Nutritional Quality of Rabbit Meat as Affected by Cooking Procedure and Dietary Vitamin E

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ABSTRACT: The effect of two dietary treatments (50 as opposed to 200 mg kg^{-1} of α-tocopheryl acetate) on rabbit meat, cooked by different procedures (boiling, frying, roasting), was evaluated. Cooking caused an increase of pH, shear force, lightness, hue and chroma, and a decrease of water holding capacity. The change in nutritional value was slight but lipid oxidation was increased and the fat quality worsened. Boiled meat had the lowest nutrient retention and the highest TBARS values. Supplemental vitamin E was effective in reducing oxidative processes and increasing the amount of n-3 polyunsaturated fatty acids also during cooking.

Key Words: rabbit, cooked meat, vitamin E

Introduction

THE NUTRITIONAL QUALITY OF MEAT IS INFLUENCED BY NUmerous factors. Among them, cooking has an important role because heat and oxygen promote lipid oxidation that greatly affect the nutritional and sensory traits and safety of the meat. The modifications caused by heat depend on cooking procedures (temperature, rate, duration, moist or dry heat) and packaging system (vacuum, modified atmosphere, aerobic conditions), which can both affect lipid oxidation (Kingston and others 1998), cooking yield, and nutrient retention (Unklesbay and others 1983; Renk and others 1985; Lyon and Lyon 1993).

The rate and extent of the peroxidative processes depend on the lipid characteristics and on the balance between the pro-oxidant and the antioxidant components present in the meat.

α-Tocopherol is the most important inhibitor of meat lipid peroxidation (Sheldon and others 1997) and its concentration is strictly correlated with its dietary intake as verified in beef (Engeseth and others 1993), sheep (Wulf and others 1995), poultry (Wen and others 1996), fish (Silva and others 1994), pig (Monahan and others 1990), and rabbit (Lopez-Bote and others 1997; Castellini and others 1999).

Since α-tocopherol is partly destroyed by feed processing, DL α-tocopheryl acetate is generally used because it is more stable.

Few studies have evaluated the influence of cooking on the qualitative characteristics of rabbit meat enriched with vitamin E (Stepanova and others 1982). Hence, the aim of this investigation was to assess the effect of cooking procedure on the nutritional quality of rabbit meat and to evaluate the effect of DL α-tocopheryl acetate as a dietary supplement.

Materials and Methods

Animals and diets

The study was carried out at the experimental rabbitry of the Dept. of Animal Science of the Univ. of Perugia where the ambient temperature was 18.5 ± b 3.1 °C and the photoperiod was 16 light. Forty hybrid males breed, weaned at 35 d of age, were randomly assigned to 2 groups and provided ad libitum access to diets containing different DL α-tocopheryl acetate levels: 50 or 200 mg kg^{-1} (E_{50} or E_{200}). At 85 d of age rabbits were electrically stunned and killed by cutting carotids and jugulars. Carcasses were prepared according to standard procedures (Blasco and Ouhayoun 1996).

Tissue sampling

Two longissimus dorsi muscles were placed in a chilled room (4 °C per 24 h) and trimmed of external fat and epimysial connective tissue. Eighty homogeneous samples (40 for each dietary treatment) of about 20 g each (6 × 2 × 2 cm) were obtained; twenty samples chosen at random were analyzed (fresh meat), while the others were cooked using three different procedures: boiling, frying and roasting.

Cooking procedures

The cooking systems had been standardized in a previous investigation (Cyril and others 1996) by analyzing the trend of the temperature increases and the time needed to reach an internal temperature of 80 °C.

The protocol used was: boiling-each sample was placed in a vacuum sealed polyethylene bag and totally immersed in an agitated water bath at 100 ± 1 °C for 8 min; frying-meat pieces were totally immersed in sunflower oil at 175 ± 5 °C for 3 min; roasting-samples were placed in aluminium pans and put in an electric oven preheated to 200° ± 10 °C for 15 min.

Following cooking, the samples were cooled for 30 min at 15 °C and immediately analyzed.

Analytical determinations

The chemical composition of the muscles was determined according to AOAC (1995) procedures. In order to obtain a direct comparison of the results analytical data were respectively referred to 100 g of fresh muscle and to the residual amount of muscle after cooking (cooking yield = 100 - cooking loss in grams).

Water holding capacity (WHC) was estimated by centrifuging 1 g of muscle placed on tissue paper inside a tube for 4 min at 1,500 × g and drying the centrifuged material at 70 °C overnight to determine the residual water (Nakamura and Katoh 1985). The following formula was used: WHC (%) = (weight after centrifugation – weight after drying) × 100/ initial weight.

