclerosis. Currently, consumers are especially conscious of dietary intakes of animal products that contain high levels of saturated fatty acids and cholesterol, which in turn elevate serum cholesterol (Park et al., 1991). A high blood cholesterol level incurs an increased risk of heart disease, which is mainly related to high levels of low-density lipoprotein (LDL) cholesterol, with corresponding low levels of high-density

lipoprotein (HDL) cholesterol. Thus, any modification in diet that can decrease total and LDL cholesterol levels is associated with a reduction of heart disease.

Muscle foods are generally considered one of the most desirable sources of dietary minerals. It has been suggested that muscle foods may enhance the bioavailability of minerals in non-muscle foods that are consumed concurrently (Godber et al., 1993). Johnson et al. (1992) studied zinc and iron utilization in young women fed diets containing varying amounts of lean ground beef. The 28-day study was divided into one 7-day pre-experimental period during which the subjects were fed a vegetarian diet, and three randomly arranged 7-day experimental periods, during which the diet contained 3, 6, or 9 oz of beef, supplying varying level of zinc and iron. All diets resulted in positive effects in zinc and iron balances. They found that zinc and iron balances were significantly higher for diets containing beef rather than a vegetarian diet. As the amount of beef in the diet increased, the bioavaliability of iron and zinc increased. Even though meat provides various essential nutrients for maintaining human health, as well as certain benefits such as increased mineral bioavailability, it can be a primary source of saturated fat and cholesterol, which may be a contributing factor in cardiovascular diseases.

1.3. Nutritional Attributes of Rice Bran

Rice bran is potentially one of the most valuable by-products of rice processing, but it is underutilized. Rice production in Louisiana in 1992 was about 1.44 million tons, which accounted for 16 % of the total U.S. rice production. Rice bran was about one hundred tons of by-product from rice processing. It is used only as livestock feed

or must be disposed of. However, rice bran could be a promising new source of healthy human food in processing food products (Saunders, 1990).

Rice as harvested is referred to as paddy where the kernel is fully enveloped by the rice hull. The hull is removed to yield brown rice in the first milling stage. The rice is still enclosed by an outer brown layer. The outer brown layer is removed from the brown rice kernel to yield white rice in the second milling stage. The separated brown layer is called rice bran. The amount of rice bran is 8-12 % of the total weight of brown rice (Palipane and Swarnasiri, 1985).

Rice bran contains approximately 12-22 % oil, 11-17 % protein, 6-14 % fiber, 10-15 % moisture, and 8-17 % ash of the total weight (Saunders, 1990). The nutritional value of rice bran protein is high and its digestibility is reported to be 73%, while in the extracted concentrates, its protein digestibility is greater than 90% (Saunders, 1990). Rice bran is also rich in a variety of vitamins and minerals such as thiamin, niacin, vitamin E, phosphorus, potassium, magnesium, and silicon. In addition, the oil and fiber from rice bran have been shown to contribute to its potential in lowering serum cholesterol level (Suzuki and Ochima, 1970; Sharama et al., 1987; Raghuran et al., 1989; Godber et al., 1993).

Rice bran contains the unsaturated fatty acids, oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) and the saturated fatty acids, palmitic (C16:0) and stearic (C18:0). The major fatty acids are palmitic, oleic, and linoleic, which make up about 90 % of the total fatty acids (Sayre and Saunders, 1985). Rice bran oil is also higher in nonsaponifiable fraction than any other vegetable oil. This nonsaponifiable fraction is known to act as an antioxidant *in vivo* and *in vitro*. The nonsaponifiable fraction of rice

bran includes tocopherols and tocotrienols, γ-oryzanol, and squalene (Rukmini and Raghuram, 1991). The nonsaponifiable fraction is also generally represented by the sterol fraction of the oil. These include free sterols, sterol esters, sterylglycosides, and acylsteryl glycosides. β-Sitosterol is the most abundant sterol accounting for 50 % of total concentration of sterol (Luh, 1991). Oryzanol, present at 0.96-2.9 % of bran oil by weight (Okada and Yamaguchi, 1983), is a mixture of substances that include ferulic acid esters of sterols and other triterpene alcohols. The sterol compounds were considered to be an important component, along with tocotrienols and other nonsaponifiables, in lowering the cholesterol level (Seetharamaiah and Chandrasekhara, 1988, 1989; Saunders, 1990; Rukmini and Raghuram, 1991). Plant sterols have long been known to diminish absorption of cholesterol due to competition with cholesterol for incorporation into micelles for transport across the intestinal cell wall (Sayre and Saunders, 1985; Heinemann et al., 1986).

The fiber in rice bran contains primarily insoluble and some soluble fiber, which has been shown to lower serum cholesterol level (Godber et al., 1993). However, most of dietary fiber in the rice bran is insoluble, so the cholesterol-lowering ability of rice bran may be through different mechanism. A study by Qureshi et al. (1991) in human and various animal models suggested that tocotrienol, a nonsaponifiable component, inhibits cholesterol synthesis and lowers serum cholesterol. Also, it has been reported to influence certain parameters of homeostasis and lowers the occurrence of chemically induced tumors in rodent models (Abeywardena et al., 1991; Tan and Chu, 1991).

Even though rice bran consumption has certain health benefits, a potential health risk associated with increased rice bran consumption is the impairment of mineral

untilization. Rice bran contains high levels of fiber and phytic acid, which both have been found to reduce the bioavaliability of some minerals such as iron and zinc (Godber et al., 1993).

1.4. Antioxidants in Rice Bran

1.4.1. Vitamin E Vitamers

Tocopherols and tocotrienols are collectively referred to as vitamin E (see appendix 1). They are classified according to the saturation of the side chain. The tocopherols contain a saturated side chain whereas the tocotrienols contain an unsaturated side chain between the carbons 3' and 4', 7' and 8', and 11' and 12'. The tocopherols and tocotrienols are designated by alpha (α), beta (β), gamma (γ), and delta (δ), according to the number and position of the methyl groups in the chroman nucleus (Lang et al., 1992). The presence of chiral centers at position 2 of the chromanol ring and the 4' and 8' carbons of the terpenoid side chain result in the eight diastereomeric forms of tocopherol. Moreover, tocotrienol double bonds can form *cis* and *trans* isomers (Lang et al., 1992).

The biological activities or biopontencies of vitamin E active compounds differ according to the individual tocopherol and tocotrienol homologs and their stereoisomers (Nelis et al., 1985). α-Tocopherol among the eight naturally occurring isomers is biologically the most potent compound of vitamin E even though this compound is the weakest antioxidant for fats (Lea and Ward, 1959). The biological potency of β and γ homologs is only half as active and δ-tocopherol even less (Lang et al., 1992). Vitamin E in unesterified form is slowly oxidized by atmospheric oxygen to yield biologically inactive quinone compounds like α-tocopherylquinone. The process is accelerated by

exposure to UV light, heat, alkaline pH, and the presence of trace minerals such as iron and copper (Nelis et al., 1985; Lang et al., 1992). The richest dietary sources of vitamin E are the cereal seed oils (Yuki and Ishikawa, 1976; Syvaoja et al., 1986), but the distribution varies greatly in different plant oils. Rice bran and palm oils, especially, contain over 70 % tocotrienol in total vitamin E concentration (Wilkinson, 1987; Cottrell, 1991). The differences in antioxidant effectiveness between tocotrienol isomers are unclear at present. However, recently a study has hypothesized that α-tocotrienol may have a higher antioxidant potency than α-tocopherol due to a higher recycling efficiency from chromanoxyl radicals, a more uniform distribution in the membrane bilayer, and a membrane disrupting effect that increases the efficiency of the interaction of chromanols with lipid radicals (Duthie et al., 1992).

1.4.2. γ-Oryzanol

Ferulic acid derivatives are present in rice, especially in rice bran (Yagi and Ohishi, 1979). Because the compounds were isolated from rice bran oil (*Orysase Sativa L.*) and contained a hydroxyl group, it was called oryzanol (Graf, 1992). Oryzanol is not a single compound but a variety of ferulic acid esters called α-, β-, and γ-oryzanol. Thus, oryzanol is a mixture of ferulic acid esters with sterols and triterpene alcohols, predominantly β-sitosterol, campesterol, cycloartenol, and 24-methylene cycloartanol (Sayre and Saunders, 1985). γ-Oryzanol consists primarily of cycloartenol, 24-methylene cycloartanol, and campesterol, all of which have beneficial effects in the treatment of arteriosclerosis (Kim and Kim, 1991).

The rice bran extract, γ-oryzanol, is of substantial commercial significance in Japan as a food and medical antioxidant, particularly when used in synergy with α-

tocopherol. γ-Oryzanol markedly inhibits the oxidation of rice bran oil, malondialdehyde generation during iron-mediated microsomal lipid peroxidation, and formation of dienes during peroxidation of linoleic acid by UV irradiation (Graf, 1992). Rice bran and rice germ oils contain 1.5-2.9 % of oryzanol by weight. Compared with the other cereals, the amount of oryzanol in rice bran is significantly higher (3500 mg/g) (Norton, 1995).

1.4.3. Phytic Acid (*myo*-inositol 1,2 3,4,5,6-hexakisphosphate)

Phytic acid (Appendix 2), an abundant chemical found in seeds, has long been regarded as an antinutrient due to its ability to chelate minerals and reduce their solubility and nutritional bioavailability (Cheryan, 1980; Cosgrove, 1980). However, phytic acid (InsP6) is converted by enzymes such as phosphatases into new compounds called inositol polyphosphates, which act as antioxidants (Phillippy and Graf, 1995). Phytase, with multiple forms, is an acid phosphatase that is capable of catalyzing the stepwise removal of inorganic orthophosphate from phytic acid, which yields lower inositol pentakis-, tris-, bis-, and monophosphates (Cosgrove, 1980). These lower inositol phosphates may be not significant in a point of view of antinutritional effect (Tao et al., 1986). Therefore, phytase can improve the nutritional value of bran-based foods and phytases present in plant tissue have been considered to be an endogenous means of reducing phytate content in foods (Gibson and Ullah, 1990).

Rice bran contains a high level of phytic acid as well as a variety of phytases (Ravindran, 1994). The fiber in rice bran can be an abundant source of phytic acid. Recently, there is increased interest in phytic acid for its nutritional and functional properties. In large part, this is because phytic acid is considered to be a precursor to

natural antioxidants (Empson et al., 1991). The antioxidant capability is based on the fact that phytic acid is a potent inhibitor of iron-catalyzed radical (•OH) formation because it can chelate free iron and then block the coordination site (Graf et al., 1984). Empson et al. (1991) reported that iron-induced oxidative damage in food could be diminished by the addition of a small amount of phytic acid. Phillippy and Graf (1997) supported the hypothesis that 1, 2, 3-trisphosphate *myo*-inositol accounts for the antioxidant potential of phytic acid. The 1,2,3-trisphosphate group exhibited a much higher affinity for iron than all other isomers of *myo*-inositol trisphosphate. The increased solubility of inositol 1,2,3-trisphosphate and inositol 1,2,3,6-tetrakisphosphate, hydrolysis products of phytic acid, over the solubility of phytic acid itself could enhance their potential use as antioxidants in food preservation, nutrition, and medical therapeutics.

1.5. Lipid Oxidation and Antioxidation

Unsaturation in fatty acids makes lipids susceptible to oxygen attack, which leads to complex chemical changes that eventually manifest themselves in the development of off-flavors in food. This process is termed autoxidation. The process of autoxidation and development of rancidity/oxidation in foods involves a free radical chain mechanism proceeding via three steps, initiation, propagation, and termination. In the initiation step, lipid free radical (L•) is produced by hydrogen abstraction from unsaturated fatty acids (LH), which reacts with oxygen to form peroxy radical (LOO•) in the next step. The oxygenation of the free radical is very rapid. In the propagation step, the peroxy radical can abstract hydrogen from unsaturated fatty acids to form hydroperoxides. The reaction in the propagation constitutes a chain reaction unless

termination occurs. In the termination step, one radical reacts with another radical forming a non-radical product. As lipid oxidizes, it forms hydroperoxides, the primary oxidation products, which are susceptible to further oxidation or decomposition. The hydroperoxides are labile and readily produce a number of secondary products such as aldehydes, ketones, acids, and alcohols. Unlike primary oxidation products, the secondary oxidation products contribute undesirable sensory characteristics to food (Vercellotti et al., 1991). There are many catalytic initiators, which can oxidize lipid. These initiators include light, temperature, enzymes, and metals. The free radical mechanism of lipid oxidation is summarized below.

Initiation: $LH \rightarrow L\bullet$ (by initiators)

Propagation: $L\bullet + O_2 \rightarrow LOO\bullet$

$LOO\bullet + LH \rightarrow LOOH + L\bullet$

Termination: $LOO\bullet + L\bullet \rightarrow$ non-radical product

$LOO\bullet + LOO\bullet \rightarrow$ non-radical product

$L\bullet + L\bullet \rightarrow$ non-radical product

Lipid oxidation is a problem not only in food but also in the human body. In the body, free radicals affect lipid cell membranes and produce secondary products, which are highly reactive and react with biological components such as protein, amino acids, and DNA. The reaction may be involved in many diseases and damages tissues such as liver, heart, blood, gastrointestinal tract, and the cardiovascular system, and may be involved in the process of aging, carcinogenesis, and mutagenesis (Nawar, 1985; Cuppett et al., 1997; Shahidi, 1997).

Generally, an antioxdant (AH) is a compund that can terminate a free radical mechanism. Antioxidants can delay the onset and rate of lipid oxidation and extend the induction period. The reaction proceeds by donation of hydrogen or an electron to radicals, which convert the radicals to more stable products. First, the antioxidants react with radicals (LOO•) formed from unsaturated lipids in the propagation step and terminate the free radical reaction.

$$LOO\bullet + AH \rightarrow LOOH + A\bullet$$

$$A\bullet + LOO\bullet \rightarrow \text{non-radical product}$$

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

The free radical antioxidants may further interfere with the chain-propagation reactions by forming a peroxy antioxidant compound (LOOA) or react with a free radical of lipid to produce the original antioxidant. Plants provide a rich source of natural antioxidants. The natural antioxidants from dietary sources include phenolic compounds, vitamin C, phytic acid, carotenoids, amino acids, and enzymes. These food-grade antioxidants are involved in the control of food autoxidation and rancidity prevention. Their presence in live plants may be for the sake of protection of tissues from injurious damage. Furthermore, the beneficial effects of consuming plant foods are associated with counteracting risk of most cardiovascular diseases, cancer, and other degenerative diseases. A current trend is to use natural antioxidants as food additive because of their safety.

1.6. Cholesterol Autoxidation

Cholesterol oxidizes readily in aqueous dispersions and foods when it is exposed to air, elevated temperature, light, metal, or a combination of these (Maeker, 1987).

Thus, cholesterol can form cholesterol oxide products (COPs), which have been shown to be cytotoxic, atherogenic, mutagenic, and carcinogenic (Imai et al., 1976; Peng et al., 1978, 1979; Yan and White, 1990). COPs have been identified in a variety of foods including raw, cooked, and dehydrated meats (De Vore, 1988), dairy products (Cleveland and Harris, 1987) and egg products (Chicoye et al., 1968). These COPs are formed readily during the cooking process or during prolonged storage (Rankin and Pike, 1993).

Typical oxidation products include cholestanetriol, 25-hydroxycholesterol, 7α- and 7β- hydroxycholesterol, 7-ketocholesterol, enantiomeric 5,6-epoxides, and cholesta-3,5-dien-7-one (Smith, 1981; Park and Addis, 1985). Once ingested, cholesterol oxides are readily absorbed and incorporated into high-density (HDL), low-density (LDL), and very low-density lipoproteins (VLDL) (Simic and Karel, 1980; Emanuel et al., 1991). Cholesterol oxides are reported to inhibit the biosynthesis of cholesterol and possess remarkable cytotoxic effects on membrane cells. They could also have a direct effect on the membrane by replacing cholesterol in the membrane, which may result in a decrease in membrane cholesterol levels and lead to impaired functionality and cellular injury (Brown and Goldstein, 1974; Peng et al., 1979). The replacement effect is conceivable because the stronger polar groups on one end of the cholesterol oxide molecule and the hydrophobic group on the other end might make it possible for this molecule to integrate into the membrane easily and thereby cause dysfuction of membrane.

The mechanism of cholesterol oxidation is known as a free radical mechanism, similar to autoxidation of unsaturated fatty acids (Nawar, 1985). Generally, the

epimeric 7α- and 7β-hydroperoxides are recognized as the initial products, which decompose to 7α- and 7β-hydroxycholesterols, α- and β-epoxides, and 7-ketocholesterols, with the latter being a major product. In addition, 20- and 25-hydroxycholesterol may be formed by side-chain derivatizations, whereas cholesta-3, 5-diene, cholesta-3, 5-dien-7-one arise from an elimination reaction (Maerker, 1987). Because both lipid and cholesterol oxidation proceed by the same mechanism, compounds that prevent unsaturated fatty acid oxidation may be reasonably expected to inhibit cholesterol oxidation.

1.7. Emulsion System for Studying Cholesterol Oxidation

Because meat is a complex food, model systems have been developed to gain a fundamental understanding of oxidation processes in meat. *In vitro* approaches have used emulsion, liposome, and microsome systems. An emulsion is a relatively simple model system containing two immisicible phases and can be used to study oxidation in muscle-based foods (Zulak and Maerker, 1989; Chan et al., 1997). The emulsion system is composed of oxidizable substrates (lipid), surfactant, and aqueous solution buffer. To produce a stable emulsion, surfactants must be used. These adsorb at the lipid/aqueous boundary and serve to lower interfacial tension, which provides a physical resistance to coalescence. Sodium dodecyl sulfate (SDS), Triton X-100, or Tween 20 are commonly used surfactants. These surfactants may help generate a more homogenous micelle solution (Nawar, 1985; Chan et al., 1997). Micellar systems are relatively simple, uniform and well-understood, although they are heterogeneous. Therefore, micellar solutions provide a takeoff point for studies of more complex

membrane systems and represent a system in which antioxidants equilibrate rapidly from the aqueous phase into the model membrane (Pryor et al., 1988).

Systematic studies of cholesterol autoxidation under controlled conditions with a dispersed system have not been made in biological system such as animal tissues. Generally, cholesterol autoxidation in tissues can not be easily investigated systematically because of an inability to control artifacts due to unknown variables. Aqueous sodium stearate dispersions of cholesterol have been used as vehicles for administration of cholesterol to biological systems. However, emulsions prepared with nonionic surfactants such as Tween 20 or Tween 80 and various other detergents such as sodium dodecyl sulfate or sodium cholesterol sulfate have become useful for such work. According to Rankin and Pike (1993), SDS effectively dispersed the cholesterol and oxides throughout the sampling period at 80 °C without visual indication of instability. Pryor et al. (1988) presented data on the reactivity of a series of antioxidants with peroxyl radical from linoleic acid in SDS micelles at 37 °C. The SDS micelle allows components such as antioxidants and initiators to equilibrate rapidly into the oil phase so that quantitative kinetic data can be obtained more easily (Turro and Weed, 1993). Therefore, the incorporation of natural antioxidants into a simple model system may be useful in understanding the effectiveness of natural antioxdants in inhibiting cholesterol oxidation in food and human blood.

1.8. Research Objectives

The overall objective of the research presented in this dissertation was to establish increased nutritional value and oxidative stability in restructured beef roasts by including purified components of rice bran.

The specific objectives were:

1. To determine the oxidative stability and change in nutritive values of restructured beef roasts containing rice bran oil during refrigerated storage.
2. To establish the potential of restructured beef roasts containing rice bran oil and rice fiber as a functional food.
3. To study the change of vitamin E vitamers and oryzanol during cholesterol oxidation in a model system containing the nonsaponifiable fraction of rice bran.
4. To determine the antioxidative effect of nonsaponifiable fraction in inhibiting the formation of 7-ketocholesterol in a model system during cholesterol oxidation.

CHAPTER 2
EXPERIMENT 1

OXIDATIVE STABILITY AND NUTRITIVE VALUES INCREASED IN RESTRUCTURED BEEF ROASTS WITH ADDED RICE BRAN OIL

2.1. Introduction

The value of red meat products can be enhanced through restructuring techniques (Akamittath et al., 1990). Restructured meat products have advantages such as compositional control, convenient preparation and economical production. Therefore, these products can be formulated to meet specific consumer needs (Pearson and Tauber, 1984; Tanchotikul et al., 1989; Akamittath et al., 1990). Nevertheless, there are some major concerns associated with precooked restructured beef products, which include both lipid and cholesterol oxidation (Arganosa, 1991; Rankin and Pike, 1993). Warm-over-flavor (WOF), which is caused by lipid oxidation, is unacceptable to the consumer because of a rancid taste (Tanchotikul et al., 1989). Generally, lipid oxidation can be affected by lipid content, unsaturated fatty acids, antioxidant compounds, preparation technique, and storage time (Tanchotikul et al., 1989). In addition to lipids, cholesterol is also susceptible to oxidation even at ambient temperature when exposed to air. Cholesterol oxide products (COPs) are formed and may be toxic to cells and possibly be involved in cardiovascular disease (CVD).

Eight commonly occurring COPs are 25-hydroxycholesterol, cholestane-triol, 7α and 7β-hydroxycholesterol, 7-ketocholesterol, the α- and β-epoxides of cholesterol and cholesta-3,5-dien-7-one (Bischoff and Byron, 1977; Smith, 1981; Ansari et al., 1982). Among these COPs, 7-ketocholesterol is the predominant compound in heated tallow (Park and Addis, 1986). Sander et al. (1989) reported that 7-ketocholesterol is a toxic sterol as well as the most potent inhibitor of cholesterol biosynthesis, which is essential for cell function; it is 100 times more potent than cholesterol itself. Muscle foods contain high levels of polyunsaturated fatty acids (PUFA) and cholesterol, which may

lead to health problems (Pearson et al., 1983; Addis, 1990). Because both PUFA and cholesterol oxidation proceed by a free radical mechanism, compounds that inhibit PUFA oxidation may also inhibit cholesterol oxidation. Wild rice has recently been used in ground beef products for both nutritive and antioxidant functionality (Minerich et al., 1991). Rice bran possesses high levels of the monounsaturated fatty acid, oleic acid, and very high levels of antioxidant compounds including tocopherols, tocotrienols, and oryzanols (Godber et al., 1993). These compounds have been reported to lower serum cholesterol and the risk of coronary artery disease and to serve as natural antioxidants (Nicolosi et al., 1991). The purpose of this research was to determine the oxidative stability of restructured beef roasts containing rice bran oil during refrigerated storage and to determine the changes in nutritive values in restructured beef roasts as affected by the incorporation of rice bran oil.

