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EFFECT OF ADDING VITAMIN E WITH NON-VACUUM TUMBLING ON QUALITY OF PRECOOKED ROAST BEEF

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The purpose of this study was to explore the effect of adding exogenous vitamin E into meat system with tumbling on lipid oxidation, physical properties and microbiological growth in precooked roast beef. According to measured TBARS, adding α-tocopherol into meat with tumbling maintained oxidative stability compared to the non-tocopherol treatment. All samples had the same cooking yield for tumbled or non-tumbled samples and α-tocopherol levels. There were no significant three-way and two-way interaction of moisture, pH, shear values, total iron, heme iron and nonheme iron contents of precooked roast beef. Also, the factors of α-tocopherol and tumbling did not influence microbial growth in precooked roast beef. Moreover, one of the various forms of Vitamin E with tumbling was more effective process of lipid stability than control which is non-adding antioxidant in precooked roast beef.

Keywords: Vitamin E, Tumbling, Lipid oxidation, Roast beef

INTRODUCTION

Lipids can be oxidized by enzymatic or nonenzymatic reactions, and there were many mechanisms to explain these reactions in meat system. However, autoxidation is a continuous free-radical chain reaction that is the most important mechanism of lipid oxidation in meat (Pearson *et al.*, 1983). Phospholipids, which are located in the cell membranes, are sensitive to oxidation in meat due to the composition of many unsaturated fatty acids. Lean meat contains a high percentage of phospholipids that makes it sensitive to oxidation (Igene *et al.*, 1980). Therefore, phospholipids act as the major contributors to oxidative rancidity in meat. However, lipid oxidation is also influenced by the degree of unsaturation of the fatty acids in the phospholipid and triglyceride fractions.

Antioxidants are the chemical compounds used to delay the autoxidation or slow the speed of lipid oxidation (Min, 1998). Consumers concern about synthetic antioxidants; however, natural antioxidants have limited used in food systems due to their special color, flavor and the cost (Hettiarachchy *et al.*, 1996). Deshpande *et al.* (1996) reviewed several papers and suggested that Vitamin E as a hydrophobic antioxidant is the secondary defense for inhibiting lipid peroxidation caused by free radicals. The antioxidative ability of vitamin E is caused from being a free radical scavenger and a singlet oxygen quencher (Yang and Min, 1993).

Tumbling is a physical process that involves rotating, falling, and contacting with the metal walls and the paddles in a tumbling drum. This process provides a way of transformation with kinetic energy to extract protein that forms a binding agent for the muscle fibers (Addis and Schanus, 1979). Ockerman and Dowiercial (1980) reported that the tumbling caused more even distribution of sodium

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nitrite and sodium chloride in bacon. Lawlis *et al.* (1992) indicated that there was a linear relationship between pre-rigor tumbling, cellular disruption and color distribution in ham roasts.

The purpose of this study was to evaluate the effectiveness of vitamin E with tumbling on the quality of precooked roast beef.

MATERIALS AND METHODS

Materials and Procedures

Raw beef bottom round primal cuts, which were approximately 7 days post-mortem, were purchased from a local supermarket in Columbus, Ohio. The α-tocopherol was dissolved in distilled water. All beef bottom round were cut into uniform roasts (8 x 8 x 8 cm dimensions). The á-tocopherol solution was needle injected by 1% of green weight with a medical hypodermic syringe at 8 locations. The addition into the meat was limited to 2% of propylene glycol by regulation (as the amount of 55% cooking yield). The tumbling was conducted with an intermittent schedule, which was ten minutes of tumbling and then fifty minutes of rest per hour, for continuous 18 hours before roasting. Non-tumbled treatments were stored in the same environment as the tumbled treatments. The muscles were then roasted in a hot-air convection oven (Type EFIII, The GS Blodgett Co Inc., Burlington, VT) at 168 °C till the internal temperature (I.T.) reached 71 °C.

This study is a 4 x 2 x 4 factorial experiment with 4 levels of α-tocopherol (0, 200 ppm, 500 ppm and 1000 ppm), tumbling or not (non-tumbled or tumbled), and 4 different lengths of storage time (day 0, 2, 4 and 7). This study was repeated three times.

Analysis

Moisture Measurement

Procedure for moisture measurement was described by Oven Dry Method (Ockerman, 1985).

The pH Measurement

The pH value of the sample was measured using a Corning pH meter (Ockerman, 1985).