Shear force was evaluated on cores (1.25 cm × 2 cm) obtained from the mid-portions of the cooked samples by cut-

Table 1—Composition of fresh (g/100 g) and cooked rabbit longissimus muscles (g/g after cooking) retention coefficients

		Control				Vitamin E				SEM
		Fresh	Boiled	Fried	Roasted	Fresh	Boiled	Fried	Roasted	
Water	g	74.12^{c}	44.74^{b}	37.64^{a}	44.69^{b}	74.26^{c}	44.62^{b}	37.52^{a}	44.78^{b}	21.25
Protein (Nx6.25)	g	22.97^{b}	21.49^{a}	22.95^{b}	22.28^{ab}	22.89^{b}	21.61^{a}	22.87^{b}	22.05^{ab}	1.24
Lipid	g	1.85^{b}	1.73^{a}	1.88^{b}	1.79^{ab}	1.81^{b}	1.71^{a}	1.84^{b}	1.74	0.94
Ash	g	1.06^{b}	0.99^{a}	1.04^{b}	1.02^{ab}	1.04^{b}	0.97^{a}	1.02^{ab}	1.0	0.08
Total	g	100.00	68.95	63.51	69.7	100.00	68.91	63.25	69.57	–
Cooking loss (CL)	g	–	31.05^{a}	36.49^{b}	30.22^{a}	–	31.09^{a}	36.75^{b}	30.43^{a}	4.56
Water loss (WL)	g	–	29.38^{a}	36.48^{b}	29.43^{a}	–	29.64^{a}	36.74^{b}	29.48^{a}	5.21
WL/CL	%	–	94.62^{a}	99.97^{b}	97.38^{ab}	–	95.34^{a}	99.97^{b}	96.88^{ab}	1.54
Protein retention	%	–	93.56^{a}	99.91^{b}	96.99^{ab}	–	94.41^{a}	99.91^{b}	96.33^{ab}	6.01
Lipid retention	%	–	93.51^{a}	101.62^{b}	96.76^{ab}	–	94.47^{a}	101.66^{b}	96.13^{ab}	4.90
Ash retention	%	–	93.40^{a}	98.11^{b}	96.23^{ab}	–	93.27^{a}	98.08^{b}	96.15^{ab}	7.14

n = 20–Values with different letters within row are different–$P < 0.05$.

ting them perpendicularly to the grain direction, using a model 1011 Instron, equipped with a Warner-Blatzler Meat Shear apparatus (Instron International Limited, Bucks, U.K.).

The color parameters (L*, a*, b*) were evaluated using a tristimulus analyzer (Minolta Chroma Meter CR-200, Laghenagen, Germany), with the C.I.E. Lab Colour System (1976); hue and chroma were calculated from the chromaticity coordinates as following:

$$\text{hue} = (a^{*2} + b^{*2})^{0.5};\ \text{chroma} = \text{tang}^{-1}\, b^{*}/a^{*}.$$

α-Tocopherol was measured according to Schuep and Rettenmeier (1994) by adding 500 L distilled water and 1 mL ethanol to 500 mg minced meat, and then vortexing for 10 s. Successively, 0.2 mL hexane and BHT (0.01%) were added and the mixture was homogenized and centrifuged. An aliquot of supernatant (0.8 mL) was dried, redissolved in 0.2 mL ethanol/dioxane (1:1) and homogenized for 10 min. After adding methanol (0.3 mL) and further homogenizing, 20 μL were injected into the HPLC (CM 4000, Milton Roy, Riviera Beach, Fla., U.S.A.), using a silica column (Beckman, Fullerton, Calif., U.S.A.).

Fatty acids were determined by taking fresh samples of about 5 g, from the core of muscle, and extracting them in a homogenizer with 20 mL of 2:1 chloroform-methanol, and then filtered through Whatman No. 1 filter paper (Folch and others 1957). Fatty acids were determined as methyl esters (FAME) using a Mega 2 Carlo Erba gas chromatograph, model HRGC (Milano, Italy), with a D-B wax capillary column (25 mm dia, 30 m length). Their percentages were quantified with Chrom-Card software and the mean value of each fatty acid was used to calculate total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Atherogenic and thrombogenic indices were calculated according to Ulbricht and Southgate (1991) as follow:

$$\text{atherogenic} = (C12{:}0 + 4\text{x}C14{:}0 + C16{:}0)/[(\Sigma\text{MUFA} + \Sigma\text{PUFA (n-6) and (n-3)}];$$

$$\text{thrombogenic} = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times \Sigma\text{MUFA} + 0.5 \times \Sigma\text{PUFA (n-6)} + 3 \times \Sigma\text{PUFA (n-3)} + (n\text{-}3)/(n\text{-}6)].$$