2.2. Materials and Methods

2.2.1. Preparation of Restructured Beef Roasts

Beef rounds from carcasses that had not been graded were obtained from a local retailer in the state of Louisiana. Lean meat and fat trim were manually separated and most of the connective tissue and external fat were removed. The lean meat and fat trim were then pooled in separate containers. Half of the lean meat was manually cut into approximately 1 inch cubes while the other half of the lean meat was ground with the fat trim using a 1 cm plate (Model d5-A, Hobert, Ohio). Beef roasts were manufactured on three separate occasions, which represented three replications of the manufacturing process. The formulation of beef batter was designated to contain 30 % (w/w) fat trim, 10 % (w/w) water, 59 % lean trim and 1 % salt. The control treatment was the batter

without rice bran oil. Rice bran oil replaced part of fat trim levels at 1 or 2 %. All ingredients were mixed in a Kitchen Aid mixer (Model d5-A, Hobert, Ohio) at the speed setting of 2 for 5 min. The batter was hand-stuffed into polyethylene bags, shaped and then, vacuum-packed using a Multivac Model A300122 (Sepp Hagen-Muller Kg, Walfertschweden, West Germany). Beef roasts in the polyethylene bag were cooked in a water bath at 70 °C until an internal temperature of 149 °F was reached, requiring about 25 min. Cooked products were cooled in an ice-water bath for 20 min, then sliced 3 cm thick. Each treatment was separately placed in a polyethylene bag and stored at 4 °C. Samples were analyzed at 0, 7, and 14 d.

2.2.2. Moisture Analysis

Moisture of each roast sample was determined at 105 °C for 4 h according to the oven drying method (AOAC,1984). Moisture was determined as a percentage of the total weight from the total loss after drying.

2.2.3. Lipid Analysis

Total lipid was determined by the extraction method using chloroform and methanol, which was described by Folch et al. (1957). The results were expressed as % total lipid by weight.

2.2.4. Free-Fatty-Acid Analysis

Free fatty acids were determined by the AOCS method Ca 5a-40 (1990).

2.2.5. Thiobarbituric Acid Reactive Substance (TBARs) Analysis

The distillation method of Tarlagis et al. (1960) modified by Ockerman (1980) was used to measure the level of lipid autoxidation. Results were expressed as milligrams of thiobarbituric acid reactive substances (TBARs) / kg meat.

2.2.6. Vitamin E Vitamers Analysis

2.2.6.1. Solid-Phase-Extraction (SPE)

The analytical method used was based on Shin and Godber (1997) but modified for beef products. Finely minced roast sample (1g) was placed in a 15-mL test tube with 5 mL ethanol and 0.1g ascorbic acid. The test tube was placed in an 80 °C water bath for 10 min after which 0.25 mL of 80 % KOH was added. The sample was saponified for 15 min in an 80 °C water bath. The test tube was cooled to room temperature and 2 mL of 15 % acetic acid (v/v in water) was added. The extract was centrifuged at 537 × *g* for 4 min. The supernatant was transferred to a conditioned SPE tube (Supelco, Bellefonte, PA) and 5 mL of ethanaol and 2 mL of 15 % acetic acid were added to the test tube and mixed with a vortex mixer. The Supelclean LC-18 SPE tube was washed with 2 mL of methanol, followed by 2 mL of 1 % acetic acid (v/v in water), twice, to reestablish conditioning. The sample was centrifuged and the supernatant was transferred to a conditioned SPE tube. This procedure was repeated one more time. The extract was allowed to pass through the SPE tube at less than 2 mL/min using a calibrated visiprep™ 24 port-DI solid phase extraction vacuum manifold (Supelco, Bellefonte, PA). The tube was washed with 2 mL distilled water, 2 mL of 1% acetic acid (v/v in water), and 0.5 mL of methanol:water (50:50, v/v) to remove unwanted and unretained materials, and then dried under a stream of nitrogen. Compounds of interest were recovered by eluting the SPE tube with 0.5 mL methylene chloride 3 times. The eluate was dried under a stream of nitrogen and diluted with 1 mL mobile phase.

2.2.6.2. High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Waters (Milford, MA) Model M-45 pump, a Waters Model 680 automated gradient controller, Model 715 Ultra WISP injector, and a Model 470 scanning flurescence detector with excitation λ at 290 nm and emission λ at 330 nm ; a Supelcosil™ (Supleco Bellefonte, PA) LC-Si, 5 μm, 25 cm × 4.6 mm i.d. column was used. Mobile phase was made up with hexane: acetic acid: ethyl acetate (99: 0.5: 0.5). The flow rate was at 1.8 mL/min for the first 9 min and at 2.4 mL/min from 10 to 25 min. Quantification was accomplished using Maxima chromatography workstation (Waters, Milford, MA) with external standard curves obtained using vitamin E vitamers purified as described by Shin and Godber (1994).

2.2.7. Cholesterol and 7-Ketocholesterol Analysis

2.2.7.1. Lipid Extraction and Cold Saponification

Lipids were extracted from 5 g of roast beef into 30 mL of 2:1(v/v) chroloform/methanol (Folch et al.,1957). Ten mL of 1.5 N KOH solution in methanol was added to the lipid extract and then shaken until the mixture became free of dispersed fat particles. Saponification was conducted at room temperature overnight (18-20 h) according to Park et al. (1996) and Park and Addis (1986). Ten mL of distilled water were added to the saponified mixture, which was transferred to another test tube (25×200mm) with a Teflon-lined screw cap. Nonsaponifiables were extracted three times with 10 mL diethyl ether. The pooled diethyl ether extracts were washed twice with 5 mL of 0.5 N KOH and three times with 5 mL of distilled water, and dried over anhydrous sodium sulfate. The dried extract was obtained after filtration and solvent removal. The extract was dissolved in 1 mL of mobile phase before HPLC

analysis. The recovery of extraction for cholesterol and 7-ketocholesterol was established using homogenized roast beef samples with known sterol standards. The extraction efficiency was 95 % (n=3) for cholesterol and 89 % (n=3) for 7-ketocholesterol.

2.2.7.2. HPLC

The HPLC system was the same as above, except that a LC-Si column (25 cm× 4.6 mm i.d. Supelco Bellefonte, PA) was used for 7-ketocholesterol and a C18-5 μm 100 A column (Rainin Instrument Company, Inc.) was used for cholesterol. The mobile phase consisted of hexane/isopropyl alcohol (97.5:2.5) for 7-ketocholesterol and methanol/acetonitrile (7:3) for cholesterol. The mobile phase was pumped at 1.5 mL/min for the first 5 min and then at 2.4 mL/min for cholesterol. An UV/VIS variable wavelength detector (Hewlett Packard 1050) was used and the wavelength of detector was 230 nm for 7-ketocholesterol and 209 nm for cholesterol. A Maxima chromatography workstation (Waters, Milford, MA) was used as signal recorder and to calculate the concentration of sterol component with external standard curves.

2.2.8. Fatty Acids Analysis

2.2.8.1. Extraction

Lipid samples (0.05 g) extracted from beef roast were dissolved (w/v) in hexane (1 mL) in a 15-mL test tube and 5 % (v/v) methanolic hydrogen chloride (2 mL) and 1 mL (1 μg/μL) heptadecanoic acid methyl ester as internal standard were added. The mixture was incubated for 12 h in an 80 °C water bath, and then 5 mL of 5 % sodium chloride (w/v) in H_20 was added. The required esters were extracted with 5 mL hexane twice using Pasteur pipettes to separate the layers. The hexane layer was washed with

10 mL 5 % $KHCO_3$ (w/v) twice and dried over anhydrous sodium sulfate. The solution was filtered and then evaporated under a stream of nitrogen (Christie, 1982).

2.2.8.2. Gas Chromatography (GC)

Analysis of the fatty acid methyl esters (FAME) was carried out on a Hewlett-Packard (San Fernando, CA, U.S.A.) 5890 gas chromatograph equipped with a split/splitless capillary inlet system and a flame ionization detector with Supelco SP-2380 (Supelco, Bellefonte, PA) fused silica column (0.25 mm i.d.×30m). Operational parameters were as follows: oven temperature, 175 °C; injector temperature, 250 °C; flame ionized detector (FID) temperature, 270 °C. (A representative chromatogram is presented in appendix 5). Quantification was accomplished using Maxima software based on internal standard methods.

2.2.9. Statistical Analysis

The three experimental replications of beef roasts were applied in a randomized complete block design with treatments assigned by 3×3 factorial arrangement. Rice bran oil treatment and storage time were main treatment factors in a 3×3 factorial arrangement. Data were analyzed using a general linear model procedure of the statistical analysis system (SAS, 1990). Means were separated by the least significant difference method (LSD) at $p<0.05$.

2.3. Results and Discussion

2.3.1. Nutritive Aspects

Restructured beef roasts containing 1 or 2 % rice bran oil (RBO) had a higher total lipid content than the control (0 % RBO). All restructured beef roast samples

Table 2.1- Composition of restructured beef roasts containing different levels of rice bran oil before and after storage (14days) at 4 °C.

	Total Lipid (%)		Moisture (%)		Free-fatty-acid (%)		Cholesterol (mg/100g)		SFA[2] (%)		UFA[3] (%)		S/U[4]		Total VitaminE (mg/kg)	
Treatment	0	14	0	14	0	14	0	14	0	14	0	14	0	14	0	14
0% RBO	7.45a	8.28b	67.25a	66.08c	0.23a	0.28c	49.02a	46.73c	51.42a	52.70a	48.58b	47.30b	1.06a	1.11a	1.11a	0.75d
1% RBO	8.52b	9.39d	66.21b	65.05d	0.22b	0.26d	48.28b	46.04d	48.59a	52.06a	51.41b	47.04b	0.98a	1.09a	1.94b	1.33e
2% RBO	9.01b	9.67d	65.31d	64.68d	0.22b	0.26d	47.21b	45.96d	48.05a	48.68a	51.95b	51.32b	0.92b	0.95b	2.47c	1.71f

[1] Means (n=3) within rows and columns in 0 and 14 days with different letters are significantly different ($p<0.05$).
[2] Saturated fatty acids (wt. % of total fatty acids).
[3] Unsaturated fatty acids (wt. % of total fattyacids).
[4] Saturated fatty acids/Unsaturated fatty acids.
[5] RBO is rice bran oil treatment.

contained between 46 to 49 mg/100 g cholesterol, which is similar to the reported value for cholesterol in cooked ground beef patties, 42 to 47 mg/100 g (Kregel et al., 1986). Kritchevsky and Tepper (1961) have reported that cooking reduces the cholesterol content of meat. Significant differences in cholesterol content were not found among the rice bran oil treatment products at each storage time; while cholesterol content in products with RBO was lower than the control (Table 2.1). The decrease in total cholesterol content would give a positive impression of the cooked beef product containing rice bran oil.

The beef roasts contained 11.2 to 24.7 mg/100 g of vitamin E before storage. Significant differences in total vitamin E content were found when 1% or 2% RBO was added to the formulation of restructured beef roasts. Rice bran oil possesses unsaponifiable fraction such as tocotrienols and oryzanols, in higher concentrations than other oils (Saunders, 1990). Tocotrienols in the unsaponifiable fraction inhibit 3-hydroxyl-3methyl-glutaryl coenzyme A (HMG-Co A) reductase activity in the biosynthetic pathway of cholesterol with a resultant decrease in blood cholesterol (Quereshi et al., 1991; Rukmini and Raghuram, 1991). Recent studies indicate that tocotrienols of palmolein effectively lowered circulating cholesterol levels, despite palm oil's greater saturated fatty acid content (Hornstra et al., 1986; Rukmini et al., 1991). The concentrations of vitamin E vitamers in restructured beef roasts are shown in Fig. 2.1. Both α- and γ- vitamers were detected in all samples. At the beginning of experiment, the concentrations of α-tocopherol and γ-tocotrienol contents in 1 % and 2 % RBO treatment were significantly higher than the control. α-Tocotrienol and γ-tocopherol in products with rice bran oil were not higher than those of the control. The

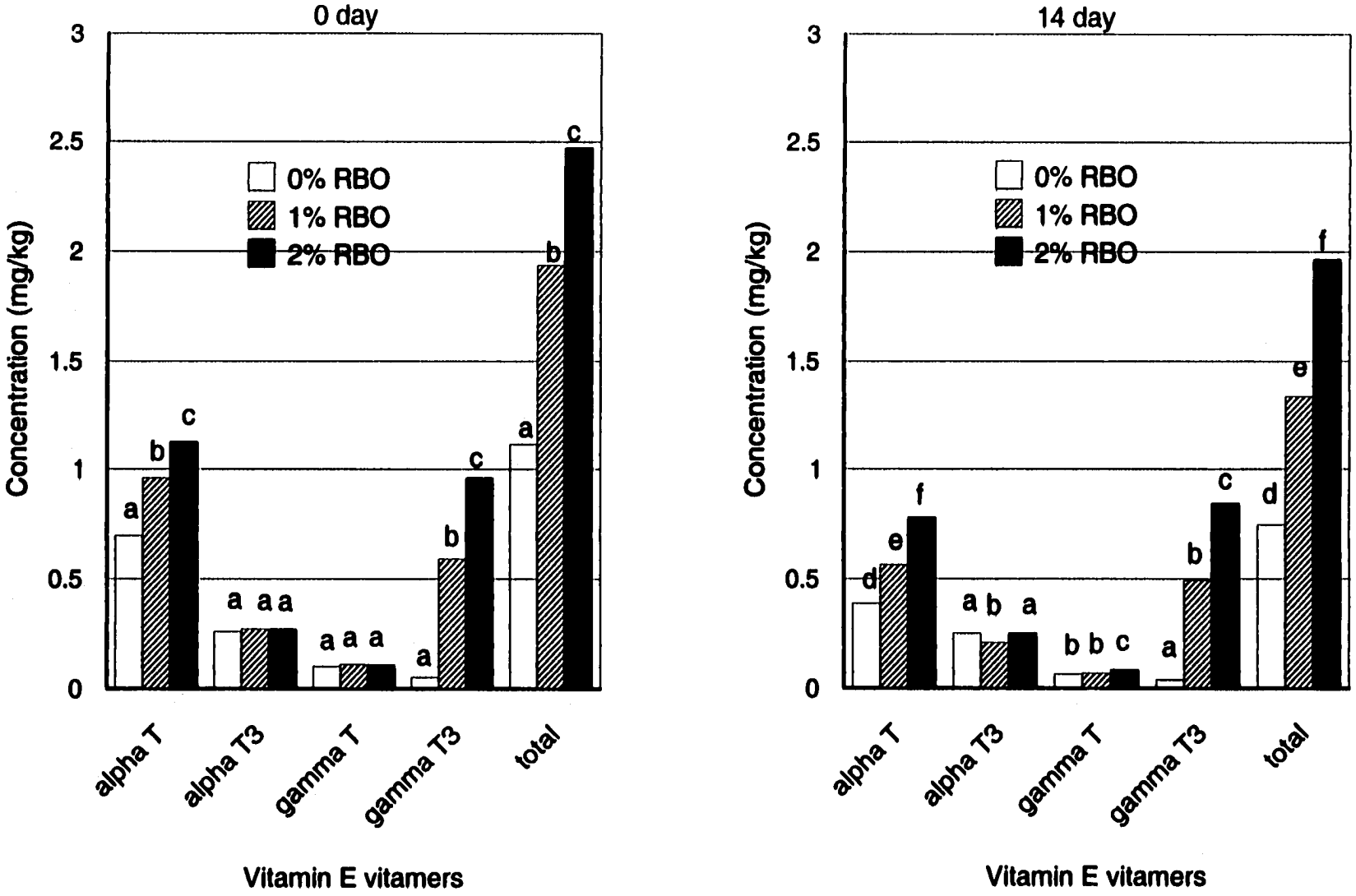


Fig. 2.1 -Concentration of vitamin E vitamers in restructured beef roasts containing different levels of rice bran oil (RBO) before and after storage (14 days) at 4 °C. Different letters in each vitamer are significantly different ($p<0.05$). T=tocopherol, T3=tocotrienol.

Table 2.2- Fatty acid profiles of restructured beef roasts containing different levels of rice bran oil (RBO).

	fatty acids (wt. % of total fatty acids)									
Treatment	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	SFA[1]	UFA[2]	S/U[3]
RBO[d]	0.13	17.53	1.33	-	44.77	34.91	1.32	18.99	81.00	0.23
0% RBO	2.85	34.75	4.22	14.46	40.99	2.73	-	52.06a	47.94b	1.09a
1% RBO	2.65	34.00	4.09	13.63	40.98	4.64	-	50.32a	49.71b	1.01a
2% RBO	2.36	33.08	3.34	12.93	41.88	4.41	-	48.37a	51.63b	0.94b

[1]Saturated fatty acids (wt. % of total fatty acids)
[2]Unsaturated fatty acids (wt. % of total fatty acids)
[3]Saturated fatty acids/Unsaturated fatty acids
Each mean (n=3) is based on % total fatty acid.
Means with different letters in each column are significantly different ($p<0.05$).

concentrations of vitamin E vitamers were lower than the values reported by Piironen et al. (1985). This may result from processing and cooking.

Table 2.2 depicts the fatty acid compositions of restructured beef roasts supplemented with rice bran oil. The major fatty acids in restructured beef roasts include the saturated fatty acids (SFA), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids, and the unsaturated fatty acids (UFA), palmitoleic (C16:1), oleic (C18:1) and linoleic (C18:2) acids. Palmitic acid (C16:0) and stearic acid (C18:0) represented a high proportion of the saturated fatty acids in the beef roasts. Although stearic acid is one of the major saturated fatty acids in beef roasts (14.46 % of total fatty acids and 27.78 % of saturated fatty acids), this fatty acid is not highly associated with cardiovascular disease (Godber, 1993) and is possibly neutral in terms of its effect on plasma cholesterol in humans (Hegsted et al., 1965; Bonanome et al., 1988). On the other hand, palmitic acid, which is the most abundant saturated fatty acid in beef roast (34.75 % of total fatty acids and 66.75 % of saturated fatty acids), raises plasma cholesterol in humans (Grundy,1986). Adding rice bran oil to the beef roast formulation changed the saturated fatty acids/unsaturated fatty acids ratio (S/U) (Table 2.2). Even though a significant rice bran oil treatment effect was not found among the total saturated fatty acids, most saturated fatty acids decreased and particularly linoleic acid (C18:2), an unsaturated fatty acid, increased as rice bran oil level increased. This is because rice bran oil contains a lower proportion of saturated fatty acids (18.99 %) and a higher proportion of unsaturated fatty acids (81.10 %). The oleic acid (C18:1) content of restructured beef roasts constitutes 40.99 % of total fatty acids and 44.77 % of the fatty acid was found in rice bran oil. Oleic acid is the major monounsaturated fatty

acids in animal and plant fatty acids. Linoleic acid (C18:2), which is the primary polyunsaturated fatty acid, is the most widespread fatty acid in most plant oils and animal fats and is also nutritionally essential. However, animal fat contains a much lower level of linoleic acid compared with plant oil. In this experiment, only 2.73 % linoleic acid was found in control of restructured beef roasts. Rice bran oil contained 34.91 % linoleic acid. Addition of rice bran oil to the formulation of beef roasts significantly increased the linoleic acid and total unsaturated fatty acids content ($p<0.05$). Linolenic acid (C18:3), which is minimal in meat, was detected but could not be quantified due to the trace amount in restructured beef roast samples. Although the addition of rice bran oil did not significantly change total saturated and total unsaturated fatty acids, as the level of rice bran oil increased, the ratio of S/U in product with 2 % RBO was lower ($p<0.05$) rather than the other two treatments.

2.3.2. Oxidative Stability

The TBARs and 7-ketocholesterol content of restructured beef roasts were determined during refrigerated (4 °C) storage and are presented in Table 2.3. After 7 and 14 days storage, TBARs values were higher for the control sample compared to initial values (Table 2.3). These values are similar to TBARs values found for cooked beef patties at 7 day of storage at 4 °C (McMillin et al., 1991). Initially there was no effect due to rice bran oil, but TBARs values significantly decreased ($p<0.05$) as the percent of rice bran oil increased. At 7 and 14 d of storage, TBARs of the control were significantly higher than those of products with the rice bran oil treatment. The RBO treatment samples showed a 50 % decrease in TBARs compared to the control sample and the % RBO did not affect the TBARs values.

Table 2.3 – Thiobarbituric acid reactive substances (TBARs) and 7-ketocholesterol concentrations in restructured beef roasts containing different levels of rice bran oil (RBO) at 4 °C for 0, 7, and 14 days.

	TBARs value[1] (mg/kg) storage days			7-Ketocholesterol[2] (μg/g) storage days		
Treatment	0	7	14	0	7	14
0% RBO	0.17a	1.61b	1.63b	2.47a	5.02c	5.27c
1% RBO	0.16a	0.82c	0.80c	1.69b	3.31d	3.51d
2% RBO	0.16a	0.79c	0.80c	1.52b	3.25d	3.45d

[1] mg TBARs/kg meat sample
[2] Each value represents triplicate HPLC determination on two lipid extracts of each treatment. Means (n=6) with different letters are significantly different ($p<0.05$).