TBARS (Thiobarbituric acid Reactive Substances) Test

The modified extraction method of TBARS was used in this study (Pensel, 1990).

Cooking Yield

Cooking yield = (final product weight/fresh meat weight)* 100%

Warner-Bratzler shear value

The cooked roast beef was tested by the Warner-Bratzler (G.K. Electric Mfg. Co., Kansas) shear value instrument (Ockerman, 1985).

Total iron (ferrozine assay from Stookey, 1970; and Clark *et al.*, 1997)

Ferrozine was used to determine total iron with wet-ashed samples.

Heme Iron

Total pigment test followed the modified method from Ockerman (1985).

Total pigments (ppm; μg/g) = A640 x 680

Heme iron was calculated by the modified technique of Clark *et al.* (1997); the iron content is calculated with the factor of 0.0882 μg/μg hematin (Merck, 1989).

Heme iron (ppm; μg/g) = total pigment (ppm; μg/g) x 0.0882

Nonheme Iron

Nonheme iron of sample = Total iron content – Heme iron content

Microbiological Tests

Total aerobic, psychrotrophilic, and thermophilic bacteria tests were conducted to detect contamination of various bacteria in precooked roast beef. Total aerobic plate count was evaluated using aerobic plate count agar (APC; Difco Laboratory, Detroit, MI) at an incubation temperature of 25 °C for 4 days (Speck, 1984). Psychrotrophile was tested by incubation using APC agar at 4 °C for 10 days, and thermophile was incubated with APC agar at 35 °C for 48 hours. All microbiological tests were conducted on day 7 of refrigerated storage. The number of bacteria was converted to log10 colony forming units per gram (log10CFU/g).

Statistical Analysis

A complete Three-Way GLM (General Linear Model) was first used to analyze each measurement. Then, a new Two-way GLM Reduced Model was conducted by SAS after the three-way interaction removed from the model if the three-way interaction was not significant. This procedure was

repeated until main effects were evaluated. However, yield was measured at day 0 and total plate counts were determined at day 7. These two tests were followed by 4x2 factorial design; 4 levels of á-tocopherol (0, 200 ppm, 500 ppm and 1000 ppm), and tumbling (nontumbled or tumbled).

RESULTS

There was no significant two-way (concentration of α-tocopherol x tumbling) for the yield. Also, all samples had the same cooking yield for tumbled or non-tumbled samples and a-tocopherol levels (Table 1).

There were no significant three-way (concentration x tumbling x storage time) and two-way (concentration x tumbling, tumbling x storage time, concentration x storage time) interaction of moisture, pH, shear values, total iron, heme iron and nonheme iron contents of precooked roast beef; therefore, main effects (concentration, tumbling and storage time) were discussed for these measurements as follows.

For the moisture content, there was no significant difference among a-tocopherol levels or tumbling (Table 2). At day 7, roast beef had the significantly lowest moisture content when compared to day 0 and 2 (Table 2). However, the exogenous addition of vitamin E in this study did not retain moisture in precooked roast beef.

The α-tocopherol levels and the tumbling process did not influence pH values of the samples. However, the pH values were significantly higher at day 4 and 7 compared to that at day 0 (Table 2).

For shear values, samples with different a-tocopherol levels had the same tenderness. The non-tumbled precooked roast beef had significantly higher values (tougher) compared to those with tumbling (Table 2). Also, the shear values of roast beef significantly increased during refrigerated storage.

Table 1: Effects of Tocopherol on Yield, Psychrotrophile, Mesophile and Thermophile in Roast Beef

Main Effect Concentration of α-tocopherol	Yield %	Psychrotrophile log_{10}CFU/g	Mesophile log_{10}CFU/g	Thermophile log_{10}CFU/g
0 ppm	54.27	1.79	2.11	1.86
200 ppm	53.99	0.91	1.25	1.59
500 ppm	54.14	1.35	1.17	1.42
1000 ppm	54.34	1.18	1.47	1.42
Tumbling				
NonTumbled	53.93	1.38	1.64	1.57
Tumbled	54.44	1.23	1.37	1.56

Note: ^{AB} Means with different uppercase superscripts within main effect of concentration are significantly different (P<0.05). ^{ab} Means with different lowercase superscripts within main effect of tumbling are significantly different (P<0.05).