Lipid oxidation was assessed by TBARS and expressed as mg of malondialdehyde (MDA) kg $^{-1}$ of tissue, using the modified method of Tarladgis and others (1960). Ten grams of minced sample were homogenized for 2 min with 95.7 mL distilled water and 2.5 mL 4N HCl. The mixture was distilled until 50 mL were obtained, and 5 mL distillate with 5 mL TBA reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid) were heated in a boiling water bath for 35 min. After cooling under running tap water for 10 min, the absorbance was measured at 538 nm against a blank. TBARS values were obtained by multiplying absorbance by 7.843.

Statistical analyses

A linear model (SAS/STAT 1990–GLM) was used to evaluate the effect of diet on differently treated meat samples (raw, cooked). Statistical significance of differences was assessed by multiple t-test (SAS/GLM option PDIFF).

Results and Discussion

Nutrient amounts and retention coefficients

Dietary vitamin E showed no effect on the composition and retention coefficients in rabbit longissimus muscles whereas cooking caused water and nutrient losses (Table 1).

The fried rabbit muscle had the highest cooking loss but retained the most nutrients because only water was lost during the very short cooking time (3 min as compared to 8 and 15 min) as shown by WL/CL ratio. This is in agreement with Fillion and Henry (1998) who reported that frying has little or no impact on the protein and mineral content of foods. Schock and others (1970) observed a higher moisture loss in fried than in roasted semimembranosus muscle of beef.

The retention percentage of nutrients in boiled and roasted meat had similar values. The protein lost in the roasted samples (3.2 to 3.9%) was similar to that (4.3%) found by Dumont and Hundzik (1983) in beef.

Physical traits

Dietary vitamin E showed no effect on any physical traits measured.

Cooking caused a significant increase in pH (Table 2) probably due to modifications of the electric charge of acid groups, separation of peptide chains and production of new compounds (Grau 1978). Sherman (1961) attributed the increase in pH to the fission of protein chains at labile linkages involving imidazole, –SH and –OH groups, followed by hydrogen bonding between carboxyl and amino groups. In fried muscle the pH increase was less than in the boiled and roasted ones, presumably due to the shorter cooking time.

It has been observed that pH variations are also dependent on the initial value in the tissue. Savic and Karan-Djurdjic (1955) found an increase when the initial value was lower than 6.4 and a decrease when the initial value was higher.

Table 2—Physical characteristics of fresh and cooked rabbit longissimus muscles

	Control				Vitamin E				SEM
	Fresh	Boiled	Fried	Roasted	Fresh	Boiled	Fried	Roasted	
Ultimate pH	5.42^{a}	5.75^{b}	5.65^{b}	5.76^{b}	5.55^{a}	5.79^{b}	5.66^{b}	5.77^{b}	0.21
Water holding capacity %	56.83^{c}	47.05^{b}	44.15^{a}	47.98^{b}	57.87^{c}	48.58^{b}	44.89^{a}	48.26^{b}	2.94
Shear force kg\cm	3.59^{a}	3.61^{a}	5.04^{b}	4.65^{b}	3.52^{a}	3.78^{a}	4.67^{b}	4.46^{b}	0.89
Color									
L*	59.92^{a}	84.57^{b}	81.54^{b}	83.51^{b}	60.01^{a}	85.45^{b}	80.15^{b}	83.64^{b}	2.01
a*	5.35^{b}	4.08^{a}	3.09^{a}	3.74^{a}	5.31^{b}	3.99^{a}	3.05^{a}	3.78^{a}	1.54
b*	1.23^{a}	6.05^{b}	6.15^{b}	5.71^{b}	1.27^{a}	5.97^{b}	6.11^{b}	5.64^{b}	2.58
Hue	12.95^{a}	56.46^{b}	63.57^{b}	56.80^{b}	13.46^{a}	56.31^{b}	63.47^{b}	56.19^{b}	10.98
Chroma	5.49^{a}	7.38^{b}	7.15^{b}	6.82^{b}	5.46^{a}	7.18^{b}	6.83^{b}	6.78^{b}	0.74

n = 20–Values with different letters within row are different–$P < 0.05$.

Bouton and others (1971) reported an increase of 0.4 units with a value near 7.