The decrease of TBARs values in restructured beef roasts with added rice bran oil may be due to the increase of the ratio of other components like tocopherol, tocotrienol, and any other nonsaponifiable fraction in the products that come from rice bran oil. Vitamin E vitamers act as antioxidants by reacting with free radicals (Tappel, 1962). Several studies have found natural antioxidant effectiveness in restructured meat products (Stoick et al., 1990; Addis et al., 1998).

Park and Addis (1985) found no detectable 7-ketocholesterol in raw beef purchased from a local market and cooked hamburgers purchased from a fast food restaurant. However, in this study 7-ketocholesterol was detected in restructured beef roasts and it markedly increased during 7 days of storage, increasing two fold from 0 d to 7 d of storage (Table 2.3) and there was no significant difference between 7 and 14 d. At 7 and 14 d of storage, 7-ketocholesterol in products with added rice bran oil was approximately 34 % lower rather than those of the control and there were no significant differences between the RBO treatment products. The correlation between 7-ketocholesterol and TBARs values was 0.96 (r^2) as shown in Fig. 2.2.

Polyunsaturated lipids are susceptible to oxidation and may form highly reactive free radicals. Membranes are the major source of polyunsaturated fatty acids and cholesterol (Igene etal., 1980; Rhee et al., 1982). Because PUFA and cholesterol are integral components of membrane structure and are susceptible to autoxidation, free radicals formed by phospholipid oxidation may initiate cholesterol oxidation in the tissue membranes of restructured beef roasts. Storage was the main effect on the cholesterol concentration of restructured beef roasts (Table 2.1). Cholesterol content of the samples significantly decreased ($p<0.05$) after 14 d of refrigerated storage. The

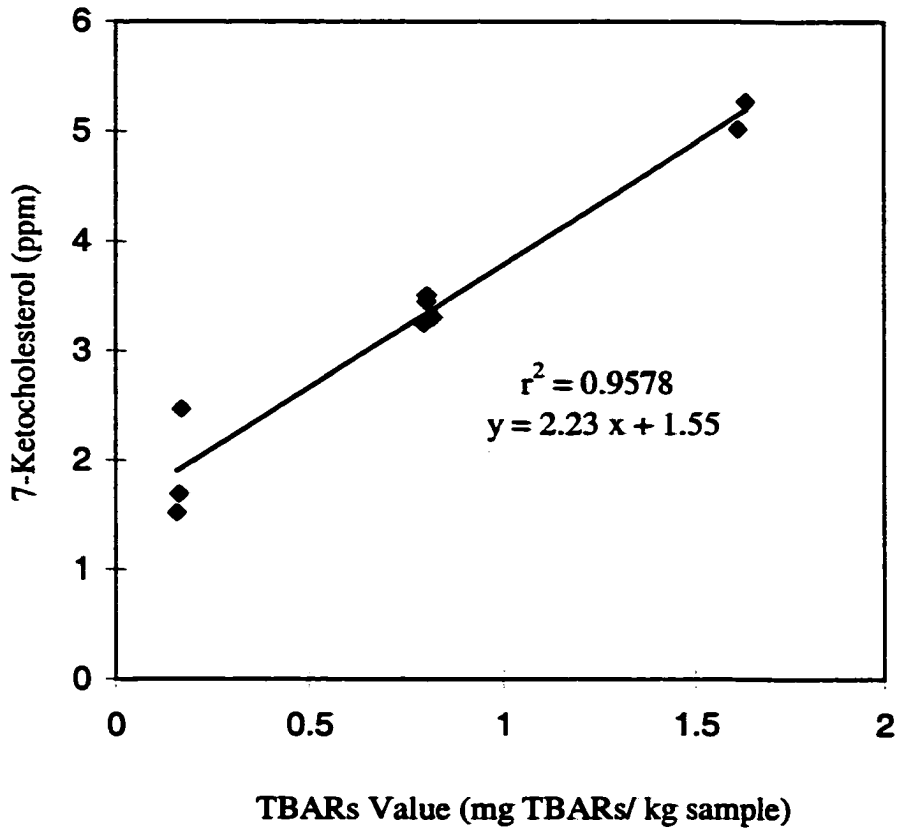


Fig. 2.2- Correlation of thiobarbituric acid reactive substances (TBARs) value with 7-ketocholesterol content of restructured beef roasts stored 0, 7, and 14 days at 4 °C.

decrease of cholesterol content during storage of the samples may be due to cholesterol oxidation. Cholesterol can be oxidized to cholesterol oxide products (COPs) when exposed to high temperature, air, light and metal ions. COPs have been reported to be carcinogenic, mutagenic and atherogenic (Bischoff et al., 1977; Ansari et al., 1982; Nawar, 1985). Smith (1980) indicated that cholesterol autoxidation was a free radical mechanism similar to autoxidation of unsaturated fatty acids. Therefore, antioxidants, which may inhibit free radical production, may improve cholesterol oxidation stability. Rankin et al. (1993) reported the inhibition of cholesterol autoxidation by several natural antioxidants. The γ- and δ-tocopherol vitamins were more effective, while α-tocopherol was less effective. Because rice bran oil contains all tocopherol isomers, cholesterol in beef roast samples containing rice bran oil was more stable to autoxidation than the control samples.

The effect of refrigerated storage on fatty acids in restructured beef roasts is shown in Table 2.1. No significant changes were found in SFA, UFA, and S/U before and after storage ($p>0.05$). Moreck and Ball (1974) followed the changes of polyunsaturated fatty acids in meat in order to monitor primary changes in the initial stages of lipid oxidation. SFA and S/U increased, while UFA decreased after 14 d of storage. The decrease in the degree of UFA of lipid may be related to an increase in oxidative degradation of the meat caused by free radicals formed from the double bond when initiators like metal ions or oxygen are added to the double bond.

The loss of total vitamin E during storage is presented in Table 2.1. Initial contents of total vitamin E in the samples were 1.12, 1.94, and 2.47 ppm for control and samples with 1 and 2% RBO, respectively. After 14 d of storage, those values

decreased to 0.75, 1.33, and 1.97 ppm for control and the samples with 1 and 2% RBO, respectively. The order of total vitamin E loss was 0% RBO (33%)> 1% RBO (31%)> 2% RBO (21%). α-Tocopherol and γ-tocopherol significantly decreased compared to α-tocotrienol and γ-tocotrienol during storage (Fig. 2.1). α-Tocotrienol and γ-tocotrienol were not significantly lower after 14 d of storage. The order of α-tocopherol retention was 2 % RBO (69.2 %)> 1 % RBO (58.6 %)> 0 % RBO (55.8 %). Rice bran oil treatment had no effect on γ-tocopherol even though the concentration of γ-tocopherol decreased significantly after 14 d of storage. α- and γ- Tocopherols were more easily destroyed compared to other two isomers and less stable during storage. α-Tocotrienol and γ-tocotrienols were the more stable vitamin E vitamers in the samples. According to Chow and Draper (1969), the order of antioxidant activity of natural tocopherol was γ >δ>β>α. The loss of vitamin E vitamer may be related to the amount of free fatty acids hydrolyzed by lipolytic enzymes in rice bran. Lipoxygenase in bran and meat catalyzes the peroxidation of polyunsaturated fatty acids (Galliard, 1986; Angelo, 1996), which causes the loss of the vitamin E vitamers. In addition, vitamin E vitamers are directly oxidized through lipid oxidation, which is dependent on moisture content, temperature, humidity, and microbial growth during storage (Loeb and Mayne, 1952; Slover and Lehman, 1972; Galliard, 1986). The results presented have illustrated that tocopherols and tocotrienols in restructured beef roast supplemented with rice bran oil had a protective effect against lipid oxidation during refrigerated storage.

2.4. Conclusions

This study demonstrated that the addition of rice bran oil to the formulation of restructured beef roast can improve the nutritive value and oxidative stability of the

product during refrigerated storage. The addition of rice bran oil produced a lower SFA/UFA ratio, higher vitamin E vitamer concentrations, and lower cholesterol and 7-ketocholesterol concentrations. After refrigerated storage, TBARs values were significantly lower in beef roasts with rice bran oil. The oxidative stability of beef roast containing rice bran oil was probably due to the vitamin E vitamers in rice bran oil. The results of this investigation indicated that rice bran oil may be a source of natural antioxidant for restructured beef roast because it improved the nutritive values and prevented oxidative degradation during storage. Ultimately, we would like to develop a restructured beef product possessing rice bran components, which maximize sensory and nutritional properties.

CHAPTER 3
EXPERIMENT 2

FUNCTIONAL BEEF PRODUCT CONTAINING RICE BRAN OIL AND FIBER INFLUENCES CHOLESTEROL OXIDATION AND NUTRITIONAL PROFILE

3.1. Introduction

Currently many consumers desire additional health benefits from food beyond that of meeting basic nutritional needs. Such foods are called functional foods and refer to processed foods containing ingredients that in addition to being nutritious aid specific bodily functions (Hasler, 1998). Therefore, health-conscious consumers are increasingly seeking functional foods to control their own health and well-being. The beef industry is continually developing new products to improve health and nutrition. These products must meet the demand of today's consumers in having high nutritive value and desirable taste (Godber and Mullins, 1990). Several studies have stated that rancidity is a major deterrent to taste in precooked beef products (Arganosa et al., 1989; 1991; Tanchotikul et al., 1989). Addition of antioxidants would be an approach to reduce rancidity. Several chemicals found in rice bran have functional potential as natural antioxidants, including tocopherols, tocotrienols, oryzanols, and inositol phosphates.

Beef has highly available forms of minerals and increases the biological value of non-meat nutrients by increasing nutrient absorption when consumed together. However, beef also contains factors perceived as unhealthy such as saturated fat and cholesterol, which are related to cardiovascular disease. Rice bran, on the other hand, has certain health benefits such as reducing serum cholesterol, but it also has the drawback of impairing mineral bioavaliability due to high levels of fiber and phytic acid (Gordon and Godber, 1989; Godber, 1990; Qiao et al., 1992). Therefore, mineral bioavailability may be enhanced in rice bran when beef is incorporated concurrently in

the diet (Godber, 1990). Combination of these nutritional properties into a single product might be an ideal prospect.

Rice products have been used as extenders in meat loaf products for many years. Recently, wild rice has been incorporated into meat products for both nutritive and antioxidant functionality (Minerich et al., 1991; Addis et al., 1998). Addis et al. (1998) suggested that the inclusion of barley bran by itself or in combination with wild rice to a meat system can prevent the development of warmed over flavor. Rice bran typically contains 7-22 % oil by weight and the main component of crude rice bran oil, the triglycerides, makes up approximately 80% of the total oil. Rice bran oil is more stable than other vegetable oils under frying conditions (Yuki and Ishikawa, 1976) due to a balance between linoleic and oleic acids, a low level of linolenic acid, and a high level of antioxidants, both vitamin E vitamers and oryzanols (Sayre and Saunders, 1990). In addition, rice bran contains both insoluble and soluble fibers. Soluble fibers in brans bind to cholesterol and bile acids in the gastrointestinal tract to reduce the absorption of cholesterol (Chen and Anderson, 1986; Godber et al., 1993). The nutritive value of beef and resistance to oxidative degradation would be increased by incorporation of natural antioxidants into beef products. Our objective was to investigate oxidative stability of restructured beef roasts containing rice bran oil and rice fiber, and establish the functional potential of this product.

3.2. Material and Methods

3.2.1. Sample Preparation

Roasts were prepared from the muscles of beef inside rounds obtained from a local retailer. Lean meat and fat trim were separated after removing external fat and

excessive connective tissue. The lean meat and fat trim were then pooled into the separate containers. Half of the lean meat was manually cut into approximately 1 inch cubes while the other half was ground with the fat trim using a 4-arm blade Butcher Boy (model A52.HF, Hobart, Ohio) grinder. The roasts were formulated (Table 3.1) and mixed with a Kitchen-Aid mixer (Model D5-A, Hobart, Ohio), using a mixing type paddle at the intermediate speed. The lean meat and all required ingredients such as water, salt, and rice bran extracts were added during the first five minutes of mixing. Afterwards, fat trim was added and mixing was continued for an additional five minutes. The resulting meat dough of each treatment was hand-stuffed and shaped in a polyethylene bag, and vacuum-packaged (Multivac Model A300122, Sepp Hagen-Muller kg, Walfertchweden, West Germany). Roasts were immersed into a preheated (70 °C) constant temperature water bath for 25 min until the internal temperature at the center of a representative roast reached 65 °C. The roasts were removed and cooled for 20 min in cold water. The cooked products were placed in polyethylene bags and stored at 4 °C for 4 and 8 days. After refrigerated storage, they were subdivided into six portions, vacuum packaged and frozen (-20 °C) for future analyses. Portions were used for moisture, lipid, thiobarbituric acid reactive substances (TBARs), vitamin E, cholesterol and 7-ketocholesterol, and fatty acids analyses. Determinations were made at 0, 4, and 8 days. Rice fiber and rice bran oil used in the formulation of restructured beef roasts (Table 3.1) were obtained from Food Ex. (Eldorado Hills, CA) and Riceland Foods (Stutgart, AR), respectively.

Table 3. 1- Formulation of restructured beef roasts containing no additive (CON), rice fiber (RF), and rice bran oil (RBO) and a combination of RF and RBO.

	Meat (g)		Ingredients (g)				
Treatment	lean	fat trim	salt	RF	RBO	H_2O	Total
Control	147.5	75	2.5	-	-	25	250
3% RF	140	75	2.5	7.5	-	25	250
2% RBO & 3% RF	140	70	2.5	7.5	5	25	250
% (w/w)	56-59	28-30	1	3	2	10	100

3.2.2. Chemical Analysis

3.2.2.1.Moisture

Moisture was determined at 105 °C by the oven drying method (AOAC, 1984) and expressed as percentage of the total weight.

3.2.2.2. Lipid Analysis

Total lipid was extracted using chloroform and methanol (2:1, v/v) as described by Folch et al. (1957). The results were expressed as weight % of total lipid.

3.2.2.3. Thiobarbituric Acid Reactive Substances (TBARs)

The distillation method of Tarladgis et al. (1960) modified by Ockerman (1980) was used to measure the level of lipid autoxidation. Results were expressed as milligrams of TBARs / kg of meat.

3.2.3. Vitamin E Vitamers Analysis

3.2.3.1. Solid-Phase-Extraction (SPE)

The analytical method reported by Shin et al (1997) was used with modification for meat products. The roast sample (1g) was chopped and placed in a 15-mL test tube with 5 mL ethanol and 0.1g ascorbic acid. The test tube was mixed for one min using a vortex mixer. The test tube was then placed in an 80 °C water bath for 10 min and 0.2 mL of 80% KOH was added. The sample was saponified for 15 min and cooled to room temperature. After that, 2 mL of 15% acetic acid (v/v in water) was added. The extract was centrifuged at 537*g* for 4 min. Supelclean LC-18 SPE (Supelco, Bellefonte, PA) tubes were conditioned by washing with 2 mL of methanol which was followed by 2 mL of 1% acetic acid (v/v in water) twice. The supernatant was then transferred to the conditioned SPE tube. Five mL of ethanol and 2 mL of 15% acetic acid were added

and mixed using a vortex mixer. After centrifugation, the supernatant was transferred to a conditioned SPE tube as above. This procedure was repeated one more time. The extract was allowed to pass through the SPE tube at less than 2 mL/min using a calibrated visiprep™ 24 port-Dl solid phase extraction vacuum manifold (Supelco, Bellefonte, PA). The tube was washed with 2 mL distilled water, 2 mL of 1% acetic acid (v/v in water), and 0.5 mL of methanol:water (50:50, v/v) to remove unwanted and unretained materials, and then dried thoroughly under a stream of nitrogen. Compounds of interest were recovered by eluting the SPE tube with 0.5 mL of methylene chloride, 3 times. The eluate was dried under a stream of nitrogen and diluted with 1 mL mobile phase.

3.2.3.2. High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Model-45 pump (Waters, Milford, MA), automated gradient controller Model-680, Ultra WISP injector Model-715, and scanning fluorescence detector Model-470 with excitation λ at 290 nm and emission λ at 330 nm. A Supelcosil™ (Supleco Bellefonte, PA) LC-Si, 5μm, 25cm × 4.6mm i.d. column was used. The mobile phase was hexane :acetic acid: ethyl acetate (99: 0.5: 0.5), with a flow rate of 1.8 mL/min for the first 9 min and 2.4 mL/min afterwards for a total of 28 min. Quantification was accomplished using Maxima chromatography workstation (Waters, Milford, MA) with external standard curves obtained using vitamin E vitamers purified as described by Shin and Godber (1994).

3.2.4. Cholesterol and 7-Ketocholesterol Analysis

3.2.4.1. Lipid Extraction and Cold Saponification

Lipids were extracted from 5g of roast into 30 mL of 2:1(v/v) chloroform/methanol (Folch et al.,1957). Ten mL of 1.5N KOH solution in methanol was added to the sample, and then the sample was shaken until the mixture became free of dispersed fat particles. Saponification was conducted at room temperature overnight (18-20 h) according to Park et al. (1996) and Park and Addis (1986). Ten mL of distilled water was added to the saponified mixture, and then transferred to another test tube (25×200mm) with a Teflon-lined screw cap. Nonsaponifiables were extracted three times with 10 mL of diethyl ether. The pooled diethyl ether extracts were washed twice with 5 mL of 0.5N KOH and three times with 5 mL of distilled water, and then dried over anhydrous sodium sulfate. After filtration and solvent removal, the dried extract was obtained.

3.2.4.2. HPLC

The HPLC system was as described. An LC-Si column (25cm×4.6mm i.d. Supelco Bellefonte, PA) was used to determine 7-ketocholesterol; whereas, a C18, 5μm, 100 A column (Rainin LC and Supplies, Waburn, MA.) was used for cholesterol. The mobile phase consisted of hexane/isopropyl alcohol (97.5:2.5) for 7-ketocholesterol and methanol/acetonitrile (7:3) for cholesterol. The mobile phase was pumped at 1.5 mL/min for the first 5 min and then at 2.4 mL/min. The detection wavelength of the UV-VIS variable wavelength detector (Hewlett Packard 1050) was 230 nm for 7-ketocholesterol and 209 nm for cholesterol. Quantification was accomplished using Maxima chromatography workstation (Waters, Milford, MA) with external standards (a

Sigma, St Louis, MO). Recovery was established by using homogenized roast samples with known sterol standards. The recovery was 95 % (n=3) for cholesterol and 89 % (n=3) for 7-ketocholesterol.

3.2.5. Fatty Acids Analysis

3.2.5.1. Extraction

Lipid sample (0.05g) extracted from beef roast was dissolved in 1 mL of hexane and 2 mL of 5% methanolic hydrogen chloride, and 1mL of heptadecanoic acid methyl ester (1μg/μL) was added as the internal standard. The mixture was incubated for 12 h in an 80 °C water bath after which 5 mL of sodium chloride solution (5% w/v) was added. The required esters were extracted twice using 5mL of hexane. The hexane layer was washed twice with 10 mL of 5% potassium bicarbonate ($KHCO_3$), and dried over anhydrous sodium sulfate. The solution was filtered and evaporated under a stream of nitrogen (Christie, 1982).

3.2.5.2. Gas Chromatography (GC)

Analysis of the fatty acid methyl ester (FAME) was carried out on a gas chromatograph (Model-5890, Hewlett-Packard, San Fernando, CA), which was equipped with a split/splitless capillary inlet system and a flame ionization detector (FID) with fused silica column, 0.25mm i.d.× 30m, in Supelco SP-2380 (Supelco, Bellefonte, PA). Operation parameters were oven temperature at 175 °C, injector temperature at 250 °C, and FID temperature at 270°C. Quantification was accomplished using Maxima software based on internal standard methods.

3.3.6. Consumer Sensory Analysis

Untrained consumers (n = 41) without differentiation of age and sex were randomly recruited from the Louisiana State University. This consumer panel evaluated control samples without additives, samples with 3% RF, and samples with the 2% RBO and 3% RF for acceptability of appearance, texture, flavor, and color, and overall liking, using a 9-point hedonic scale (1=dislike extremely, 5=neither dislike nor like, and 9=like extremely). The samples were coded with three–digit random numbers, then served at room temperature in partitioned booths illuminated with white fluorescent lights. Water and expectoration cups were provided for consumers to use during the test to minimize any residual effects between samples. Two sessions (before storage and after 4 d of storage) were conducted with the same panels for each session (before storage and after storage).

3.2.7. Statistical Analysis

A randomized complete block design with 3×3 factorial arrangement was used. Replications (n = 3) were blocked, but since no difference ($p>0.05$) was found among blocks, the block effect was not considered. The General Linear Model (GLM) was applied at $\alpha = 0.05$ for statistical significance (SAS, 1990). Means were separated using Least Significant Difference (LSD). Analysis of variance (ANOVA) was performed to determine differences in consumer sensory data (SAS, 1990). Tukey's studentized range test was performed for post-hoc multiple comparisons.

3.3. Results

The main effects of treatment and storage on moisture content (%) and total lipid (%) of restructured beef roasts are reported in Table 3.2. There was a storage

Table 3. 2- Main effect of storage and treatment in variables of restructured beef roast.