Table 2: Main Effect (Concentration of Tocopherol, Tumbling and Storage Time) on TBARS Values, Moisture, pH, Shear Values, Total Iron, Heme Iron, and Nonheme Iron of Roast Beef

Main Effect Concentration of α-tocopherol	TBARS Values	Moisture %	PH	Shear Values (kg)	Total Iron (<g/g)	Heme Iron (<g/g)	Nonheme Iron (<g/g)
0		50.05	5.65	4.49	45.26	22.18	23.06
200 ppm		50.34	5.62	4.28	45.56	22.85	22.71
500 ppm		50.46	5.63	4.35	45.56	23.38	22.18
1000 ppm		50.4	5.65	4.24	46.52	23.77	22.76
Tumbling							
Nontumbled	0.56	50.45	5.64	4.57^{a}	45.99	23.09	22.89
Tumbled	0.57	50.17	5.64	4.11^{b}	45.46	23	22.46
Storage Time							
Day 0		51.38^{W}	5.61^{X}	3.92^{y}	44.81^{W}	23.79	21.02^{X}
Day 2		50.74^{WX}	5.63^{WX}	4.08^{y}	44.09^{X}	22.89	21.20^{X}
Day 4		49.75^{XY}	5.66^{W}	4.49^{x}	46.04^{W}	23.22	22.82^{WX}
Day 7		49.39^{Y}	5.65^{W}	4.87^{w}	47.95^{W}	22.28	25.66^{W}

Note: ^{abc} Means with different lowercase superscripts within a column, and within main effect of tumbling are significantly different (P<0.05). ^{WXYZ} Means with different uppercase superscripts within a column, and within main effect of storage time are significantly different (P<0.05).

There was insignificant difference due to a-tocopherol levels and tumbling for total iron, heme iron and nonheme iron in precooked roast beef (Tables 2). The heme iron of roast beef was not significantly decreased during refrigerated storage. The total iron content of roast beef was not significantly different when comparing day 0 to day 7. It seems that there was no consistent trend in total iron levels during storage. For non-heme iron, it showed that the non-heme iron values were significantly higher at day 7 compared to that at day 0 as expected (Table 2).

For TBARS values, there was insignificant three-way interaction (concentration x tumbling x storage time) in this study. Only a-tocopherol level and storage time had significant two-way interaction; therefore, main effect is discussed for tumbling. TBARS values of nontumbled roast beef had the same TBARS value as tumbled samples (Table 2). Table 3 shows that from day 2 through day 7, roast beef with 0 ppm tocopherol had significantly higher TBARS values; on the other hand, samples with different levels of

tocopherol usually (except day 4) had similar values when compared at the same storage intervals. At days 4 and 7, roast beef injected with 0 ppm of tocopherol had significantly higher TBARS values than those treatments with tocopherol at any days. During refrigerated storage, lipid oxidation still increased in all treatments. Again, there was no salt or phosphate added to increase the ionic strength during tumbling in this non-salt meat system; therefore, the performance of the tumbling process on TBARS values was not significant. Moreover, the tumbling process probably could be more effective with a water-soluble antioxidant than with a fat-soluble antioxidant even though it is dissolved in a liquid carrier, such as propylene glycol.

For microbiological growth, there was no significant two-way interaction (concentration x tumbling) of total plate counts at 7, 25, and 35 °C. Also, the factors of α-tocopherol and tumbling did not influence microbial growth in precooked roast beef (Table 1).

DISCUSSION

Mitsumoto *et al.* (1995) indicated that vitamin E supplementation reduced drip loss because á-tocopherol maintained stability of cellular membranes and reduced leakage of sarcoplasmic components. In this study, the exogenous addition of vitamin E did not retain moisture in precooked roast beef by measuring moisture content.

Non-heme iron content was increased during refrigerated storage and it is probably the reason for the positive relationship between non-heme iron and the speed of lipid oxidation. Chan *et al.* (1995) reported that metmyoglobin formation of longissimus lumborum (LL) muscle was decreased by vitamin E supplementation. A strong negative correlation between α-tocopherol concentration and metmyoglobin was also found (Mitsumoto *et al.*, 1993; and Chan *et al.*, 1995).

Table 3: TBARS Values (mg of Malonaldehyde/Kg of Muscle) of Precooked Roast Beef During Refrigerated Storage at Different ~-tocopherol Levels

Concentration of α-tocopherol	Days of Storage			
	0	2	4	7
0	0.22^{H}	0.74^{C}	0.86^{B}	1.04^{A}
200 ppm	0.32^{FG}	0.42^{EF}	0.48^{DE}	0.67^{C}
500 ppm	0.30^{GH}	0.47^{E}	0.56^{D}	0.73^{C}
1000 ppm	0.34^{FG}	0.40^{EF}	0.69^{C}	0.76^{C}

Note: ^{ABCDEFG} All means with different uppercase superscripts are significantly different (P<0.05).

