Studies on Vitamin E and Meat Quality. 2. Effect of Feeding High Vitamin E Levels on Chicken Meat Quality

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The objective of this work was to study the influence of vitamin E (as *all-rac*-α-tocopheryl acetate) supplementation on the sensory quality of frozen chicken meat. Two dietary treatments were compared as follows: (1) the control group received a diet containing respectively 30, 20, and 15 mg of α-tocopheryl acetate/kg of feed for the age periods of 0−20, 21−38, and 38−45 days; (2) the supplemented group received a starter diet containing 30 mg of α-tocopheryl acetate/kg of feed from 0 to 20 days and a finisher diet containing 200 mg/kg of feed from 21 to 45 days. HPLC analyses showed that muscle α-tocopherol levels of the chickens on the supplemented diet were 6−7-fold higher than those of the chickens on the control diet. Meat quality was evaluated by sensory as well as by instrumental techniques, such as induced thiobarbituric acid values (TBA) and gas chromatography−mass spectrometry. It was shown that vitamin E supplementation had a beneficial effect on the sensory data and the oxidative stability of the meat as measured by TBA. GC−MS analyses also showed that the concentration of aldehydes, which are considered to be responsible for rancid off-flavors, was much more important in the control samples compared to the supplemented samples.

Keywords: *Chicken meat; sensory quality; volatiles; vitamin E*

INTRODUCTION

Lipid oxidation is a major cause of quality deterioration in meat and meat products and can give rise to rancidity and the formation of undesirable odors and flavors. These undesirable developing flavors are caused primarily by the rapid oxidation of the highly unsaturated protein-bound phospholipid fraction, and their formation is catalyzed by iron. Phospholipids are composed of high levels of polyunsaturated fatty acids (Gray and Pearson, 1987).

In chicken meat, the phospholipids constituted approximately 70−75 g/100 g of the total lipid content of the subcellular fractions (mitochondrial and microsomal). The neutral lipids (25−30 g/100 g) represented the minor structural components. Seventeen fatty acids were identified in the phospholipids of the mitochondrial and microsomal membranes isolated from dark and white chicken meat. In general, C_{18} (oleic $C_{18:1}$ and linoleic $C_{18:2}$) and C_{20} (arachidonic $C_{20:4}$) were the dominant unsaturated fatty acids and accounted for about 45.8% of the total (Lin, 1988). These membranes are believed to be the sites where oxidative changes are initiated in raw meat (Gray and Pearson, 1987). Consequently, chicken meat is very susceptible to oxidation, particularly during and after frozen storage.

Dietary vitamin E has been reported to reduce or prevent the process of lipid oxidation occurring in broiler meat during storage. Several studies have revealed a positive effect of high α-tocopherol levels on the oxidative stability of broiler meat (Asghar et al., 1989, 1990; Frigg et al., 1991; Blum et al., 1992; Bartov and Frigg, 1992).

Evaluation of the flavor quality (freshness) and the oxidation stability of meat can be performed by both sensory and instrumental techniques, such as the TBA method and gas chromatography−mass spectrometry of meat volatiles. In a first approach organoleptic tests with panels should be used to determine the effect of feeding high vitamin E levels on the oxidative stability of meat as a function of storage time.

Instrumental measurement of oxidative changes in muscle foods can be performed by the measurement of secondary degradation products. The TBA method measures a red-colored complex between malonaldehyde and thiobarbituric acid. However, in the case of classical TBA measurements as a function of storage of raw or frozen meat (without forced oxidation), only very low absorbance values are obtained. Stimulated lipid peroxidation and determination of so-called induced TBA values can provide more reliable results (Kornbrust and Mavis, 1980).

With modern analytical methods, such as a combination of suitable isolation procedures and gas chromatography−mass spectrometry, it is also possible to measure a whole series of volatiles (aldehydes, ketones, alcohols...) formed as a function of oxidation. Especially the volatile-saturated and -unsaturated aldehydes, which have low odor threshold values, are known to impart rancid off-flavor notes to meat.

The aim of this study was to evaluate the effect of increased dietary vitamin E on the oxidative stability and the sensory quality of different parts (leg and breast) of chicken meat.