Variables	Storage Days	Treatment		
		Control	3% RF	2% RBO & 3% RF
Moisture (w/w %)	0	67.02a	66.56a	63.65b
	8	66.11c	65.82c	63.28b
Total Lipid (w/w %)	0	5.09a	5.02a	7.59b
	8	5.55a	5.19a	7.61b
Cholesterol (mg/100 g)	0	45.90a	45.46a	45.39a
	8	45.01a	44.34a	44.19a
7-keto cholesterol (μg/g)	0	1.29a	1.34a	1.63a
	4	2.10b	1.69a	1.88a
	8	8.02c	2.24ab	1.91a
TBARs (mg TBARs/kg)	0	0.15a	0.06d	0.08c
	4	0.19d	0.10e	0.09ce
	8	0.39f	0.12e	0.11e

For each variable, values within each row and each column with different letters are significantly different ($p < 0.05$). Each value represents a mean of experimental replication (n=3) with duplicates of each sample.

effect for the moisture content ($p=0.0093$) in which material declined during storage, but not for total lipid ($p=0.1599$) (Table 3.2). Roast samples with 3% RF were not significantly different from the control for both moisture and total lipid content (%). However, total lipid in the mixture of 2% RBO and 3% RF was higher ($p<0.05$), while moisture content was lower ($p<0.05$) due to the addition of RBO. Table 3.2 shows that at 0 d, the TBARs value for control was higher ($p<0.05$) than those of the samples with RF and RF/RBO. After 4 d of storage at 4 °C, the TBARs values increased ($p < 0.05$) in the control samples and RF treated samples but not in RF/RBO samples. After 8 d of storage, TBARs were higher than at 0 d for treated samples, but were much lower than controls.

There was no treatment effect on cholesterol content ($p<0.05$); cholesterol content in samples treated with RF and RBO were similar to the control regardless of the storage time. The 7-ketocholesterol levels of samples treated with RF and RBO were slightly higher than the control sample at 0 d; however, a much lower concentration was observed after 8 d of storage. The trends in TBARs and 7-ketocholesterol contents were similar during storage.

Both α-tocopherol and γ-tocotrienol decreased ($p<0.05$) during storage, while α-tocotrienol and γ-tocopherol remained stable until 4 d of storage, then decreased ($p<0.05$) at 8 d of storage (Table 3.3). Total vitamin E content was higher ($p<0.05$) in samples with the mixture of 2% RBO and 3% RF (Table 3.3). Unsaturated fatty acid content in control and samples with the mixture of 2% RBO and 3% RF decreased ($p<0.05$) during storage, whereas that in samples with 3% RF remained stable (Table 3.4). Saturated fatty acids were higher ($p<0.05$), while unsaturated fatty acids were

Table 3. 3- Changes during storage at 4° C in vitamin E vitamer content (μg/g) in restructured beef roasts.

Storage day	Treatment	α-T	α-T3	γ-T	γ-T3	Total
0	Control	2.61a	0.29a	0.04a	0.27a	3.21a
	3% RF	2.58a	0.30a	0.07b	0.29a	3.24a
	2% RBO & 3% RF	2.77b	0.30a	0.17c	2.28b	5.52b
4	Control	1.17c	0.31a	0.03a	0.08b	2.13c
	3% RF	1.95c	0.29a	0.06b	0.11b	2.43c
	2% RBO & 3% RF	2.25d	0.28a	0.16c	0.73c	3.42e
8	Control	1.09e	0.14b	0.03d	0.01c	1.28d
	3% RF	1.25e	0.06b	0.04d	0.05c	1.40d
	2% RBO & 3% RF	1.64f	0.21b	0.13f	0.50d	2.48f

For each storage period and each column, values with different letters are significantly different ($p < 0.05$). RBO is rice bran oil; RF is rice fiber; T=tocopherol; T3=tocotrienol.

Table 3. 4- Fatty acid profiles during storage in restructured beef roasts.

Treatment	Storage Days	Fatty Acid (wt. % of total fatty acids) C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	C22:0	SFA[1]	UFA[2]	S/U[3]
Control	0	2.25	30.59	4.49	11.64	44.29	4.51	6.63	1.21	0.42	44.89a	55.11a	0.81a
	8	2.61	31.81	5.08	12.05	42.97	4.71	-	0.77	-	46.48d	53.52d	0.87a
3% RF	0	2.22	30.15	4.11	10.87	44.49	6.59	0.65	0.71	0.19	43.44b	56.56a	0.77a
	8	2.43	29.54	4.50	11.18	45.08	6.91	-	0.37	-	43.14b	56.86a	0.76a
2% RBO	0	1.81	23.52	3.35	8.70	46.00	14.76	0.91	0.79	0.16	34.19c	65.81b	0.53c
+ 3% RF	8	1.91	28.04	3.19	8.42	46.10	12.35	-	-	-	38.36f	61.64f	0.62d

[1] Saturated fatty acids (wt. % of total fatty acids)
[2] Unsaturated fatty acids (wt. % of total fatty acids)
[3] Saturated fatty acids / Unsaturated fatty acids
For each sample and each column, values with different letters are significantly different ($p<0.05$).

Table 3. 5- Sensory attribute data in restructured beef roasts.

Treatment	Storage days	Sensory attributes mean score					Acceptability (%)
		Appearance	Flavor	Color	Texture	Overall Liking	
Control	0	4.88a (1.54)	5.66a (1.57)	5.07a (1.27)	5.66a (1.65)	5.46a (1.71)	73.2
	4	4.83a (1.75)	5.39a (1.95)	4.83a (1.58)	5.54a (1.78)	5.32a (1.84)	61.0
3% Fiber	0	5.10a (1.37)	5.41a (1.76)	4.98a (1.49)	5.44a (1.43)	5.47a (1.50)	58.5
	4	5.34a (1.48)	5.12a (1.69)	5.29a (1.36)	5.41a (1.56)	5.32a (1.51)	61.0
2% Oil	0	5.12a (1.76)	6.05a (1.48)	5.34a (1.53)	5.44a (1.86)	5.66a (1.61)	73.2
	4	5.24a (1.51)	5.76a (1.26)	5.34a (1.39)	5.71a (1.42)	5.66a (1.26)	80.5
2% Oil & 3% Fiber	0	5.10a (1.34)	5.39a (1.66)	4.85a (1.53)	5.51a (1.49)	5.37a (1.55)	61.0
	4	5.37a (1.50)	5.29a (1.43)	5.29a (1.40)	5.46a (1.451)	5.29a (1.35)	68.3

Numbers in parentheses refer to standard deviation of 41 consumer responses. A 9-point hedonic scale was used (1=dislike extremely, 5=neither dislike nor like, 9=like extremely). Mean values with common letters in the same column are not significantly different ($p<0.05$).

lower ($p<0.05$) in samples containing 3% RF or 2% RBO/3%RF compared to the control sample. This caused the ratio of S/U to be lower ($p<0.05$).

Table 3.5 indicates that there were no differences ($p<0.05$) for any sensory attribute. Based upon 50% agreement, panelists accepted all samples (Table 3.5). Also there was no difference for any sensory attributes before or after storage ($p<0.05$). Even though there was no difference ($p<0.05$) among treatments, samples with 2% RBO had the highest score for overall liking.

3.4. Discussion

3.4.1. The Effect of Rice Bran Oil and Fiber in Oxidative Stability

The higher TBARs values of the control (Table 3.2) may be due to an increase of reaction between substrate and catalyst during storage and the cooking process. Such processes may result in increased degradation of heme compounds, which increases free and lower-molecular-weight iron compounds that are hypothesized to be responsible for lipid peroxidation (Kanner et al., 1988; Decker and Hultin, 1990). TBARs values of samples with either 3% RF or the mixture of 2% RBO and 3% RF were not significantly different during 4 and 8 d refrigerated storage and those values were lower than those of the control (Table 3.2). This may result from phytic acid in rice fiber which acts as an antioxidant. The value of phytic acid in food systems has been recognized in several studies (Empson et al., 1991; Lee and Hendricks, 1995). The addition of phytic acid to beef products reduced TBARs value to levels lower than other antioxidants such as ascorbate, butylated hydroxyanisole (BHT) and ethylenediaminetetraacetic acid (EDTA), and prevented off-flavor formation (Lee and Hedricks, 1995). Phytic acid is a powerful inhibitor of iron-driven hydroxyl radical formation because it can form a

unique iron chelate, which becomes catalytically inactive (Graf et al., 1987). In addition, inositol 1,2,3 trisphosphate and inositol 1,2,3,6-tetrakisphosphate, which can be produced by dephosphorylation of phytic acid by either chemical or enzymatic hydrolysis, have been reported to be responsible for the inhibition of iron-catalyzed hydroxyl radical formation (Phillippy and Graf, 1997). This results from higher solubility of hydrolysis compounds of phytic acid and affinity for iron of those isomers, resulting in enhanced potential as antioxidants in food preservation.

Rice bran oil contains high levels of vitamin E vitamers and oryzanol components, which act as natural antioxidants (Sayre and Saunders, 1990). Moreover, it contains a relatively high level of tocotrienols, an analog of tocopherol, compared with other vegetable oils. Others have reported that the addition of vitamin E inhibited lipid oxidation to some extent in pork (Miles et al., 1986; Whang et al., 1986), ground beef (Benedict et al., 1975), beef steak (Okayama et al., 1987) and restructured pork (Miles et al., 1986). The loss of total vitamin E during storage (Table 3.5) may be due to the interaction between vitamin E and the free radicals generated during lipid oxidation. Tocopherol has been identified as a sacrificial inhibitor of the propagation stage of lipid oxidation (Jadhav et al., 1995). The significant decrease of α-tocopherol in all samples during storage (Table 3.3) was also seen by Aoyama et al. (1987). α-Tocopherol contributes a more biologically active vitamin E potency to diets than any other tocopherol isomers (Bieri, 1984). However, α-tocopherol is unstable in many food products during storage. The effects of tocopherols at different concentrations on the oxidative stability of ethyl linoleate and tocopherol-stripped oils were investigated under microwave heating conditions (Yoshida et al., 1991; 1993). The antioxidant effect of

tocopherols decreased in the order $\alpha > \beta = \gamma > \delta$ at each level, for all substrates. α-Tocopherol was consumed first, followed by β- or γ-tocopherol, and δ-tocopherol at a very slow rate. Our results show similar trends in antioxidant activity. In our experiment, α-tocopherol was depleted first during storage and γ-tocopherol was destroyed more slowly (Table 3.3). On the other hand, α-tocotrienol was not different until 4 d of storage, similar to γ-tocopherol (Table 3.3). Yamaoka and Komiyama (1994) reported that the induction period and consumption of α-tocotrienol was longer and greater than those of α-tocopherol during oxidation in a model system even though the estimated concentration of α-tocopherol was greater than that of α-tocotrienol.

Cholesterol oxidizes readily in solution, aqueous dispersions, and in foods when it is exposed to air, elevated temperatures, free radical initiators, light, or combinations of these (Maerker, 1987). As a result of cholesterol oxidation, foods containing cholesterol can form cholesterol oxide products (COPs) that may be toxic to cells (Bischoff and Byron, 1977; Ansari et al., 1982). Also, cholesterol oxidation may decrease the concentration of cholesterol in food during storage. However, as seen in the Table 3.2, there was no significant change in the content of cholesterol after 8 d of storage even though cholesterol oxidation may occur. 7-ketocholesterol is one of the first COPs to form and one of the oxidized compounds found in highest abundance in food (Penazzi et al., 1995). Several studies have identified that the loss of cholesterol and the formation of COPs occurs in food during heating or storage (De Vore, 1988; Sander et al., 1989). Without exception, 7-ketocholesterol significantly increased ($p<0.05$) during storage for control samples (Table 3.2). The increase in 7-

ketocholesterol during storage was similar to that of TBARs values. These trends suggest that lipid oxidation and cholesterol oxidation possess similar mechanisms. Smith (1980) indicated that cholesterol autoxidation was a free radical mechanism, similar to autoxidation of unsaturated fatty acids (Nawar, 1985). The onset of lipid and cholesterol oxidation may not only be affected by the same factors, but also inhibited similarly by antioxidants. Addis (1990) suggested that research be directed at protecting cholesterol from oxidation in foods. The inclusion of RF and RBO showed a significant and beneficial effect in suppressing cholesterol oxidation, the formation of 7-ketocholesterol and lipid oxidation in restructured beef roasts (Table 3.2).

3.4.2. The Effect of Rice Bran Oil and Fiber on Nutritive Aspects

The total vitamin E content was significantly higher ($p<0.05$) in roasts with the addition of 2% RBO and 3% RF. There was no difference between the control and the sample with 3% RF (Table 3.2). Rice bran oil contains 0.1-0.14% vitamin E vitamers and 0.9-2.9% oryzanol (Okada and Yamaguchi, 1983; Sayre and Saunders, 1990), which are known as potent antioxidants. Rice bran oil contains a relatively high level of tocotrienols, which inhibit cholesterol synthesis and lowers serum cholesterol (Qureshi et al., 1986). The tocotrienols are different from tocopherols (vitamin E) in that they have double bonds at 3', 7', and 11' position in the isoprenoid chain. This characteristic appears to be essential for the inhibition of cholesterogenesis (Qureshi et al., 1986). Dietary *d*-α-tocotrienol was reported to suppress hepatic HMG-Co A reductase activity, the first rate-limiting enzyme in the synthesis of cholesterol (Brown and Goldstein, 1980; Qureshi et al., 1986; Suzuki et al., 1993), which results ultimately in the lower serum LDL cholesterol level. The hypocholestemic properties of rice bran oil might be

due to its high content of tocotrienols, oryzanol, β-sitostenols and other unsaponifiables (Seetharamaiah and Chandrasekhara, 1988; 1989). Those specific chemical compounds also have another important beneficial effect on human health in that they act as antioxidants to protect oxidative stability of lipids *in vivo* and *in vitro*.

Rice bran oil contains three major fatty acids: palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) which account for more than 90% of total fatty acids. Inclusion of 2% rice bran oil in beef roasts increased the content of linoleic acid. There was no difference in oleic acid content among samples despite the addition of rice bran oil (Table 3.4). Palmitic (C16:0) and stearic (C18:0) acids contributed to the high proportion of saturated fatty acid in sample without additives. Palmitic acid is the major saturated fatty acid in control products (30.6% of total fatty acids and 68.4% of saturated fatty acids) (Table 3.4). Stearic acid is another of the major saturated fatty acids in control products (11.6% of total fatty acids and 25.9% of saturated fatty acids) (Table 3.4), although it does not appear to cause increased plasma cholesterol (Bonanome and Grundy, 1988). Recently research has been undertaken to reduce saturated fatty acids in meat products. This has been done by increasing the monounsaturated fatty acid content through modification of animal diets or by direct incorporation of vegetable oil, high in monounsaturated fatty acids, during processing (Rhee, 1992).

Rice bran oil is more stable under frying conditions than other common vegetable oils. This is due to a greater balance between linoleic and oleic acid, a lower level of linolenic acid, and a higher level of nonsaponifiable fractions that include vitamin E vitamers or oryzanols (Yuki and Ishikawa, 1976; Sayre and Saunders, 1990).

Rice bran also contains both insoluble fiber such as cellulose and soluble fiber such as hemicellulose. Soluble fiber in bran binds to cholesterol and bile acids in the gastrointestinal tract to reduce the absorption of cholesterol (Chen and Anderson, 1986; Ranhotra et al., 1989). Therefore, soluble dietary fiber from rice bran and the nonsaponifiable fraction from rice bran oil have a positive effect on blood cholesterol levels (Wells, 1993).

3.4.3. Consumer Acceptability

The sensory attributes of a food are very important to overall acceptance (Cardello, 1994). An effect of the treatment on sensory attributes was not detected ($p>0.05$) among samples (Table 3.5). This indicates that the samples containing additives may be as acceptable as the control. Nevertheless, samples containing rice fiber, either 3% RF or the mixture of 2% RBO and 3% RF, had slightly lower acceptability compared to the control and samples with only added 2% RBO. This may be due to the cereal flavor note from fiber that could be imparted to the beef products. Sharp and Kitchens (1990) indicated that the addition of rice bran into bakery products imparted an unpleasant cereal flavor.

There were no effects on sensory attributes after 4 d of storage (Table 3.5). This indicates that oxidized flavor was not evident in cooked meat during 4 d of refrigerated storage. Oxidative changes in meat can be divided into two categories, (1) that which occurs in meats following cooking and (2) the other that occurs during frozen storage (Reineccius, 1979). The off-flavor that occurs after the cooking of meats is generally called warmed-over-flavor (WOF) and is due to lipid oxidation (Tims and Watts, 1958). Kerler and Grosch (1996) reported that WOF is not only the result of peroxidation of

unsaturated lipids, but is also caused by the loss of desirable odorants such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone over 48 h of refrigeration of the cooked beef. However, our data indicates that TBARs values in the range of 0.08-0.19 do not affect the sensory attributes. The use of either 3% RF or the mixture of 2% RBO and 3% RF in beef roast formulations resulted in similar TBARs values after 4 d of refrigerated storage. TBARs data indicate that the addition of RBO and RF to beef roast may effectively inhibit oxidation. Nevertheless, our sensory data showed that there were no significant differences in acceptability of the beef roast containing RF/RBO compared to the control.

Utilization of various vegetable protein products such as soy proteins to extend beef products has been shown to exhibit superior functional properties such as increased water binding and cooking yield as well as serve as antioxidants (Pratt and Birac 1979; Ray et al., 1981; Arganosa et al., 1991). Minerich et al. (1991) reported that the use of 15% and 30% wild rice in ground beef did not influence the preference of consumers.

In summary, this study has demonstrated that oxidative stability and nutritional properties of precooked restructured beef roasts can be improved by the addition of semi-purified rice bran components such as rice bran oil and fiber. In addition, the product has been shown to be acceptable to consumers. This research addresses a link between primary plant and animal foods, which can provide benefits for human health. Thus, this rice fiber and oil augmented beef product may be classified as a functional food in that it incorporates several components related to nutraceutical or health-promoting ingredients, especially vitamins, minerals, and phytochemicals.

CHAPTER 4
EXPERIMENT 3

INHIBITION OF CHOLESTEROL AUTOXIDATION BY THE NONSAPONIFIABLE FRACTION IN RICE BRAN IN AN AQUEOUS MODEL SYSTEM

4.1. INTRODUCTION

Crystalline cholesterol and aqueous cholesterol dispersions readily undergo oxidation at relatively mild temperatures when exposed to air and these oxidation reactions produces a variety of products (Smith, 1981). Some cholesterol oxidation products (COP) have been shown to be cytotoxic, atherogenic, mutagenic and carcinogenic when ingested by laboratory animals (Yan and White, 1990). These COPs are formed in foods containing cholesterol during the cooking process or during prolonged storage (Rankin and Pike, 1993). Eight common autoxidation products of cholesterol have been identified: 25-hydroxycholesterol, cholestane-triol, 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, the α- and β-epoxides of cholesterol and cholesta-3,5-dien-7-one, the last compound being an artifact derived from 7-ketocholesterol (Smith, 1981; Park and Addis, 1985a). The 7-ketone derivative of cholesterol, 7-ketocholesterol, is a COP found at high concentration in certain foods like muscle tissue (De Vore, 1988). It is known to be a toxic sterol and the most potent inhibitor of cholesterol biosynthesis, which is essential for cell function. Therefore, 7-ketocholesterol inhibits not only the cell growth (Brown and Goldstein, 1974) but also significantly reduces the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-Co A) reductase, a regulatory enzyme involved in cholesterol biosynthesis (Peng et al., 1979).

Addis (1990) suggested, since there appears to be no practical method to lower the cholesterol in certain foods like muscle tissue, that research should be directed to protect against cholesterol oxidation in food. Techniques are being developed to the lower cholesterol oxide content of food products. Because both unsaturated fatty acid

autoxidation and cholesterol autoxidation proceed by a free radical process, compounds that inhibit unsaturated fatty acid oxidation may reasonably be expected to inhibit cholesterol oxidation.

Rice bran contains approximately 12-22% oil, 11-17% protein, 6-14% fiber, 10-15% moisture and 8-17% ash by weight (Saunders, 1990). The unsaponifiable fraction present in rice bran (4.2% by weight) includes tocopherols and tocotrienols (0.08%),γ-oryzanol (1.6%), and squalene (0.32%). High levels of tocopherols and tocotrienols in rice bran are important in the protection against oxidation of rice bran oil. In addition, γ-oryzanol has been reported to possess antioxidant activity in stabilizing lipids (Duve and White, 1991; Sonntag, 1979). A number of studies have reported rice bran oil to be effective in lowering blood cholesterol. The cholesterol lowering ability of rice bran oil may be due to oryzanol and to other components of the unsaponifiable matter (Seetharamaiah and Chandrasekhara, 1989). Seetharamaiah et al. (1990) suggested hypercholesterolemia, which is associated with increased platelet aggregation, may be decreased by a diet containing oryzanol. Compared with the level of oryzanol in other cereals, rice bran has a high level (3500 μg/g) (Norton, 1995). Currently, in the food industry there is a trend in to use natural antioxidants compounds rather than synthetic antioxidants (Resurreccion and Reynolds, 1990). Thus, inclusion of purified nonsaponifiable fraction of rice bran into food products may be a compelling way to obtain natural antioxidants.

An emulsion of fatty acids and aqueous buffer is a relatively simple model system for studying lipid oxidation of muscle-based foods (Zulak and Maerker, 1989;

Chan et al., 1997). Systems containing biological membranes such as red blood cells are quite complex, but micellar systems are relatively simple, uniform and well-understood, although heterogeneous (Pryor et al., 1988). In addition, colloidally dispersed cholesterol may be a more suitable model of the state of cholesterol in foods and in the aqueous fluids of animal tissues (Maerker and Bunick, 1986). For this reason, the incorporation of natural antioxidants into a simple model system that represents a state of cholesterol in food and human tissue may have utility in studying the effectiveness of natural antioxidants in inhibiting cholesterol autoxidation. The objectives of this research were (1) to study the change of vitamin E vitamers and oryzanol in a cholesterol dispersion containing various level of rice bran nonsaponifiable fraction, (2) to study quantitatively the disappearance of cholesterol and the formation of 7-ketocholesterol, and finally (3) to determine the efficacy of natural antioxidants from rice bran in suppressing the formation of 7-ketocholesterol during cholesterol autoxidation.