The WHC of meat was reduced by the thermal treatment. It is well known that protein denaturation and coagulation by heating reduce the space within the myofibrillar protein network with a consequent decrease of water (Hamm 1969) and lowering of the WHC (Honikel 1998).

The shear force increased in the fried and roasted meat due probably to the shortening and shrinkage of myofibrils by heat, whereas in the boiled meat jelly formation and the swelling of the connective tissue increased the tenderness by spacing out the muscle fibers (Grau 1978). A similar trend was reported by Leander and others (1980) in beef and Cyril and others (1996) in rabbit which observed a shear force of boiled meat similar to that of raw meat.

Regarding color, an increase in lightness was observed in all treatments as a consequence of water loss that caused the muscle structure to open and scatter light (Cross and others 1986).

Also hue angle and chroma increased due to the decrease in redness and increase in yellowness. Color variations during cooking are probably caused by myoglobin denaturation as stated by Cabanes-Roiron and others (1994).

α-Tocopherol, oxidative stability and fatty acid composition

Cooking caused a reduction in the α-tocopherol level that was different in relation to both the dietary treatment and the cooking method. Boiled meat showed an average reduction of 39% in the control fed group and 41% in the group fed with supplemented vitamin E; fried meat showed respective reductions of 12% and 21%; roasted meat showed reductions of 14% and 22% respectively. The good resistance of vitamin E to heat (Liu and others 1994) was confirmed for fried and roasted meat.

The trend of TBARS was opposite to that of α-tocopherol, and values were higher in boiled than in fried and roasted meat. In fact, the slower cooking rate in boiling seemed to be very favorable to peroxidation with a greater degradation of vitamin E. Kingston and others (1998) found lower degree of oxidation with a fast cooking rate and Chen and others (1984) suggested that in fried sample the quick increase in temperature could cause a rapid coagulation of iron-containing proteins with a lower release of catalytic free iron.

The lower TBARS value of the roasted meat as compared to the boiled sample, in spite of the longer cooking time, could be due to the moderate and gradual temperature increase as result of the lower conductivity of the heating fluid (air against water; Woodams and Nowery 1968) and the heat transfer resistance, which is a consequence of water evaporation at the muscle surface and a greater thickness of the limit layer (Laroche 1990).

A comparison of meat from the supplemented animals and the control showed that the higher intake of vitamin E (50 against 200 IU) affected α-tocopherol concentration and TBARS levels. Both fresh and cooked muscles, independent of the cooking procedure, had higher amounts of antioxidant and lower values of TBARS in the supplemented animals. In raw muscle the α-tocopherol was 1.7 times greater and this led to greater oxidative stability during cooking. The TBARS reduction varied according to the procedure; in fried and roasted meat the reduction was about 54% while in the boiled sample it was only 26%. Monahan and others (1990) found a similar reduction in boiled pork, but their TBARS values were lower than ours (0.66 against 11.16 control; 0.49 against 8.19 vitamin E) probably because the unsaturation level in pork meat was much lower than in rabbit.

The fatty acid composition of muscles was also affected by cooking and vitamin E administration. Cooking decreased n-3 fatty acids. This caused an increase in the thrombogenic index due to the fact that Ulbricht and Southgate ascribed a considerable weight to these fatty acids in the index itself. Such importance is justified by the reduction of platelet aggregability determined by the action of n-3 fatty acids.

Fried samples, although only the core of muscle was analyzed, absorbed some frying oil, as showed by the highest amount of linoleic acid and by the retention coefficient of lipids which was superior to 100 (Table 1).

Duckett and Wagner (1998) found an increase in stearic acid in the total lipid of beef after cooking with a concomitant decrease in linoleic, linolenic and arachidonic acids. Smith and others (1989) and Harris and others (1992) reported no changes in the fatty acid composition of beef steak after cooking. These different findings could be due to the fact that in analyzing total lipids, instead of the two fractions some variations could be overlooked, especially when the phospholipid level of meat is low. Supplemental vitamin E improved fat quality, by lowering SFA and the thrombogenic index and raising long chain n-3 PUFA in both raw and cooked meat.

It is concluded that cooking caused a slight reduction of the nutritional value only in boiled rabbit meat but always altered the fat quality by reducing the level of n-3 PUFA and worsening the oxidative state. In boiled meat TBARS reached a very high value, but it is worth noting that rabbit meat is generally not eaten boiled.