The incorporation of dietary vitamin E supplement on improving meat quality was related to lipid oxidation, color stability, and moisture retention (Faustman *et al.*, 1998). Many researchers have studied the use of vitamin E supplement to improve color stability of fresh meat; but the exact mechanism of the lipid-soluble α-tocopherol maintaining oxymyoglobin which is a water-soluble substance is still not clear. Radicals formed from autoxidation accelerate pigment oxidation, which is a change from deoxymyoglobin or oxymyoglobin to metmyoglobin. The proposed mechanism is that α-tocopherol directly retardeds lipid oxidation and maintained color stability indirectly. The water-soluble products from lipid oxidation could be pro-oxidants for reducing color stability; therefore, α-tocopherol encouraged stabilization of oxymyglobin by inhibiting lipid oxidation (Faustman *et al.*, 1998).

Basically, lipid oxidation occurs with unsaturated fatty acids of mitochondrial and microsomal membranes in meat. Arnold *et al.* (1993) indicated that α-tocopherol levels of mitochondria, microsome, cytoplasm, connective tissue, and the remainder fractions were increased by dietary vitamin E. Usually, vitamin E supplement at the feedlot is used to improve and extend the color shelf life of meat. The free radicals occurring with the initiation of lipid oxidation were originally formed in the mitochondrial and microsomal membranes (Monahan et al. 1990). The accumulation of higher levels of α-tocopherol is critical to maintaining the color in muscle (Arnold *et al.* 1993). Some researchers also indicated that dietary vitamin E was superior with exogenous addition in meat from a lipid stability standpoint (Mitsumoto *et al.*, 1993; and O'Grady *et al.*, 2000). Jung and Min (1990) reported that the critical concentrations of α-, β-, γ-tocopherols were 100, 250, and 500 ppm, respectively, using a soybean oil model. The higher doses of those compounds acted as a pro-oxidant in this model. Generally, some food additives were added to regenerate antioxidative activity of vitamin E. Lund *et al.* (2007) reported that rosemary extract could regenerate or protect α-tocopherol to inhibit lipid oxidation but not protein oxidation. The vitamin E acting as a chain breaker or free radical scavenger maintained the oxidative stability of minced beef by pressure treated at 20 °C, 40 °C and 60 °C (Ma *et al.*, 2007) .

In fact, the results of these studies are reasonable if compared with both methods based on the same retained

level of vitamin E in meat. Vitamin E is a lipid soluble (hydrophobic) antioxidant that works in the hydrophobic environment, and it is difficult to be dissolved in brine when using direct injection to delivery it into meat. Even using solvents in food, such as propylene glycol, the distribution of antioxidants is still a problem for meat products. Therefore, tumbling could improve the distribution of vitamin E in roast beef that is a whole muscle product.

For microbiological growth, Asghar *et al.* (1991) reported that the number of psychrotrophiles and mesophiles increased when the dietary α-tocopherol level increased in fresh pork because higher drip loss occurred in lower dietary α-tocopherol levels that could reduce the bacteria load on the meat surface. However, in this study there is insignificant difference of moisture contents of precooked roast beef; therefore, microbial growth was not influenced by addition of α-tocopherol in cooked meat product. Also, Georgantelis *et al.* (2007) reported that all microbial counts increased in the samples containing á-tocopherol alone during 20 days of chilled storage as compared to the control group.

Greer *et al.* (1998) reported that there could be animal-to-animal variation due to aggressive feeders when evaluating dietary vitamin E supplementation. Also, Liu *et al.* (1994) indicated that cooking denatured membranes and protein releasing metal ions would decrease the benefit of dietary vitamin E, but the amount of α-tocopherol did not change. Vitamin E of muscle can be accumulated from the diet or by supplementation because animals do not synthesize vitamin E.

The relationship of vitamin E and microbial growth as proposed by Asghar *et al.* (1991) was that the amount of psychrotrophiles and mesophiles increased when the dietary α-tocopherol level increased in pork. The reason could be that higher drip loss occurred in lower dietary α-tocopherol levels that could reduce the bacteria load on the meat surface. However, Asghar *et al.* (1991) indicated that α-tocopherol could further prevent the microbial membranes from being attacked by free radicals.

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