4.2. Material and Methods

4.2.1. Purified Nonsaponifiable Component Extraction

A solid phase extraction (SPE) method has been reported by Shin and Godber (1997) and adapted to extract a purified nonsaponifiable fraction from rice bran using C18-SPE tubes. Rice bran (500 mg) was placed in a 15-mL test tube with 5 mL of ethanol and 0.1g ascorbic acid. The test tube was incubated in an 80 °C water bath for 10 min and then 0.2 mL of 80 % KOH (w/v in water) were added. The rice bran was saponified for 15 min, and 15% acetic acid (v/v in water) was added to the saponified sample tube and the extract was centrifuged at 537×*g* for 6 min. A 3-mL Supelclean

LC-18 tube was washed with 2 mL methanol, followed by 2 mL of 1% acetic acid (v/v in water) prior to use. A reservoir (20 mL) was connected to the SPE tube. The supernatant in the test tube was transferred to the SPE reservoir. Ethanol and acetic acid was added to the test tube and mixed with a vortex mixer for 30 seconds. The test tube was centrifuged and the supernatant was transferred to the reservoir as above. This procedure was repeated one more time. The extract was allowed to pass through the SPE tube at less than 2 ml/min using a solid phase extraction vacuum manifold. The solution eluted from the SPE tube was discarded. The tube was washed sequentially with 2 mL water and 2 mL of 1% acetic acid (v/v in water) and methanol : water (50:50, v/v) to remove unwanted and unretained materials. The packing in the tube was dried throughly under high purity nitrogen and compounds of interest were recovered by eluting the SPE tube with 0.5 mL of a mixture of ethyl acetate and hexane (20:80, v/v) three times.

4.2.2. Cholesterol Dispersion Preparation

The dispersion system was a modification of methods described by Maerker and Bunick (1986) and Rankin and Pike (1993). The buffer used for making the aqueous dispersion was 0.025 M histidine buffer prepared with deionized water. The buffer solution (312 mL) was heated to 80 °C, and approximately 312 mg of sodium dodecyl sulfate (SDS), previously dissolved in absolute ethanol (5 ml), was added to the vigorously stirred solution. Cholesterol (500 ppm) dissolved in ethanol and the nonsaponifiable fraction at different concentrations (0, 700, 1400, and 2100 ppm) were added to the mixture of SDS and buffer solution; pH was adjusted to 5.5 (the typical pH of meat products, Hultin, 1985) with 2 N HCL at room temperature (≈24 °C). The

dispersion was incubated at 80 °C to increase the rate of cholesterol autoxidation over time (0, 4, 8, 12, and 16 h). Additionally, 50 μL of copper II sulfate (0.005 M) was added to promote autoxidation. An aliquot of cholesterol dispersion (5 mL) was pipetted into a 15-mL test tube with stopper and the samples were incubated at 80 °C in a hot water bath. 7-ketocholesterol and cholesterol were determined to compare the degree of cholesterol oxidation stability in the cholesterol dispersion solutions containing different final concentrations of the nonsaponifiable fraction (0, 700, 1400, and 2100 ppm) and cholesterol (500 ppm) at 4 h intervals over a 16 h period.

4.2.3. Sample Extraction and Preparation

A five mL aliquot of cholesterol dispersion was extracted three times with 10 mL diethyl ether by liquid-liquid extraction and was washed with 5 mL water twice to yield sterol compounds such as cholesterol and 7-ketocholesterol, vitamin E vitamers, and oryzanol. The recovery of extraction for cholesterol and 7-ketocholesterol was examined using dispersions with known sterol standards. The extraction recovery was 80 % (n=3) for cholesterol and 87 % (n=3) for 7-ketocholesterol. Collected extracts were filtered through anhydrous sodium sulfate (Na_2SO_4). These extracts were evaporated completely using a stream of nitrogen at 45°C, and then diluted in 1 mL mobile phase before high performance liquid chromatography (HPLC) analysis.

4.2.4. High Performance Liquid Chromatography (HPLC)

HPLC was performed using a Model-510 pump (Waters, Milford, MA), automated gradient controller Model-680, Ultra WISP injector Model-715, and scanning fluorescence detector Model-470 with excitation λ at 290 nm and emission λ at 330 nm

for vitamin E vitamers and oryzanol and UV-VIS variable wavelength detector (Hewlett Packard 1050) with 230 nm for 7-ketocholesterol and 211 nm for cholesterol. A Supelcosil ™ (Supleco Bellefonte, PA) LC-Si, 5μm, 25 cm × 4.6 mm i.d. normal phase column was used for vitamin E vitamers and oryzanol and the mobile phase was hexane: ethly acetate: acetic acid: dimethoxypropane (DMP) (98.1: 0.5: 0.5: 0.1). The mobile phase was pumped at 1.8 mL/min for the first 9 min and at 2.4 mL/min from 10 to 26 min using a gradient controller. The same column was used for 7-ketocholesterol; whereas, a C18 5 μm 100A reverse phase column (Rainin Instrument Company, Inc.) was used for cholesterol. The mobile phase consisted of hexane: isopropyl alcohol (97.5: 2.5) for 7-ketocholesterol and methanol: acetonitrile (7: 3) for cholesterol. The mobile phase was pumped at 1.5 mL/min for the first 5 min and then at 2.5 mL/min from 6 min to 14 min for 7-ketocholesterol and at 1.3 mL/min for cholesterol (Representative chromatograms are presented in appendix 3 and 4).

4.2.5. Experimental Design and Statistical Analysis

Three separate studies were performed in a randomized complete block design with treatments assigned in a 4 × 5 factorial arrangement. Replications (n=3) were blocked and the nonsaponifiable fraction treatment at different levels (0, 700, 1400, and 2100 ppm) and oxidation time at different levels (0, 4, 8, 12, and 16) were the main treatment factors. Each treatment combination had duplicate samples for each replicate determination. General Linear Model (GLM) was applied to the data with a level of 0.05 for statistical analysis (SAS, 1990) and Least Significance Difference (LSD) was used to compare the mean difference between each treatment combination.

4.3. Results and Discussion

4.3.1. Changes of Vitamin E Vitamers and Oryzanol during Oxidation Time

The changes of vitamin E vitamers and oryzanol in a model system with cholesterol and nonsaponifiable fraction from rice bran during oxidation are shown in Table 4.1. Increased incubation times reduced ($p<0.05$) total vitamin E vitamers in the aqueous model system. The different concentrations of nonsaponifiable fraction from rice bran affected the decrease of total vitamin E vitamers. The order of loss of total vitamin E vitamers in the dispersions within 16 h cholesterol oxidation was 700 ppm (97.59 %) > 1400 ppm (95.66 %) > 2100 ppm (87.44 %) (Table 4.1). Vitamin E vitamers are quite stable at elevated temperature in the absence of oxygen. The rate of oxidation of vitamin E vitamers, however, is accelerated by heat under aerobic conditions (Ball, 1995; Lang et al., 1992). Vitamin E vitamers are lost if peroxidizing lipids are present (Hakansson and Jagerstad, 1990). Therefore, vitamin E vitamers may be oxidized by the co-oxidation reaction of cholesterol in an aqueous dispersion system. Fig. 4.1 shows the time course for the loss of individual vitamin E isomers. The isomers had similar trends with time. The decomposition of vitamin E isomers increased with the increasing oxidation time. The order of decomposition of vitamin E isomers in the dispersions during 16 h incubation was γ-T3 (88.65 %) > α-T (87.75 %) > α-T3 (86.24 %) = γ-T (86.16 %) > β-T (80.67 %) > δ-T (78.63 %) > δ-T3 (77.14 %) (Table 4.2). Miyagawa et al. (1991) reported that the decomposion rate of tocopherols decreased with increasing heating time in tempura-frying oil. Ten % of α-tocopherol was lost after frying oil was heated for 28 min, whereas when the frying oils were heated for 9 hr, 17 % of α-tocopherol was lost. The decomposition rates of vitamin E

Table 4.1-Changes of vitamin E vitamers and oryzanol in a model system during a 16 h incubation at 80 °C (Unit = ppm).

	Vitamin E vitamers			Oryzanol		
Time (h)	700ppm (Nonsaponifiable fraction)	1400ppm	2100ppm	700ppm (Nonsaponifiable fraction)	1400ppm	2100ppm
0	8.30 a	17.06 a	24.60 a	255.00 a	523.47 a	739.88 a
4	5.36 b	10.76 b	18.53 b	168.17 b	401.73 b	618.61 b
8	1.61 c	4.16 c	10.10 c	118.12 c	311.35 c	613.18 b
12	0.47 d	1.34 d	6.30 d	92.46 d	234.63 d	599.92 b
16	0.20 d	0.74 d	3.09 d	73.70 d	224.65 d	528.23 b

For each column, means with different letters are significantly different ($p<0.05$).
Each value represents mean of replication ($n=3$) with duplications of each sample.

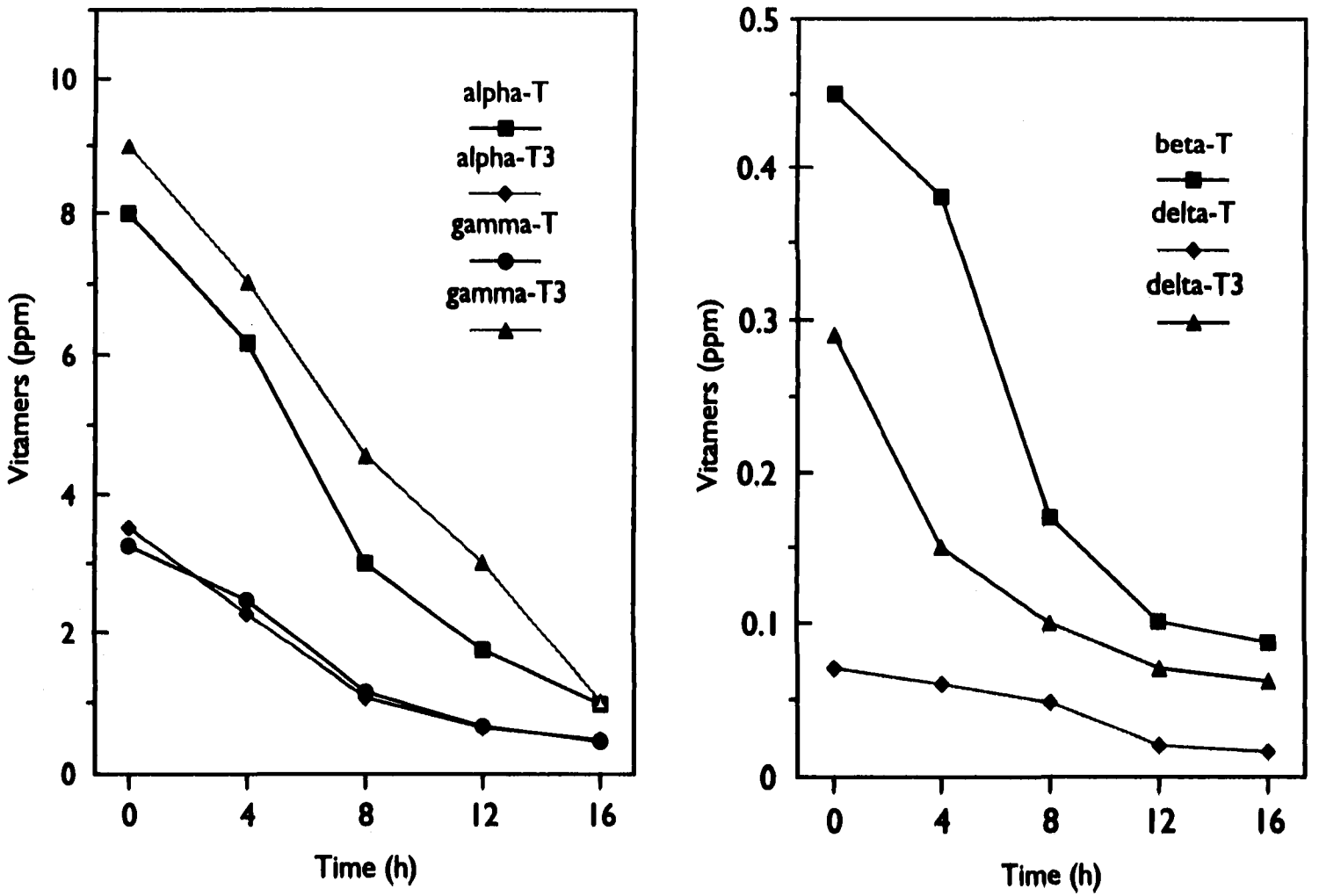


Fig. 4.1- Time course of changes of tocopherols and tocotrienols in a cholesterol aqueous dispersion at 80 °C.

Table 4.2- Retention of vitamin E vitamers and oryzanol remaining in 2100 ppm nonsaponifiable fraction dispersion during 16 h incubation at 80 °C (Unit = ppm).

Time (h)	α-T	α-T3	β-T	γ-T	γ-T3	δ-T	δ-T3	Oryzanol
0	8.00	3.54	0.45	3.25	8.99	0.07	0.29	739.88
4	6.17	2.27	0.38	2.47	7.03	0.06	0.15	618.61
8	3.00	1.07	0.17	1.15	4.56	0.05	0.10	613.18
12	1.75	0.64	0.10	0.66	3.01	0.02	0.07	599.92
16	0.98	0.47	0.09	0.45	1.02	0.02	0.06	528.23
%	12.25	13.75	19.33	13.84	11.35	22.86	21.37	71.42

For each value represents mean of replication (n=3) with duplicates of each sample. For the last row, the values refer to percentages of retention of vitamin E vitamers and oryzanol after 16 h incubation (T=tocopherol, T3=tocotrienol).

vitamers differ with the amount of antioxidants, heating time, heating method, and food composition. Du Plessis and Van Niekerk (1981) showed that the order of decomposition of α- and γ- tocopherols changed with heating time, for example: α < γ with 26 hr heating and γ < α with 103 hr heating in peanut oil, whereas, γ < α with both 26 hr and 103 hr heating in cottonseed oil. In our study, the decomposition of vitamin E vitamers showed a similar trend during 16 h cholesterol autoxidation in a model system. Lehmann and Solver (1976) reported the relative stabilities of tocopherols and tocotrienols in methyl myristate and methyl linoleate under photolytic and autooxidative conditions. The stabilities in increasing order in methyl myristate were γ-T3 < α-T3 < δ-T <α-T < γ-T < β-T under photolytic conditions and in methyl linoleate were α-T < α-T3 ≤ γ-T3 ≤ β-T < γ-T < δ-T, whereas under autooxdative conditions, the stabilities in increasing order in methyl myristate were α-T = α-T3 < β-T3 < γ-T3 < δ-T3 < γ-T < δ-T = β-T and in methyl linoleate were α-T < α-T3 < γ-T3 < β-T < γ-T < δ-T. They found that tocopherols were much more stable during autoxidation in methyl myristate than in methyl linoleate. The exact order of antioxidant activity or stability of vitamin E vitamers in a food system might be influenced by the concentration of antioxidant rather than the absolute antioxidant activity or stability in pure system. Also, the order of loss of vitamin E vitamers in a lard system at 97 °C was γ > δ > α (Parkhurst et al., 1968). Bauernfeind (1977) had reported that the order of losses of the tocopherols was γ > δ > β > α at an equivalent concentration of 0.02 % in stripped corn oil. Koskas et al. (1984) reported the antioxidant effect of α-, γ-, and δ- tocopherols during the autoxidation of linoleic acid dispersed in an aqueous media. α-Tocopherol was completely lost,

whatever its initial concentration, during linoleic autoxidation, while γ-tocopherol underwent partial oxidation and δ-tocopherol was practically unchanged.

Oryzanol was lost slowly at the higher concentrations of the nonsaponifiable fraction. The order of loss of oryzanol from 0 to 16 h incubation period was 700 ppm (71.1 %) > 1400 ppm (57.1 %) > 2100 ppm (28.58 %), which means less relative loss at a higher concentrations of the nonsaponifiable fraction from rice bran (Table 4.1). The concentration of oryzanol was significantly changed at both the 700 and 1400 ppm nonsaponifiable fraction treatment. At the higher concentration of 2100 ppm of the nonsaponifiable fraction, the concentration of oryzanol remained constant after 4 h of oxidation compared to those of the other two concentrations (Table 4.1). Oryzanol is relatively stable at high temperature (Okada and Yamaguchi, 1993). However, Kim and Kim (1991) reported that heat-treated rice bran oil had a different composition of oryzanol from control rice bran oil. Cycloartenyl ferulates were relatively less stable than steryl ferulates. Marinova and Yanishlieva (1992) investigated the antioxidant activity of ferulic acid on purified lard triacylglycerol at 25, 50, 75, and 100 °C. The ferulic acid remained constant with increasing temperature.

4.3.2. Cholesterol Autoxidation in a Model System

Table 4.3 lists the amounts of cholesterol lost and 7-ketocholesterol formed during a 16 h incubation at 80 °C. Cholesterol was significantly autoxidized in all of the dispersion samples during the 16 h incubation at 80 °C. 7-Ketocholesterol was formed at the level of 41.50 % (0 ppm) > 15.34 % (700 ppm) > 7.56 % (1400 ppm) > 3.45 % (2100 ppm) based on the initial concentration of cholesterol (500 ppm) during

Table 4.3- Time course of autoxidation of cholesterol in aqueous dispersions with nonsaponifiable fraction at 80 °C and pH 5.5 (unit = ppm).

Time (h)	Treatment							
	0 ppm		700 ppm		1400 ppm		2100 ppm	
	Cholesterol	7-Ketocholesterol	Cholesterol	7-Ketocholesterol	Cholesterol	7-Ketocholesterol	Cholesterol	7-Ketocholesterol
0	500,00 ± 0,00	0.00 ± 0,00	500,00 ± 0,00	0,00 ± 0,00	500,00 ± 0,00	0,00 ± 0,00	500,00 ± 0,00	0,00 ± 0,00
4	277,32 ± 7,68	14,09 ± 2,82	442,06 ± 9,63	2,33 ± 0,26	459,95 ± 3,10	2,23 ± 0,71	477,83 ± 6,85	0,03 ± 0,02
8	161,22 ± 5,62	52,91 ± 2,27	231,16 ± 6,42	12,91 ± 1,43	427,63 ± 5,79	11,23 ± 1,43	448,55 ±10,95	2,13 ± 0,61
12	97,76 ± 2,20	223,59 ± 6,70	182,64 ± 5,82	53,88 ± 1,59	191,86 ± 4,14	24,59 ± 1,23	307,90 ± 5,06	10,68 ± 1,61
16	36,66 ± 1,67	207,78 ± 6,16	155,94 ± 2,81	76,69 ± 1,47	155,34 ± 2,03	37,80 ± 1,27	168,46 ± 3,15	17,23 ± 1,60

Each value refers to mean ± SD of replication (n=3) with duplications of each sample.

16 h incubation (Fig. 4.2). An increase in the concentration of the nonsaponifiable fraction resulted in a decrease in the formation of the cholesterol oxide product (7-ketocholesterol). Cholesterol oxidation is initiated by hydrogen abstraction, predominantly at C-7, which is most sensitive to molecular attack by oxygen. As a result, 7-hydroperoxides, which are unstable thermodynamically, are converted to 7-ketocholesterol and 7-hydroxycholesterols, the ratio of 7-ketone to combined hydroxy compounds being 2:1 (Maerker, 1987). Thus, 7-ketocholesterol is the principal oxidation product (Baranowski et al., 1982; Zulak and Maerker, 1989) and amounts to about one-half of the original cholesterol concentration at 12 h incubation in the sample with 0 ppm of nonsaponifiable fraction (Table 4.3). In contrast to the relative stability of the crystalline cholesterol, cholesterol undergoes rapid autoxidation when dispersed in the liquid phase (Norcia and Janusz, 1965; Cadenhead et al., 1982). Smith (1981) reported that when an aqueous solution of sodium stearate at pH 8.5 was diluted with an ethanol solution of cholesterol and heated at 85 °C for 5 h, extensive autoxidation occurred. Autoxidation of cholesterol has been reported as an air/water interface phenomenon (Kamel et al., 1971). Cadenhead et al.(1982) also reported the same observation and found that oxidation of cholesterol was autocatalytic as long as the oxidation products remained miscible with cholesterol. Maerker and Bunnick (1986) reported that 7-ketocholesterol and 5,6-epoxides were the major oxidation products in aqueous dispersion with sodium stearate or Triton surfactants regardless of pH or temperature. Because cholesterol autoxidation is a free radical mechanism, similar to autoxidation of unsaturated fatty acids, factors affecting rate and onset of lipid oxidation may be the same as for cholesterol autoxidation (Smith, 1980; Nawar, 1985).