Whereas the increase in muscle α-tocopherol by dietary means had no effect on the retention coefficient or the phys-

Table 3—α-tocopherol content, oxidative stability and fatty acid amounts in fresh (mg/100g) and cooked rabbit longissimus muscles (mg/g after cooking)

	Control				Vitamin E				SEM
	Fresh	**Boiled**	**Fried**	**Roasted**	**Fresh**	**Boiled**	**Fried**	**Roasted**	
α-tocopherol mg kg^{-1}	2.15^{b}	1.31^{a}	1.89^{ab}	1.85^{ab}	3.85^{c}	2.28^{b}	3.04^{c}	2.99^{c}	1.25
TBARS mg MDA kg^{-1}	2.48^{ab}	11.16^{d}	7.31^{c}	8.15^{c}	1.57^{a}	8.19^{c}	3.38^{b}	3.72^{b}	3.58
Fatty acids									
C12:0	13.7	11.5	11.8	11.5	11.6	11.0	11.9	11.4	0.9
C14:0	47.6	45.3	46.8	46.2	46.1	43.6	44.8	43.9	1.6
C16:0	537.9	518.3	543.7	535.4	496.3	482.9	499.6	490.8	89.2
C18:0	102.3	104.3	113.1	109.9	99.3	105.0	110.3	107.6	51.6
Others	24.7	22.6	22.6	22.6	23.3	21.8	23.1	22.7	7.5
Σ SFA	726.2^{b}	702.0^{b}	738.0^{b}	725.6^{b}	676.6^{a}	664.3^{a}	689.7^{a}	676.4^{a}	142.4
C16:1(n-7)	58.1	53.2	55.0	54.1	58.5	55.4	56.3	55.3	21.3
C18:1(n-9)	312.1	329.6	361.2	340.0	308.3	325.6	354.8	332.4	12.1
Others	22.6	22.2	22.2	22.1	21.3	21.2	19.6	20.2	5.1
Σ MUFA	392.8	405.0	438.4	416.2	388.1	402.2	430.7	407.9	21.2
C18:2(n-6)	310.9^{ab}	294.1^{a}	358.0^{b}	305.9^{ab}	320.2^{b}	300.9^{ab}	361.8^{b}	304.4^{ab}	46.2
C20:4(n-6)	44.8	43.4	43.3	44.1	42.8	47.2	47.2	46.9	2.1
Others	7.7	6.7	5.2	6.2	7.1	7.7	7.8	7.8	1.1
Σ (n-6)	363.4	344.2	406.5	356.2	370.1	355.8	416.8	359.1	11.9
C18:3(n-3)	49.2^{b}	24.0^{a}	25.3^{a}	25.1^{a}	51.4^{b}	26.7^{a}	28.2^{a}	28.3^{a}	15.2
C20:5(n-3)	2.0^{c}	0.7^{a}	1.4^{b}	1.5^{b}	5.7^{c}	1.9^{c}	2.6^{d}	2.7^{d}	1.0
C22:5(n-3)	5.8^{c}	0.9^{a}	1.3^{a}	1.2^{a}	8.1^{d}	3.0^{b}	3.6^{b}	3.6^{b}	2.0
C22:6(n-3)	6.3^{c}	1.9^{a}	2.6^{a}	3.3^{a}	11.9^{d}	6.1^{b}	7.0^{b}	7.1^{b}	5.1
Others	3.5^{b}	1.4^{a}	2.5^{a}	2.4^{a}	3.7^{b}	3.1^{b}	4.1^{b}	3.6^{b}	2.2
Σ (n-3)	66.8^{d}	28.9^{a}	33.1^{a}	33.5^{a}	80.8^{e}	40.8^{b}	45.5^{b}	45.3^{b}	20.8
Σ PUFA	430.2	373.1	439.6	389.7	450.9	396.6	462.3	404.4	89.4
Atherogenic index	0.90	0.91	0.91	0.91	0.82	0.84	0.84	0.83	0.19
Thrombogenic index	1.19^{b}	1.45^{c}	1.42^{c}	1.42^{c}	1.0^{a}	1.26^{b}	1.24^{b}	1.24^{b}	0.97

n = 20–Values with different letters within row are different–$P < 0.05$.

ical traits of rabbit meat, it provided protection against oxidation during cooking, and improved the fat quality by decreasing SFA and increasing n-3 PUFA. This fact has positive implications in human nutrition since higher intake of n-3 fatty acids without adequate antioxidant protection could result in increased free radicals and oxidative by-products.

These findings confirm that supranutritional doses of vitamin E should be administered to protect meats rich in PUFA, such as that of rabbit.

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MS 20001579

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