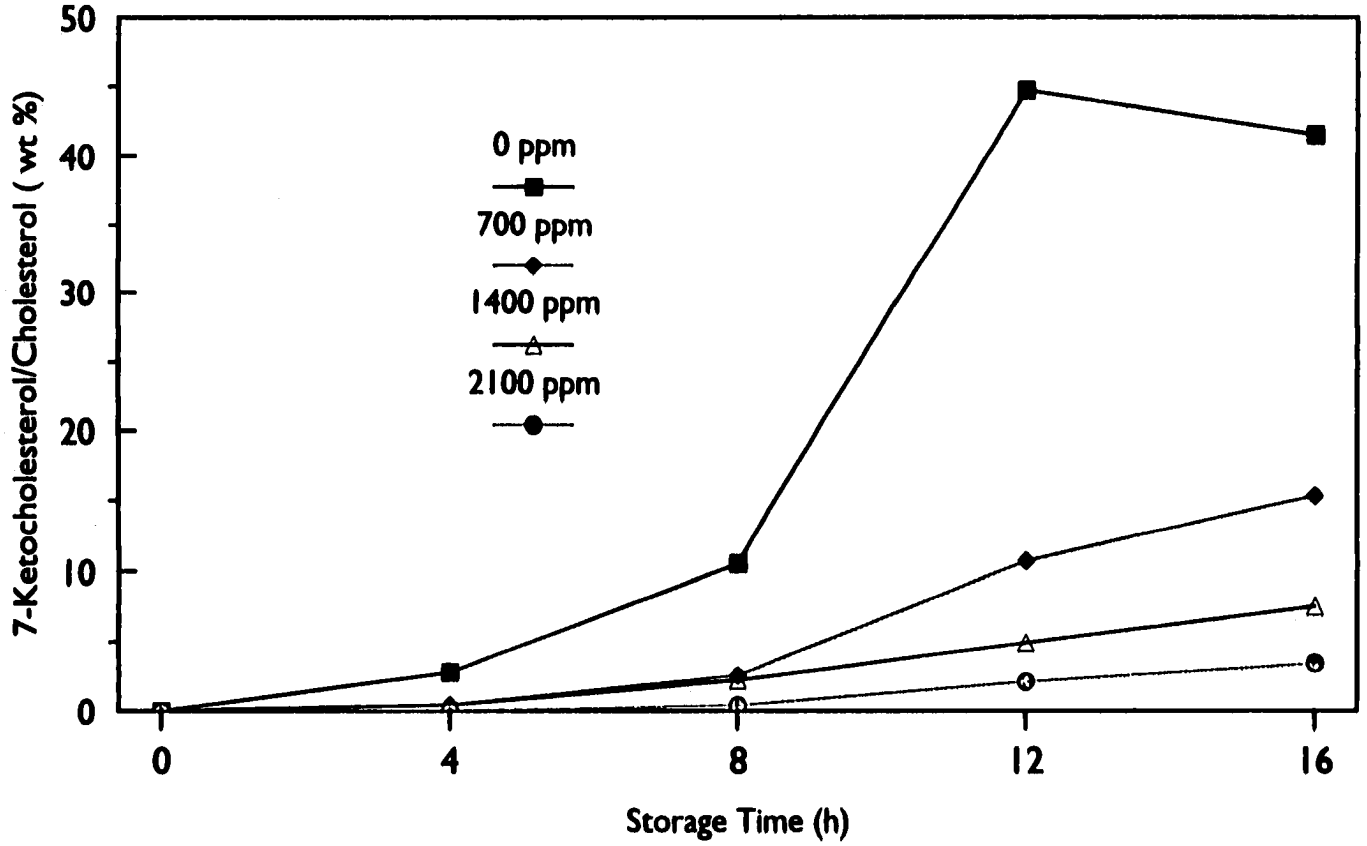


Fig.4.2- Time course of cholesterol autoxidation in aqueous dispersions with different concentrations of nonsaponifiable fraction of rice bran.

It is known that lipid and cholesterol oxidation is dependent on the exposure to air, high temperature, light, metal ions, and other compounds such as chlorophyll (Nawar, 1985). As our result showed (Table 4.2), 7-ketocholesterol gradually increased as the oxidation time increased. 7-ketocholesterol formed during 12 h was at the level of 60 to 70 % of total amount formed during 16 h in samples treated with the nonsaponifiable fraction except the untreated sample. 67 to 69 % of the cholesterol was lost during 16 h oxidation at 80 °C in nonsaponifiable fraction treatment samples. However, the loss of cholesterol in the dispersion samples without nonsaponifiable fraction was around 92 % after 16 h oxidation (Table 4.3). This indicates that during the incubation time at high temperature, thermal degradation of cholesterol occurred, which was accelerated by metal ion, air, and pH in an aqueous model. It also reflects that prolonged heating time of cholesterol may influence the loss of cholesterol and the formation of cholesterol oxide products (COPs). Yan and White (1990) determined the loss of cholesterol and the formation of COPs in lard during heating at 180 °C. They reported that 7-ketocholesterol was one of the predominant COPs formed and the amount of COPs formed did not total the amount of cholesterol lost. Osada et al. (1993) reported that various oxidized cholesterol derivatives were produced during heating above 120 °C within a relatively short time and the composition of the COPs differed depending on temperature and the length of time of heating. When cholesterol was heated at 150 °C, the production of oxidized cholesterol was maximum and 7-ketocholesterol was the most predominant oxidized product. At 16 h, without the nonsaponifiable fraction treatment (Table 4.3), the amount of 7-ketocholesterol decreased compared to the

amount of 7-ketocholesterol at 12 h cholesterol oxidation. The degradation of 7-ketocholesterol may be due to inherent thermal instability of cholesterol oxide products and pH change resulting from extended cholesterol autoxidation under high temperature. It is known that the 7-ketocholesterol is highly sensitive to base and is subject to thermal dehydration yielding 3, 5-cholestadien-7-one (Chicoye et al., 1968; Ansari et al., 1982). Kim and Nawar (1993) investigated the effects of temperature, time, presence of water, pH, type of buffer and form of substrate used on cholesterol oxidation. 7-Ketocholesterol/7-hydroxycholesterol dropped significantly with increasing pH, but 7-ketocholesterol underwent further decomposition during heating. They reported that solid cholesterol films were found to be resistant to autoxidation in the dry state, but when the cholesterol films were in aqueous suspensions and heated at 125 °C, the film fragments of cholesterol were oxidized at a faster rate and a sudden increase in oxidation rate occurred. In aqueous suspensions, changes in the resistance to cholesterol oxidation were not significant within the pH range 6.0-7.4, except for the early stages of oxidation. In addition, dehydration of the 7-hydroperoxide to yield 7-ketocholesterol occurred readily at lower pH, whereas the 7-ketocholesterol was particularly sensitive to alkaline conditions (Chicoye et al., 1968; Park and Addis, 1985b; Maerker, 1987). Our aqueous dispersion model system had conditions favorable to oxidation. These conditions included oxygen-saturation, high temperature, and a copper catalyst, which accelerated cholesterol autoxidation within 16 h.

4.3.3. Antioxidative Properties of Nonsaponifiable Fraction from Rice Bran

Fig. 4.3 shows percent retention of total vitamin E vitamers and oryzanol during cholesterol autoxidation in a model system. The trends of retention of total vitamin E

vitamers and oryzanol in different concentrations of the nonsapnifiable fraction during the cholesterol autoxidation at 80 °C were similar. However, in case of the higher concentration (2100 ppm) of nonsaponifiable fraction, the retention remained more stable after 4 h cholesterol autoxidation compared to the other two concentrations in which there was a significant decrease in total vitamin E during oxidation. This may be due to the relatively high concentration of oryzanol, which is more heat-stable, and a sacrificed consumption of vitamin E vitamers during the initial stage of oxidation. The molar concentration of oryzanol in rice bran is about five times higher than total vitamin E in rice bran (Shin et al., 1997) and the nonsaponifaible fraction (4.2 %) present in rice bran shows total vitamin E vitamers at 0.08 % and oryzanol at 1.6% (Saunders, 1990).

Fig. 4.2 illustrates the decrease of percentage of 7-ketocholesterol/cholesterol due to the increasing concentration of nonsaponifiable fraction. In contrast to the nonsaponifiable fraction treatment, the percentage of 7-ketocholesterol/cholesterol in the control sample increased greatly during cholesterol oxidation. Okada and Yamaguch (1983) reported that in a 0.01 % α-tocopherol and oryzanols solution, α-tocopherol had higher antioxidant activity than oryzanol. Increasing the concentration of α-tocopherol up to 0.5 % did not increase the antioxidant activity, but with a smilar increase in oryzanols the activity increased proportionally. An antioxidative function of oryzanol has been reported (Sonntag, 1979; Duve and White, 1991). Oryzanol is a mixture of ferulate esters with sterols and triterpene alcohols, predominantly β-sitosterol, campasterol, cycloartenol, and 24-methylene cycloartenol (Sayre and Saunders, 1985; Sietz, 1989; Norton, 1995). It has been suggested that the phenolic

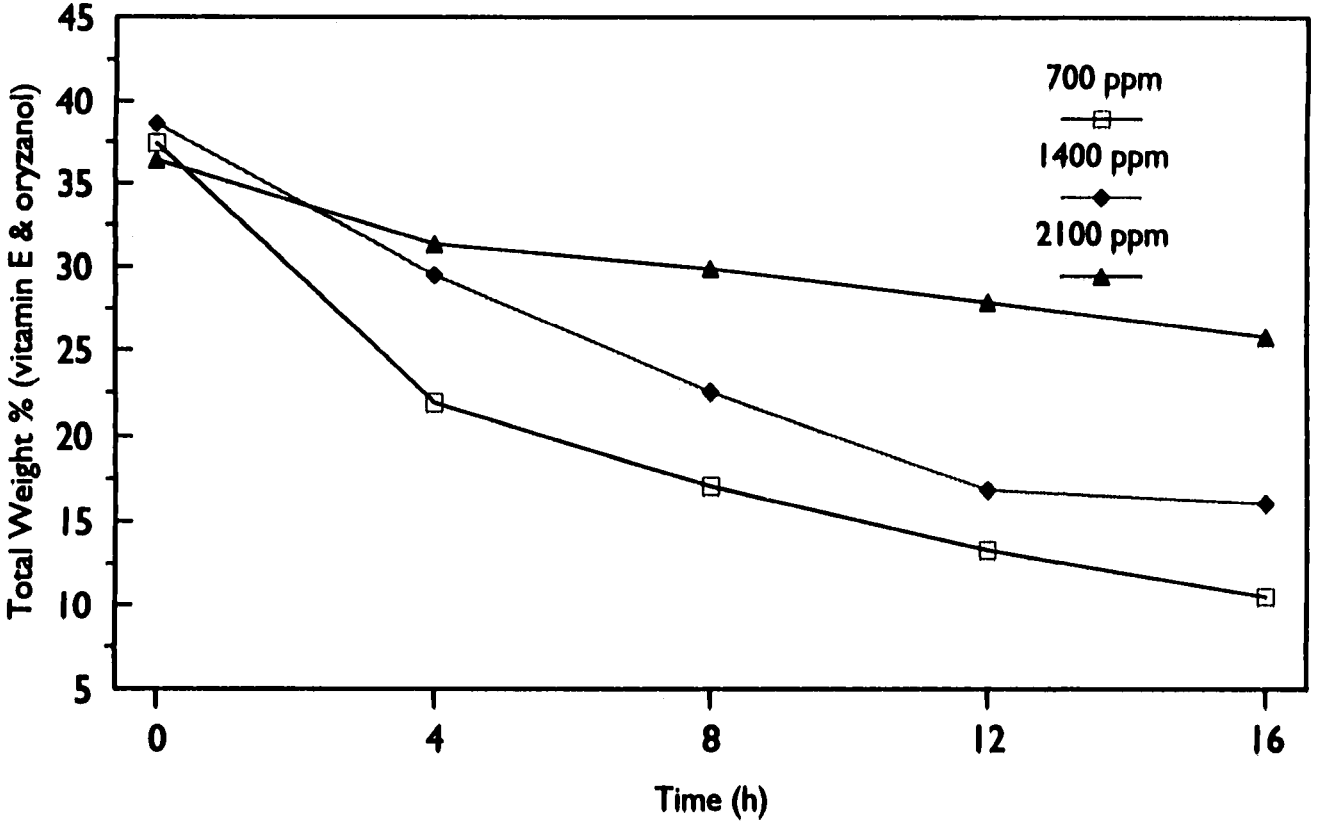


Fig. 4.3-Time course of changes of total vitamin E vitamers & oryzanol (wt %) in dispersions with different concentrations of nonsaponifiable fraction at 80 °C.

hydroxyl group in the ferulate esters of oryzanol might be responsible for its antioxidative function. Generally, phenolic antioxidants inhibit lipid oxidation by trapping the peroxy radical to yield the hydroperoxide. They prevent the reaction of the peroxy radical to produce a lipid radical and propagate a free radical chain reaction. Chimi et al. (1991) reported that the rates of reaction of aroxyl radical, which is produced after penolic acid donates a hydrogen proton to a peroxy radical, exceed the rates of reaction of another aroxyl radical, which produces free radicals. This mechanism may be related to oxidation inhibition by phenolic compounds.

Huang et al. (1994) evaluated antioxidant activity of α- and γ-tocopherols in bulk oils and in 10 % oil-in-water emulsions. α-Tocopherol had maximun antioxidant activity at 100 ppm in bulk oil and at 250-500 ppm in emulsions, while γ-tocopherol showed the maximum activity at 250-500 ppm in bulk oil but showed no significant difference in antioxidant activity between 250 and 1000 ppm in emulsions. α-Tocopherol had a slight, initial pro-oxidant activity at 500 ppm or higher concentrations in emulsions, whereas γ-tocopherol had no prooxidant activity. According to Koskas et al. (1984), α-tocopherol at lower concentrations (0.38 and 0.038 %) and γ- and δ-tocopherols at high concentration (3.8 %) in linoleic acid aqueous media were antioxidants. Our model system contained around 3.4-9.9 ppm of α-tocopherol from a relative low concentration treatment (700 ppm) to a relative high concentration treatment (2100 ppm) of the nonsaponifiable fraction from rice bran.

In this study, the model system was composed of 1.66-4.92 % of vitamin E relative to the oxidizable substrate, cholesterol, which had no prooxidant effect on the

formation of 7-ketocholesterol. As seen in Fig. 4.2, this indicates that additions of 700 to 2100 ppm of nonsaponifiable fraction from rice bran had an antioxidant effect in an aqueous model system during the 16 h incubation at 80 °C. Vitamin E vitamers and oryzanol in the nonsaponifiable fraction from rice bran may inhibit the formation of 7-hydroperoxy cholesterols formed at initial stage of cholesterol oxidation and reduce the formation of 7-ketocholesterol. There is some evidence that the hydrophilic-lipophilic properties of antioxidants may influence their effectiveness (Nawar, 1986). Based on the greater antioxidative effect at higher concentration of nonsaponifiable fraction (Fig. 4.3), cholesterol autoxidation in aqueous model system may require a more lipophillic antioxidant.

The addition of natural antioxidants, like nonsaponifable fraction in rice bran, at high level into a food system may bring several benefits. It may increase nutritional value and play a role as a functional food as well as inhibit cholesterol oxidation. Though this study shows an antioxidative effect of nonsaponifiable fraction from rice bran in suppressing cholesterol autoxidation, it does not indicate the antioxidant effects of each compound from vitamin E isomers and oryzanols. The effects of each compound on cholesterol autoxidation need to be further researched to understand more completely how they inhibit the formation of COPs. In addition, it would also be helpful to establish the actual concentrations of each compound needed for prevention of cholesterol autoxidation during processing and storage.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Beef is nutrient dense food that supplies a high proportion of many essential nutrients, such as protein, iron, and vitamin B-12. However, beef is a significant source of fats, especially saturated fatty acid and cholesterol, which are related to cardiovasucular disease. Rice bran, on the other hand, has certain health benefits such as reducing serum cholesterol level, but it contains high levels of fiber and phytic acid that have been found to reduce the bioavailability of some minerals. Muscle food such as beef, however, may enhance the bioavailability of minerals in non-muscle foods when the two are eaten together. Therefore, combining beef and rice bran may provide a way to counteract negative nutritional properties of both. However, meat quality depends on lipid stability and lipid oxidation causes rancidity, a major deterrent to taste in precooked meat products, which contributes to consumer rejection of the meat product.

Rice bran is a rich source of natural antioxidants such as tocopherols, tocotrienols, oryzanol, and inositol polyphosphates. Use of these natural antioxidants in beef products may reduce the rancidity and increase the nutritional value as well. The antioxidant compounds in rice bran mainly come from the nonsaponifiable fraction of rice bran oil, except for inositol polyphosphates. Both the nonsponifiable fraction present in rice bran oil and inositol polyphosphates are known to have hypocholesteremic properties. Thus, the development of a beef product containing such natural antioxidants in rice bran might meet the demand of today's health-conscious consumers seeking functional foods. The value of such meat products could be further enhanced through restructuring techniques due to several advantages such as compositional control, convenient preparation and economical production.

As an unsaturated lipid, cholesterol is sensitive to oxidation by free radical processes in the same manner as unsaturated lipid and their esters. Recent expanding interests in the potential biological activities of cholesterol oxidation products (COP) makes it essential that cholesterol autoxidation, its possible occurrences in foods and potential role in endogeneous metabolism be fully understood. 7-Ketocholesterol is a COP found at the highest level in beef products as well as being one of the initial oxide products formed after cholesterol oxidation. It is a toxic sterol as well as the most potent inhibitor of cholesterol biosynthesis, which is essential for cell function.

Since cholesterol oxidation as well as lipid oxidation follow a similar mechanism, the nonsaponifiable fraction from rice bran oil might theoretically inhibit cholesterol oxidation. The presence of 7-ketocholesterol in meat products might be prevented by using appropriate methods of processing, preservation, and storage.

The effect of rice bran oil on nutritional value and oxidative stability was studied in restructured beef roasts during refrigerated storage. The addition of rice bran oil to the formulation resulted in a lower level of saturated fatty acids and increased level of unsaturated fatty acids in the beef product, resulting in lower SFA/UFA ratio. Total vitamin E concentrations significantly increased with the increased level of rice bran oil. Especially, the concentrations of α-tocopherol and γ-tocotrienol in rice bran oil treatment products were siginificantly higher than the no oil treatment product. Significant differences in cholesterol concentrations were not found among the rice bran treatment roasts, but cholesterol concentrations in oil treatment roasts were lower. On the other hand, 7-ketocholesterol concentration was higher in control roasts at 0 d of storage than in oil treatment roasts. 7-Ketocholesterol concentration increased in all

treatments during refrigerated storage, but oil treatments remained lower than controls. The TBARs values showed a similar trend in the oil treatment roasts during refrigerated storage. Those two values were highly correlated, which might reflect the fact that unsaturated fatty acid and cholesterol are oxidized by the same mechanism. The oxidative stability of beef roasts containing rice bran oil was probably due to the vitamin E vitamers in rice bran oil. The results of this study indicated that rice bran oil might be a natural antioxidant for restructured beef roasts in that it improved the nutritional value and oxidative stability during storage.

The improved value of restructured beef roasts containing semi-purified rice bran components such as rice bran oil and rice fiber was studied to establish its potential as a functional food. Beef roasts containing either rice fiber or rice fiber/rice bran oil showed higher oxidative stability ($p<0.05$) during storage than did beef roast without additives. Beef roasts with either 3% rice fiber or the mixture of 3% rice fiber and 2% rice bran oil were not significantly different in TBARs and 7-ketocholesterol levels. The inhibition of unsaturated fatty acid and cholesterol oxidation in restructured beef roasts with additive treatment might be due to vitamin E vitamers from rice bran oil or phytic acid and its hydrolysis compounds from rice fiber. This result indicated that both components might be powerful inhibitors of iron-catalyzed oxidation in beef products. Vitamin E vitamer concentration and unsaturated fatty acid levels significantly increased in roasts with a mixture of rice fiber and rice bran oil. On the other hand, saturated fatty acid level decreased significantly as the additives were used. In addition, sensory attribute data showed that the beef roast products with semi-purified components from rice bran were not significantly different compared to the no additive

roasts, and were deemed acceptable by consumers. Thus, this rice fiber and oil augmented beef product might serve as a functional food in that it contains several components related to nutraceutical or health promoting ingredients in addition to its stability against oxidative degradation.

The antioxidative effect of nonsaponifiable fraction in rice bran was studied by using an aqueous model system during cholesterol autoxidation. The change of vitamin E vitamers and oryzanol from nonsaponifiable fraction in rice bran was examined during 16 h cholesterol autoxidation at 80 °C. Increased oxidation time reduced ($p<0.05$) total vitamin E vitamers and oryzanol concentration in a model system. The rate of decomposition of individual vitamin E vitamers increased with the increasing oxidation time. Oryzanol was lost minimally at higher concentration of nonsaponifiable fraction. An increase in nonsaponifiable fraction resulted in a decrease in the formation of cholesterol oxide product (7-ketocholesterol). A model without nonsaponifiable fraction showed a decrease of 7-ketocholesterol after 12 h oxidation possibly due to further decomposition. The percent of 7-ketocholesterol/cholesterol increased throughout the oxidation time. Even though all treatments of nonsaponifiable fraction (700, 1400, and 2100 ppm) showed antioxidant effect in aqueous system during cholesterol autoxidation at high temperature, the 2100 ppm treatment had a higher antioxidant activity compared to the other two concentrations in a model system. This study indicated that vitamin E vitamers and oryzanol compounds of nonsaponifiable fraction in rice bran might inhibit the formation of 7-hydroperoxy cholesterol formed at the initial stage of cholesterol autoxidation.

In conclusion, the addition of natural antioxidants in rice bran into food containing a high level of lipid and cholesterol might bring several benefits in that it increases nutritional value, improves oxidative stability, and provides many phyto-chemicals related to nutraceutical or health-promoting ingredients. This study shows the antioxidative effects of semi-purified compounds such as RBO and RF and nonsaponifiable fraction from rice bran, but it does not indicate the antioxidant effects based on each compound from vitamin E isomers and oryzanols in inhibiting the lipid and cholesterol oxidation. The effects of each compound need to be further researched to understand its mechanism more completely in preventing the oxidation of food with high levels of lipid and cholesterol during processing and prolonged storage.

REFERENCES

Abeywardena, M. Y., McLennan, P. L., and Charnock, J. S. 1991. Changes in myocardial eicosanoid production following long-term dietary lipid supplementation in rats. Am. J. Clin. Nutr. 53: 1039.

Addis, P. B. 1990. Coranary heart disease: an update with emphasis on dietary lipid oxidation products. Nutr. Rev. 62: 7.

Addis, P. B., Epley, R. J., and Katsanidis, E. 1998. Innovative antioxidants systems for muscle foods. Book of Abstracts. p.10. IFT Annual Meeting.

Akamittath, J. G., Brekke, C. J., and Schanus, E. G. 1990. Lipid Oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55: 1513.

Angelo, A. J. St. 1996. Lipid oxidation in food. Critical Reviews in Food Science and Nutrition. 36: 175.

Ansari, G. A. S., Walker, R. D., Smart, V. B., and Smith, L. L. 1982. Further investigation of mutagenic cholesterol preparations. Food Chem. Toxic. 20: 35.

A.O.A.C. 1984. *Official Methods of Analysis.* 14 ed. Assoc. of Official Analytical Chemists, Washington, D.C.

A.O.C.S. 1990. *Official Methods and Recommended Practices of the American Oil Chemists' Society.* 4th ed. American Oil Chemists' Society, Champaign, IL.

Aoyama, M., Maruyama, T., Niiya, T., and Akatsuk, S. 1987. Antioxidant effects of tocopherols on palm oil by frying tests. J. Jpn. Soc Food Sci. Technol. 34: 714.

Arganosa, G. C., Godber, J. S., Tanchotikul, U., McMillin, K. W., and Shao, K. P. 1989. Cooking temperature as a processing variable affecting the oxidative and textural stability of restructured beef roasts. J. Food Sci. 54: 1072.

Arganosa, G. C., Godber, J. S., Tanchotikul, U., McMillin, K. W., and Shao, K. P. 1991. Processing ingredients affecting oxidative and textural stability of restructured beef roasts. J. Food Sci. 56: 1480.

Ball, G. F. M. 1995. High performance liquid chromatography (HPLC). In *Fat-Souble Vitamin Assays in Food Analysis*, 8. Anonymous, p.1. Elsevier Science Publishing Co., Inc., New York, NY.

Baranowski, A., Adams, C. W. M., High, O. B. B., Bowyer, D. B., 1982. Connective tissue response to oxysterols. Atherosclerosis. 41: 225.

Bauernfeind, J. C., 1977. The tocopherol content of food and lipids and influencing factors. CRC Crit. Rev. Food Sci. Nutr. 8: 337.

Bender, A. E. 1974. Meat. In *Nutritional value of meat,* D. J. A. Cole, and R. A. Lawrie (Eds.), Butterworth, London.

Benedict, R. C., Strange, E. D., and Swift, C. E. 1975. Effect of lipid antioxidants on the stability of meat during storage. J. Agric. Food Chem. 23: 167.

Bieri, J. G. 1984. Source and consumption of antioxidants in the diet. J. Am. Oil Chem. Soc. 61: 1917.

Bischoff, R. and Byron, G. 1977. The pharmacodynamics and toxicology of steroids and related products. Adv. Lipid Res. 15: 61.

Bonanome, A. and Grundy, S. M. 1988. Effects of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318: 1244.

Brown, M. S. and Goldstein, J. L. 1974. Suppression of 3-hydroxyl-3-methyl-glutaryl coenzymes A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. Biol. Chem. 249: 7306.

Brown, M. S. and Goldstein, J. L. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J. Lipid Res. 21: 505.

Cadenhead, D. A., Kellner, B. M. J., and Balthasar, D. M. 1982. Cholesterol oxidation and the behaviour of 5a-hydroperoxy-cholesterol at the air/water interface. Chem. Phys. Lipids. 31: 87.

Cardello, H. V. 1994. Consumer expectations and their role in food acceptance. Ch.10. In *Measurement of Food Preferences,* H. J. H. Macfile and D. M. H. Thomson (Eds.), p.253. Blackie Academic & Professional, London, UK.

Chan, W. K. M., Faustman, C., and Renerre, M. 1997. Model system for studyingpigment and lipid oxidation relevant to muscle-based foods. In *Natural Antioxidants: Chemistry, health effects, and applications*, Ch.20. p319. American Oil Chemist Society (AOAC) Press. Champaign, IL.

Chen, W. J. L. and Anderson, J. W. 1986. Hypocholesterol effects of soluble fibers. In *Dietary fiber Basic and Clinical Aspects*, G. V. Vahauny and D. Kritchevsky (Eds.), p.275. Plenum Press, New York.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Critical Reveiws in Food Science and Nutrition. 12: 297.

Chicoye, E., Dowrie, W. D., and Fennema, O. 1968. Photoxidation of cholesterol in spray-dried egg yolk upon irradation. J. Food Sci. 33: 581

Chimi, H., Cillard, J., Cillard, P., and Rahmani, M. 1991. Peroxyl and hydroxyl radical scavenging activity of some radical phenolic antioxidants. J. Am. Oil Chem. Soc. 68: 307.

Christie, W. W. 1982. The preparation of derivatives of lipid. In *Lipid Analysis*, p.53. Pegramon Press, New York.

Chow, C. K. and Draper, H. H. 1969. Effect of artificial drying on tocopherols andfatty acids of corn. J. Agric. Food Chem. 17: 1316.

Cillard, J. and Cillard, P. 1980. Behavior of alpha, gamma, and delta tocopherols with linoleic acid in aqueous media. J. Am. Oil Chem. Soc. 57: 39.

Cleveland, M. Z. and Harris, N. D. 1987. Oxidation of cholesterol in commercially processed cow's milk. J. Food Protect. 50: 867.

Cosgrove, D. J. 1980. Inositol Phosphates: Their Chemistry, Biochemistry, and Physiology. Elsevier Scientific. Amesterdam.

Cuppett, S., Schnepf, M., and Hall, C. 1997. Are they a reality? In *Natural Antioxidants: chemistry, health effects, and application.* p.12. AOAC Press. Champaign, IL.

Decker, E. A. and Hultin, H. O. 1990. Factors influencing catalysis of lipid oxidation by soluble fraction of mackerel muscle. J. Food Sci. 55: 947.

De Vore, V. R. 1988. TBA values and 7-ketocholesterol in refrigerated raw and cooked ground beef. J. Food Sci. 53: 1058.

Du Plessis, L. M. and Van Niekerk, P. J. 1981. Evaluation of peanut and cottenseed oils for deep frying. J. Am. Oil Chem. Soc. 58: 575.

Duthie, G. G., McPhail, D. B., Morrice, P. C., and Arthur, J. R. 1992. Antioxidant effectiveness of tocopherol isomers. In *Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications,* A. S. H. Ong and L. Packer (Eds.), Birkhauser Verlag. Basel, Switzerland.

Duve, J. K. and White, P. I. 1991. Extraction and identification of antioxidants in oats. J. Amer. Oil Chem. Soc. 68: 365.

Emanuel, H. A., Hassel, C. A., Addis, P. B., Bergmann, S. D., and Zavoral, J. H. 1991. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. J. Food Sci. 56: 843.

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

Folch, J., Lees, M., and Sloane-Stanely, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497.

Food and Nutrition Board. 1980. Recommended Dietary Allowances. 9th ed. National Academy of Sciences, National Research Council, p161. Washington, D.C.

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

Galliard, T. 1986. Oxygen consumption of aqueous suspensions of wheat-white meal, bran, and germ: Involvement of lipase and lipoxygenase. J. Cereal Sci. 4: 33.

Gibson, D. M. and Ullah, A. B. J. 1990. *Inositol Metabolism in Plants*. p. 77-92. Wiley-Liss Inc., New York, NY.

Godber, J. S. 1990. The intrinsic role of muscle foods in nutrient bioavailability. Annual Meeting of the AOAC, Baltimore, MD. Inform 1, 276 abst. No. A4.

Godber, J. S. 1993. Nutritional value of muscle foods. In *Muscle Foods*, D. M. Kinsman and A.W. Kotula (Eds.), Van Nostrand Reinhold, New York, NY.

Godber, J. S. and Mullins, A. M. 1990. Processing beef for today's consumers. Louisiana Cattleman Magazine. 23: 13.

Godber, J. S., Qaio, D., Windhauswer, M., and Hegsted, M. 1993. Combining rice bran and beef for improved food quality through complimentary nutritional properties. Louisiana Agriculture. 36: 16.

Gordon, D. T. and Godber, J. S. 1989. The enhancement of nonheme iron bioavailability by beef protein in the rat. J. Nutr. 119: 446.

Gould, R. G., and Taylor, C. B. 1950. Effect of dietary cholesterol on hepatic cholesterol synthesis. Federation Proc. 9: 179.

Graf, E. 1992. Antioxidant potential of ferulic acid. Free Radical Biology & Medicine. 13: 435.

Graf, E., Empson, K. L, and Eaton, J. W. 1987. Phytic acid: A natural antioxidant. J. Biol. Chem. 262: 11647.

Graf, E., Mahoney, J. R., Bryant, R. G., and Earon, J. W. 1984. Iron-catalyzed hydroxyl radical formation: Stringent requirement for free iron coordination site. J. Biol. Chem. 259: 362.

Grundy, S. M. 1986. Cholesterol and coronary heart diseases - a new era. J. Am. Med. Assoc. 256: 2849.

Hakansson, B. and Jagerstad, M. 1990. The effect of chemical inactivation of lipoxygenase on the stability of vitamin E in wheat. J. Cereal Sci. 12: 177.

Hasler, C. M. 1998. Functional Foods: Their role in disease prevention and health promotion. Food Technol. 52: 63.

Haung, S. W., Frankel, E. N., and German, J. B. 1994. Antioxidant activity of α- and γ-tocopherols in bulk oils and in oil-in-water emulsions. 42: 2108.

Hegsted, D. M., McGandy, R. B., Myers, M. L. and Stare, F. J. 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17: 281.

Heinemans, T., Leiss, O., and Von Bergmann, K. 1986. Effect of low-dose sitosterol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis. 61: 219.

Hornstra, G., Hennissen, A. A., Tan, D. T., Kalafusz, R. 1986. Beneficial effects of palm oil on arterial thrombosis (rat) and atheroscreosis (rabbit). Palm Oil Research Institute of Malaysia. Kuala Lumpur, Malaysia.

Hultin, H. O. 1985. Characteristics of muscle tissue. Ch. 12 *in Food Chemistry.* O. R. Fennema (Ed.), p.725. Marcel Dekker, New York.

Hwang, K. T. and Maerker, G. 1993. Quantitation of cholesterol oxidation products in unirradiated and irradiated meats. J. Am. Oil Chem. Soc. 70: 371.

Igene, J. O., Pearson, A. M., Dugah, L. R., and Price, J. F. 1980. Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. Food Chem. 5: 213.

Imai, H., Werthessen, N. T., Taylor, C. B., and Lee, K. T. 1976. Angiotoxicity and arteriosclerosis due to contaminants of USP-grade cholesterol. Arch. Pathol. Lab. Med. 100: 565.

Jadhav, S. J., Nimbalker, S. S., Kulkarin, A. D., and Madhovi, D. L. 1995. Lipid oxidation in biological and food systems, In *Food Antioxidants,* p.5. Marcel Dekker, Inc., New York.

Johnson, J. M., and Walker, P. M. 1992. Zinc and iron utilization in young women consuming a beef-based diet. J. Am. Diet. Assoc. 92: 1474.

Kahlon, T. S., Saunders, R. M., Chow, F. I., and Berschart, A. A. 1989. Effects of rice bran and oat bran on plasma cholesterol in hamsters. Cereal Foods World. 34: 768.

Kanner, J., Shegalovich, I., Harel, S., and Hazan, B. 1988. Muscle lipid peroxidation dependent on oxygen and free metal ions. J. Agric. Food Chem. 36: 409.

Kamel, A. M., Wiener, N. D., Felmeister, A. 1971. Autoxidation of cholesterol monolayer films. J. Colloid Interface Sci. 35: 163.

Kerler, J. and Grosch, W. 1996. Odorants contributing to warmed-over-flavor (WOF) of refrigerated cooked beef. J. Food Sci. 61: 1271.

Kim, I. H. and Kim, C. J. 1991. Separation of oryzanol from the refining by-product of rice bran oil. Korean J. Food Sci. Technol. 23: 76.

Kim, S. K. and Nawar, W. W. 1993. Parameters influencing cholesterol oxidation. Lipids. 28: 917.

Koskas, J. P., Cillard, J., and Cillard, P. 1984. Autoxidation of linoleic acid and behavior of its hrdroperoxides with and without tocopherols. J. Am. Oil Chem. Soc. 61: 1466.

Kregel, K. K., Prusa, K. J. and Hughes, K. V. 1986. Cholesterol content and sensory analysis of ground beef as influenced by fat level, heating and storage. J. Food Sci. 51: 1165.

Kritchevsky, D., and Tepper, S. A. 1961. The free and ester sterol content of various foodstuffs. J. Nutr. 74: 441.

Lang, J. K., Schillaci, M., and Irvin, B. 1992. Vitamin E. In *Modern Chromatographic Analysis of Vitamins*, 2nd ed. 3. A. P. De Leenheer, W.E. Lambert, and H. J. Nelis (Eds.), p.153. Marcel Dekker, Inc. New York, NY.

Lea, C. H. and Ward, R. J. 1959. Relative antioxidant activities of the seven tocopherols. J. Agric. Food Sci. 10: 537.

Lee, B. J. and Hendricks, D. G. 1995. Phytic acid protective effect against beef round muscle lipid peroxidation. J. Food Sci. 60: 241.

Lees, A. M., Mok, H. Y. I., Lees, R. S., McCluskey, M. A., and Grundy, S. M. 1977. Plant sterols as cholesterol-lowering agents: Clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis. 28: 325.

Lehmann, J. and Slover, H. T. 1976. Relative autoxidative and photolytic stabilities of tocols and tocotrienols. Lipids. 11: 853.

Liu, R. 1995. Use of stabilized full-fat rice bran in low fat grouond beef: Effect on nutritional composition, sensory properties and oxidative stability. M.S. Thesis, LSU, Baton Rouge, LA.

Loeb, J. R. and Mayne, R. T. 1952. Effect of moisture on the microflora and formation of free fatty acids in rice bran. Cereal Chem. 29: 163.

Machlin, L. J. 1991. Vitamin E. In *Handbook of Vitamins*, L. J. Machlin (Ed.), p.99. Marcel Dekker, New York, NY.

Maerker, G. 1987. Cholesterol autoxidation - current status. J. Am. Oil Chem. Soc. 63: 771.

Maerker, G. and Bunick, F.J. 1986. Cholesterol oxides II. measurements of the 5,6-epoxides during cholesterol oxidation in aqueous dispersions. J. Am. Oil Chem. Soc. 63: 771.

Marinova, E. M., and Yamashlieva, N. V. 1992. Effect of temperature on the antioxdative action of inhibitors in lipid antioxidation. J. Sci. of Food and Agric. 60: 313.

McMillin, K. W., Bidner, T. D., Felchle, S. E., Dugas, S. M., and Koh, K. C. 1991. Flavor and oxidative stability of ground beef patties as affected by source and storage. J. Food Sci. 56: 899.

Miles, R. S., McKeith, F. K., Bechtel, P. J., and Novakofski, J. 1986. Effect of processing, packaging and various antioxidants on lipid oxidation of restructured pork. J. Food Protection. 49: 222.

Minerich, P. L., Addis, P. B., Epley, R. J., and Bingham, C. 1991. Properties of wild rice/ ground beef mixtures. J. Food Sci. 56: 1154

Miyagawa, K., Hirai, K., and Takezoe, R. 1991. Tocopherol and fluorescence levels in deep-frying oil and their measurement for oil assessment. J. Am. Oil Chem. Soc. 68: 163.

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

Nawar, W. W., 1985. Lipids. Ch. 4. In *Food Chemistry*, O. R. Fennema (Ed.), p.139. Marcel Dekker, New York, NY.

Neils, H. J., De Bevere, V. O. R. C., and De Leenheer, A. P. 1985. Vitamin E: Tocopherols and Tocotrienols. In *Modern Cromatographic Analysis of the Vitamins*, 1st ed. 3. A. P. De Leenheer, W. E. Lambert, and M. G. M. De Ruyter (Eds.), p.129. Marcel Dekker, Inc. New York, NY.

Norcia, L. N., Janusz, W. F. 1965. Autoxidation of cholesteryl linoleate in aqueous colloidal suspension. J. Am. Oil Chem. Soc. 42: 847.

Norton, R. A. 1995. Quantitation of steryl ferulate and ρ-coumarate esters from corn and rice. Lipids. 30: 269.

Ockerman, H. W. 1980. *Chemistry of Meat Tissue.* 9th ed. Dept. of Animal Science, Ohio State University and Ohio Agricultural Research & Development Center, Columbus, OH.

Okada, T., and Yamaguchi, N. 1983. Antioxidant effect and parmacology of oryzanol. Yukaku. 32: 305.

Okayama, T., Imai, T., and Yamanoue, M. 1987. Effect of ascorbid acid and alpha-tocopherol on storage stability of beef steaks. Meat Sci. 21: 267.

Osada, K., Kadama, T., Yamada, K., and Sugano, M. 1993. Oxidation of cholesterol by heating. J. Agri. Food Chem. 41: 1198.

Palipane, K. B. and Swarnasiri, C. D. P. 1985. Composition of raw and parboiled rice bran from common Sri Lankan varieties and from different types of rice mills. J. Agric. Food Chem. 33: 732.

Park, P. W., Gaurdiela, F., Park, S. H., and Addis, P. B. 1996. Kinetic evaluation of 3 β-hydroxycholest-5-en-7-one (7-ketocholesterol) stability during saponification. J. Am. Oil Chem. Soc. 73: 623.

Park, S. W., and Addis, P. B. 1985. HPLC determination of C-7 oxidized cholesterol derivatives in foods. J. Food Sci. 50: 1437.

Park, S. W, and Addis, P. B. 1986. Identification and quantitative estimation of oxidized cholesterol derivatives in heated tallow. J. Agri. Food Chem. 34: 653.

Park, Y. W., Kouassi, M. A., and Chin, K. B. 1991. Moisture, total fat, and cholesterol in goat organ and muscle meat. J. Food Sci. 56: 1191.

Parkhust, K. M., Skinner, W. A., and Sturm, P. A. 1968. The effect of various concentrations of tocopherols and tocopherol mixtures on the oxidative stability of a sample of lard. J. Am. Oil Chem. Soc. 45: 641.

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

Penazzi, G. Caboni, M. F., Zunin, P., Evangelelisti, F., Tiscornia, E., Gallina Toschi, T., and Lercker, G., 1995. Routine high-perfermance liquid chromatographic determination of free 7-ketocholesterol in some foods by two different analytical methods. J. Amer. Oil Chem. Soc. 72: 1523.

Peng, S. K., Taylor, C. B., Tham, P., Werthessen, N. T., and Mikkelson, B. 1978. Effect of autoxidation products from cholesterol on aortic smooth muscle cells. Arch. Path. Lab. Med. 102: 57.

Peng, S. K., Tham P., Taylor, C. B., and Mikkelson B. 1979. Cytotoxicity of oxidation derivatives of cholesterol on cultured arotic smooth muscle cells and their effect on cholesterol biosynthesis. Am. J. Clin. Nutr. 32: 1033.

Peirce, M. 1996. Personal communication. New Orleans, LA.

Phillippy, B. Q. and Graf, E. 1997. Antioxidant functions of inositol 1,2,3-triphosphate and inositol 1,2,3,6-tetrakisphosphate. Free Radical Biology & Medicine. 22: 939.

Piironen, V., Syvaoja, E. L., Varo, P., Salmineu, K., and Koivistoineu, P. 1985. Tocopherols and tocotrienols in Finish foods: meat and meat products. J. Agric. Food Chem. 33: 1215.

Pratt, D. E. and Birac, P. M. 1979. Source of antioxidant activity of soybeans and soy products. J. Food Sci. 44: 1720.

Pryor, W. A., Strickland, T., and Church, D. F. 1988. Comparison of the efficiencies of several natural and synthetic antioxidants in aqueous sodium dodecyl sulfate micelle solutions. J. Am. Chem. Soc. 110: 2224.

Qiao, D., Hegsted, M., Windhauser, M., and Godber, J. S. 1992. Effect of rice bran and meat on mineral absorption in rats. FASEB Journal 6: A1665, abst. No. 4221.

Quereshi, A. A., Burger, W. C., Peterson, D. M., and Elson C. E. 1986. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J. Biol. Chem. 261: 10544.

Ranhotra, G. S., Gelroth, J. A., Reevees, R. D., Rudd, M. K., Durkee, W. R., and Gardner, J. D. 1989. Short-term lipidemic responses in otherwise healthy hypercholesterolemic men consuming foods high in soluble fiber. Cereal Chem. 66: 94.

Ray, F. K., Parrett, N. A., Vanstavern, B. D., and Ockerman, H. W. 1981. Effect of soy level and storage time on the quality characteristics of ground beef patties. J. Food Sci. 46: 1662.

Raghuram, T. C. 1989. Studies on hypolipidemic effects of rice bran oil in humans. Nutr. Rep. Int. 39: 5.

Rankin, S. A. and Pike, O.A. 1993. Cholesterol autoxidation inhibition varies among several natural antioxidants in an aqueous model system. J. Food Sci. 58: 653.

Ravindran, V., Ravindran, G., and Sivalogan, S. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chemistry. 50: 133.

Reninecclus, G. A. 1979. Symposium on meat flavor: off-flavors in meat and fish- A review. J. Food Sci. 44: 12.

Resurreccion, A. V. A. and Reynolds, A. E. 1990. Evaluation of natural antioxidants in frankfurters containing chicken and pork. J.Food Sci. 55: 629.

Rhee, K. S. Dutson, T. R., and Smith, G. C. 1988. Effect of changes in intermuscular and subcutaneous fat levels on cholesterol content of raw and cooked beef steaks. J. Food Sci. 47: 1138.

Rukmini, C. and Raghuram, T. C. 1991. Nutritional and biological chemical aspects of the hypolipidemic action of rice bran oil: A review. J. Am. College Nutr. 10: 593.

Sakakida, H., Shediac, C. C., and Siperstein, M. D. 1983. Effect of endogenous and exogeneous cholesterol on the feedback control of cholesterol synthesis. J. Clin. Invest. 42: 9.

Sander, B. D., Smith, D. E., Addis, P. B., and Park, S. W. 1989. Effects of prolonged and adverse storage conditions on levels of cholesterol oxidation products in dairy products. J. Food Sci. 54: 874.

SAS Institute. 1990. *Statistical Analysis System.* Version 6.0. The SAS Institute, Cary, N.C.

Saunders, R. M. 1990. The properties of rice bran as a foodstuff. Cereal Food World. 35: 632.

Sayre, R. N. and Saunders, R. M. 1985. Rice bran and rice bran oil. Western Regional research center. ARS. USDA. Albany, California.

Sayre, R. N. and Saunders, R. M. 1990. Rice bran and rice bran oil. Western Regional Research Center. ARS. USDA. Albany, California.

Schmidt, G. R. 1986. Processing and Fabrication. In *Muscle as Food,* P. J. Bechtel, ed., p.201. Academic Press Inc., Orlando, FL.

Secrist, J. L. 1987. Restructured meat-the past and present. In *Advances in Meat Research.* Vol. 3. Restructured Meat and Poultry Products. A. M. Pearson and T. R. Dutson (Eds.), p.1. Van Nostrand Reinhold Co., Inc., New York.

Seetharamaiah, G. S. and Chandrasekhara, N. 1988. Hypocholesterolemic activity of γ-oryzanol in rats. Nutr. Rep. Int. 38: 927.

Seetharamaiah, G. S. and Chandrasekhara, N. 1989. Studies on hypocholesterolemic activity of rice bran oil. Atherosclerosis. 78: 219.

Seetharamaiah, G. S., Krishmakantha, T. P., and Chandrasekhara, N. 1990. Influence of oryzanol on platelet aggregation in rats. J. Nutr. Sci. Vitaminol. 36: 291.

Shahidi, F. 1997. Natural antioxidant: an overview. In *Natural antioxidants: chemistry, health effects, and application*, p.1. AOAC Press. Champaign, IL.

Sharama, R. D., and Rukimini, C. 1986. Rice bran oil and hypocholesterolemic in rats. Lipd. 21: 715.

Sharp, C. Q. and Kitchens, K. J. 1990. Using rice bran in yeast bread in a home baker. Cereal Food World. 35: 1021.

Shin, T. S., and Godber, J. S. 1994. Isolation of eight vitamin E isomers from a variety of natural sources by semi-preparative high performance liquid chromatography. J. Chromatography. 678: 49.

Shin, T. S, Godber, J. S., Martin, D. E., and Wells, J. H. 1997. Hydrolytic stability and changes in E vitamers and oryzanol of extruded rice bran during storage. J. Food Sci. 62: 704.

Sietz, L. M. 1989. Stanol and sterol esters of ferulic and ρ-coumaric acids in wheat, corn, rye, and triticale. J. Agric. Food Chem. 37: 662.

Simic, M. G. and Karel, M. 1980. Biological effects of some products of cholesterol autoxidation. In *Autoxidation in Food and Biological Systems*, A. A. Kandutch (Ed.), Plenum Press, New York and London.

Slover, H.T. and Lehmann, J. 1972. Effects of fumigation on wheat in storage. IV. Tocopherols. Cereal Chem. 49: 412.

Smith, L. L. 1980. The autoxidation of cholesterol. In *Autoxidation in Food and Biological Systems*, Smimic, M. G. and Karel, M. B. (Eds.), p.119. Plenum Press, New York.

Smith, L. L 1981. *Cholesterol Autoxidation.* Plenum Press, New York and London.

Sonntag, N. O. V. 1979. Composition and characteristics of individuals fats and oils. In *Bailey's Industrial Oil and Fat products*, Vol 1. 4th ed., D. Swern (Ed.), p.289. Wiley, New York, NY.

Suzuki, S. and Oshima, S. 1970. Influence of blending of edible fats and oils on human serum cholesterol level. Jap. J. Nutr. 28: 3.

Suzuki, Y. J., Tsuchiya, M., Gavil, G., Kagou, V. E., and Packer, L. 1993. Structural and dynamic membrane properties of α-tocopherol and α-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry. 32: 10692.

Syvaoja, E. L., Piironen, V., Koivistoinen, P., and Salminen, K. 1986. Tocopherols and tocotrienols in finish foods: Oils and fats. J. Am. Oil Chem. Soc. 63: 328.

Tan, B., and Chu, F. L. 1991. Effects of palm carotenoids in rat hepatic cytochrome P450-mediated benzo(a)pyrene metabolism. Am. J. Clin. Nutr. 53: 1071.

Tanchotikul, U., Godber, J. S., Arganosa, G. A., McMillin, K. W., and Shao, K. P., 1989. Oxidative stability and textual quality of restructured beef roasts as affected by end-point cooking temperatures, storage and the incorporation of surimi. J. Food Sci. 54: 280.

Tao, S. H., Fox, M. R. S., Phillippy, B. Q., Fry, B. E., and Johnson, M. R. 1986. Effect of inositol phosphates on mineral utilization. Fed Proc. 45: 819.

Tappel, A. L. 1962. Vitamin E as the biological lipid antioxidant. Vitam. Horm. 20: 493.

Tarladgis, B. G., Watts, B. M., and Younanathan, M. T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid food. J. Am. Oil Chem. Soc. 37: 44.

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

Trout, G. R. and Schmidt, G. R. 1987. Nonprotein additives. In *Advances in Meat Research*, Vol. 3. Restructured Meat and Poultry products, ed., A. M. Pearson and T. R. Dutson (Eds.), p.307. Van Nostrand Reinhold Co., Inc., New York.

Turro, N. J. and Weed, G. C. 1983. Micellar systems as "supercages" for reactions of geminate radical pairs. Magnetic effects. J. Am. Chem. Soc. 105: 1861.

Vercellotti, J. R., Sr. Angelo, A. J., and Spanier, A. M. 1991. Symposium on lipid oxidation in foods. South Regional Research Center. ARS. USDA. New Orleans, LA.

Wells, J. M. 1993. Utilization of rice bran and oil in human diets. Louisiana Agriculture. 36(3): 5.

Whang, K., Aberle, E. D., Judge, M. D., and Peng, I. C. 1986. Antioxidative activity of α-tocopherol in cooked and uncooked ground pork. Meat Sci. 17: 235.

Yan, P. S. and White, P. J. 1990. Cholesterol oxidation in heated lard enriched with two levels of cholesterol. J. Am. Oil Chem. Soc. 67: 927.

Yamaoka, M. and Komiyama, K. 1994. Antioxidative activity of tocotrienol in heterogenous system: indication of restriction within membrane by fluorescence measurement. Methods in Enzymology. 234: 320.

Yoshida, H., Kajimoti, G., and Eiucira, S. 1993. Antioxidant effects of δ-tocopherols at different concentration in oils during microwave heating. J. Am. Oil Chem. Soc. 70: 989.

Yoshida, H., Hirooka, N., and Kajimoto, G. 1991. Microwave heating effects on relative stabilities of tocopherols in oils. J. Food Sci. 56: 1042.

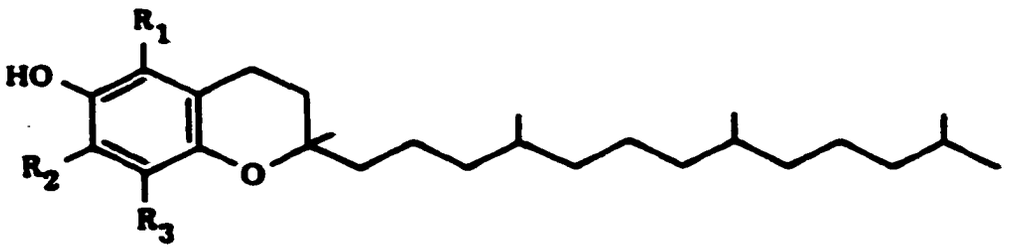
Yuki, E. and Ishikawa, Y. 1976. Tocopherol contents of nine vegetable frying oils, and their changes under simulated deep-fat frying conditions. J. Am. Oil Chem. Soc. 53: 673.

Zulak, I. M. and Maerker, G. 1989. Cholesterol oxides III. autoxidation of cholesterol in sodium stearate and sodium linoleate dispersions. J. Am. Oil Chem. Soc. 66: 1499.

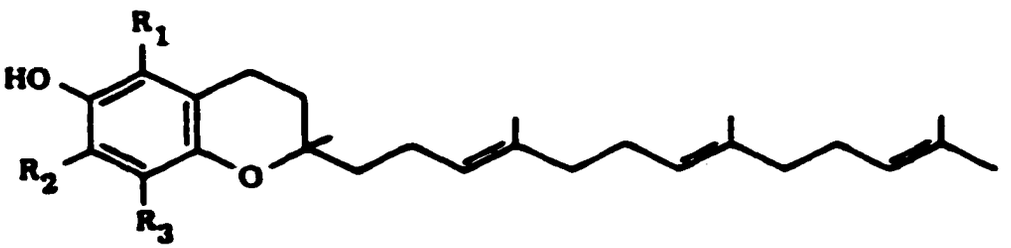
APPENDICES

APPENDIX 1: STRUCTURES OF TOCOPHEROLS AND TOCOTRIENOLS

Tocopherol

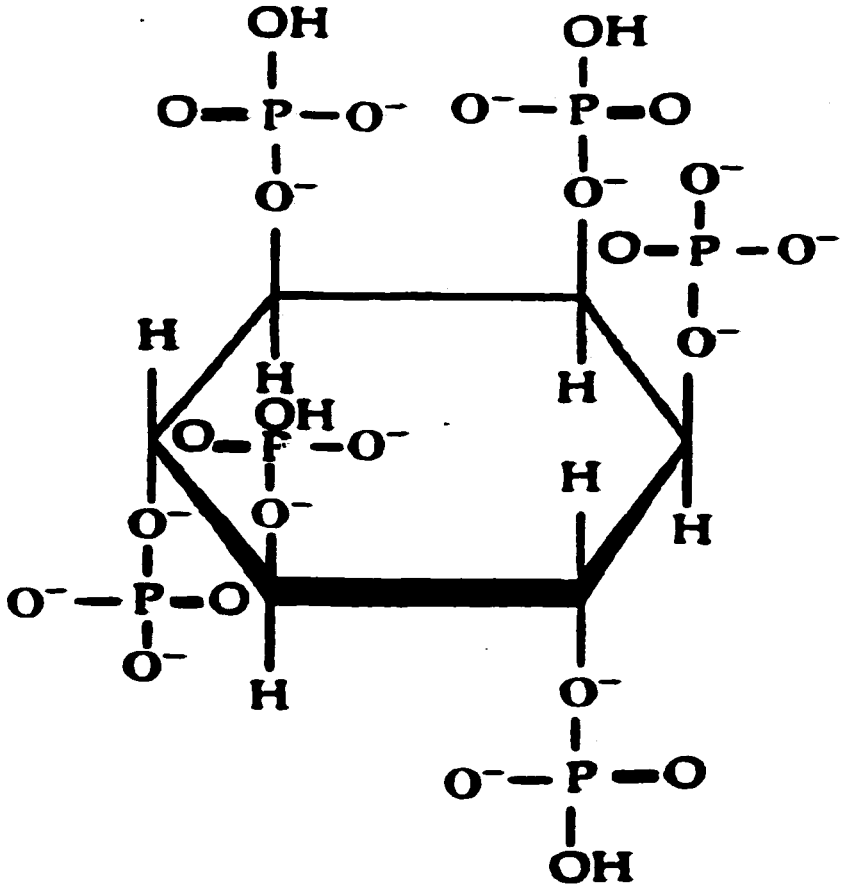


Tocotrienol

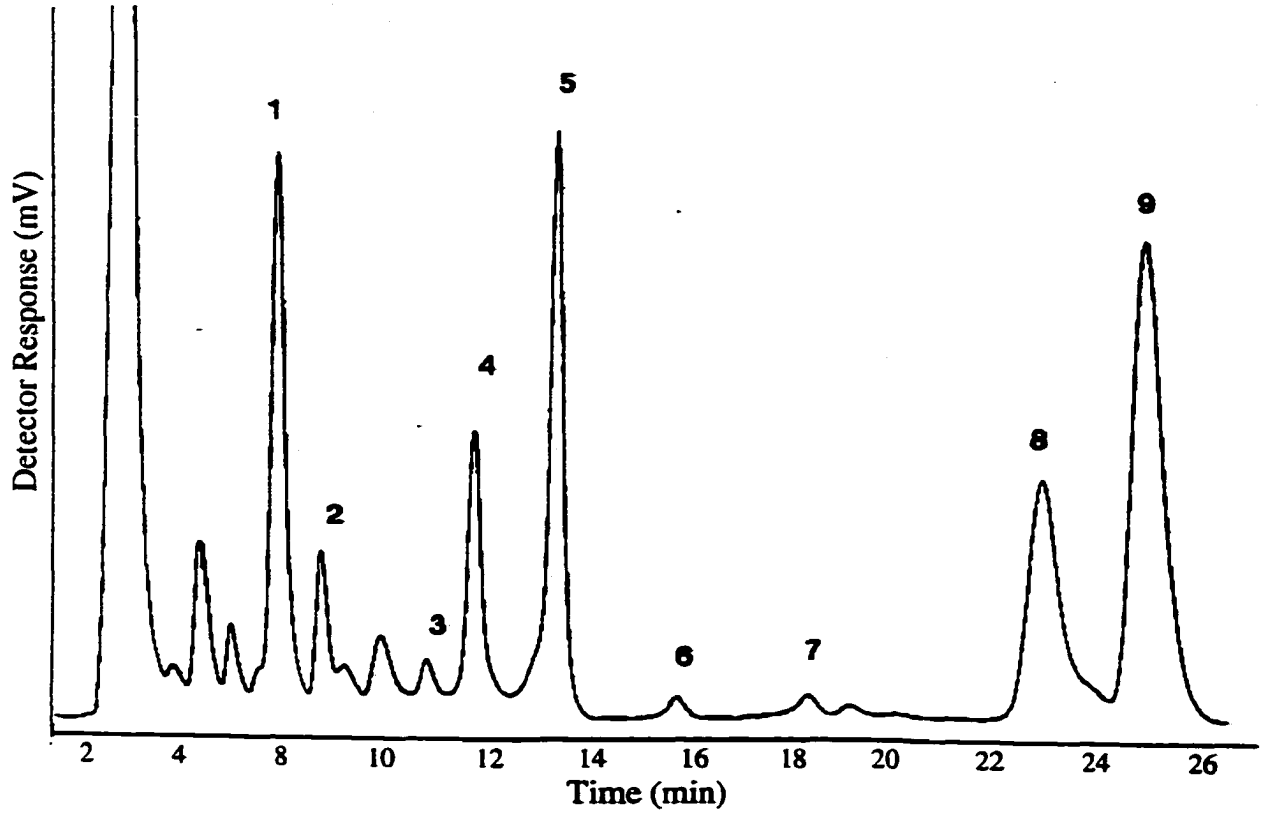


Tocol		R_1	R_2	R_3
Tocopherol	α	CH_3	CH_3	CH_3
	β	CH_3	H	CH_3
	γ	H	CH_3	CH_3
	δ	H	H	CH_3
Tocotrienol	α	CH_3	CH_3	CH_3
	β	CH_3	H	CH_3
	γ	H	CH_3	CH_3
	δ	H	H	CH_3

APPENDIX 2: STRUCTURE OF PHYTIC ACID

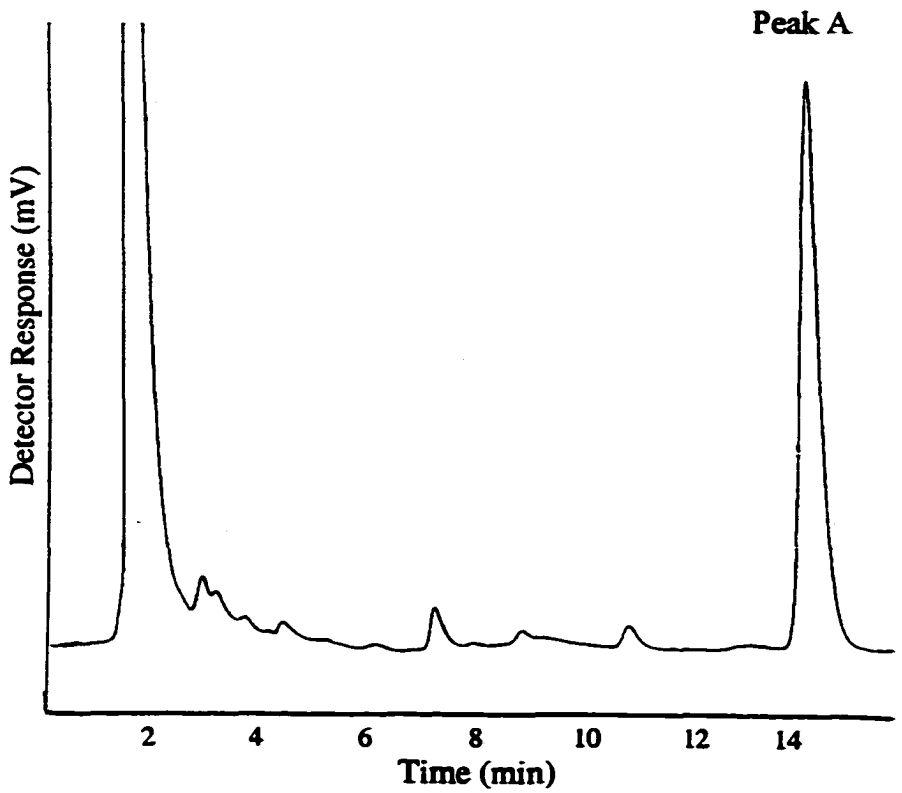


APPENDIX 3: HIGH PERFORMANCE LIQUID CHROMATOGRAM OF NONSAPONIFIABLE FRACTION IN RICE BRAN



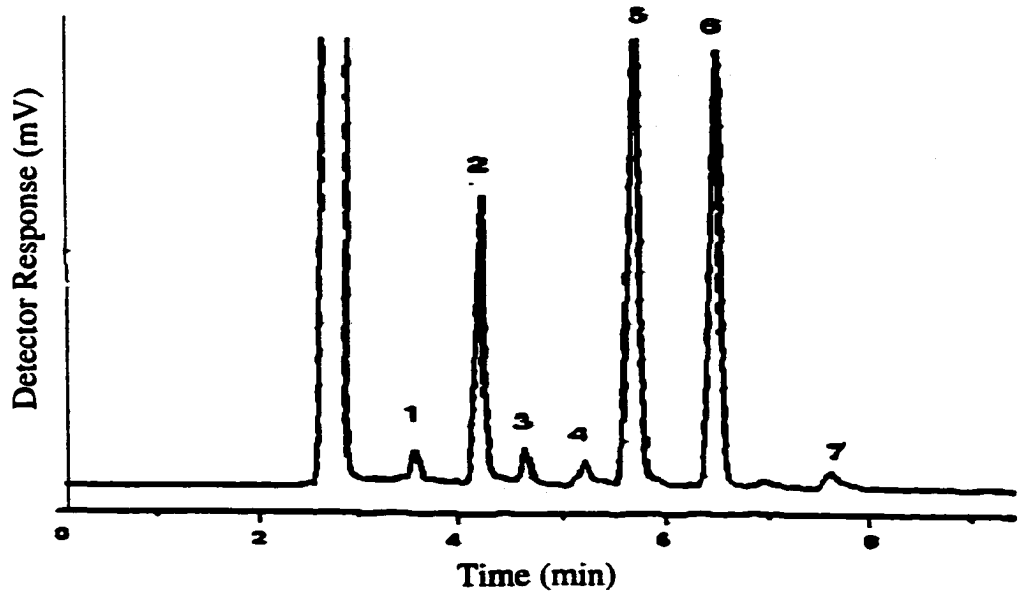
1= α-Tocopherol, 2= α-Tocotrienol, 3= β-Tocopherol, 4= γ-Tocopherol, 5= γ-Tocotrienol, 6= δ-Tocopherol, 7= δ-Tocotrienol, 8 & 9= γ-Oryzanol. (Chromatographic conditions are given in the text of experiment 3, pp.68.)

APPENDIX 4: HIGH PERFORMANCE LIQUID CHROMATOGRAM OF 7-KETOCHOLESTEROL



Peak A represents 7-ketocholesterol.
(HPLC conditions are given in the text of experiment 3, pp. 68.)

APPENDIX 5: GAS CHROMATOGRAM OF FATTY ACIDS IN RICE BRAN OIL



1= C14:0, 2= C:16:0, 3= C17:0 (Internal standard), 4= C18:0, 5= C18:1, 6= C18:2, 7= C18:3. (Chromatographic conditions are given in the text of experiment 1, pp. 28.)

APPENDIX 6: CONSUMER QUESTIONARIES FOR SENSORY EVALUATION

Name _____ Date _____

Beef Roast Sample No. _____

Please evaluate this product and check the space that best reflects your feeling about the product.

1. How would you rate the **"APPEARANCE"** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

2. How would you rate the **"COLOR"** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

3. How would you rate the **"FLAVOR"** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

4. How would you rate the **"OVERALL TEXTURE"** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

5. **OVERALL**, how do you **"LIKE"** this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

6. Is this product **ACCEPTABLE**? Yes [ ] No [ ]

7. Would you **BUY** this product if it were commercially available? Yes [ ] No [ ]

VITA

The author was born in Seoul, the Republic of Korea, on June 11, 1967. She graduated from Sang-Myung Women's University with a bachelor's degree of science in the Department of Chemistry in February 1991.

In January 1992, she entered the Graduate School of University of Tennessee, Martin. She studied in the Department of Human Environmental Science and concentrated on a dietetic program.

After graduation with a master of science degree in July 1994, she was accepted in the graduate school of Louisiana State University and completed her doctoral studies in the Department of Food Science. She worked as a graduate research assistant.

The author married Dr. Anthony, H. Y. Chung in May 1999 and she is currently a candidate for the degree of Doctor of Philosophy in food science, which will be conferred in December, 1999.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Joo-Shin Kim

Major Field: Food Science

Title of Dissertation: Increased Nutritional Value and Oxidative Stability of Restructured Beef Roasts with Purified Extracts from Rice Bran

Approved:

Samuel Godber
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Joseph F. Labuza

Soon Pyung Lee

Michael Keenan

Jo M. King

Date of Examination:

July 22, 1999