MATERIALS AND METHODS

Animals and Dietary Treatments. Forty frozen animals (20 control and 20 supplemented samples) were provided from a feeding trial of Produits Roche - Paris in cooperation with a French commercial breeder. From 0 to 20 days of age, all animals were fed a starter diet containing 30 mg of α-tocopheryl acetate/kg of feed. After 20 days the animals were separated into two groups of 20 animals each. One group was fed a control diet containing 20 and 15 mg of α-tocopheryl acetate/kg of feed respectively from 21 to 38 and from 38 to 45 days of age. The other group was fed a supplemented diet containing 200 mg/kg of feed during the whole period from 21 to 45 days of age. At the end of the 45 day feeding period, all birds were slaughtered according to standard commercial

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procedures and immediately frozen. Within 1 week the frozen samples were delivered to the lab. At arrival the animals were divided in two parts (right and left halves) while frozen. The right half was stored at −20 °C until the moment of chemical analysis. The left half was used for sensory analysis and kept frozen until required. When analyzed, samples were thawed at room temperature.

Determination of α-Tocopherol Levels. Homogenates of eight control and eight supplemented muscle samples of leg and breast meat were prepared with a mixer after partial thawing. For analysis 15 g of each homogenate was used. Extraction of α-tocopherol from the meat was carried out according to the method of Rettenmaier et al. (1992), involving a saponification step followed by a single extraction of the resulting solution with a mixture of *n*-hexane/toluene. Extracts were injected on a Varian 9002 high-pressure liquid chromatograph (HPLC), equipped with a stainless steel (15 cm × 4.6 mm i.d.) column with Bondesil Si (5 μm) as stationary phase. Detection and quantitative determination was performed using a fluorescence detector (Fluorichrom II, Varian) operating at excitation and emission wavelengths of 220 and 260 nm, respectively. The system was developed with 3% 1,4-dioxane in HPLC grade hexane at a flow rate of 1.6 mL/min. Detector signals were quantified using peak areas and a calibration curve. Standards were prepared by accurately weighing 50 mg of α-tocopherol standard, dissolved and diluted with hexane to obtain a final concentration of 25 μg/mL (stock solution). Out of the stock, standards were prepared, containing a concentration of 1, 2, 3, 4, and 5 μg of α-tocopherol/mL.

Sensory Analysis. Sensory analysis was performed in a panel room with separate booths, air-conditioning, and red light for color masking. The panel (24 panelists) was composed principally of researchers, technicians, and students of the research center. They had not been preselected in any way, but most of them were familiar with taste testing.

Sensory tests were performed on breast and leg meat of 12 control (left half) and 12 (left half) supplemented samples. The different cuts of meat (leg and breast meat) were thawed in air and stored for 4, 5, and 6 days at 4 °C. Standardized meat sample preparation consisted of roasting the meat for 2 min on each side in a commercial grill, without adding spices. The meat was manually deboned and cut in bite-sized pieces. The samples were coded, and presentation order was randomized.

A paired comparison test was used. After smelling and tasting, the panel had to select respectively the sample with the most off-odor (least fresh odor) and the sample with the most off-flavor (least fresh taste). There were comparisons of control and supplemented samples after 4, 5, and 6 days of storage at 4 °C (24 replications each day for both meat parts).

For statistical analyses of sensory data, the least intensity of a characteristic (off-odor or off-flavor) was given a 1 score and the most intensity was given a 2. The evaluations of breast and leg meat and of the 3 successive days were treated together. The means and confidence limits were calculated using variance analysis to observe differences between control and supplemented samples.

Measurement of Lipid Oxidation. The stability of the breast and leg meat after frozen storage was determined by induced thiobarbituric acid (TBA) tests on eight control and eight supplemented samples of thawed breast and leg meat. The induction of lipid oxidation was a modification of the method of Kornbrust and Mavis (1980). To avoid that oxidation proceeded too fast, the use of $FeSO_4$ as a catalyst for lipid oxidation was omitted. The determination of the thiobarbituric reactive substances was measured by the method of Buege and Aust (1978). The TBARS numbers are expressed as μg of malondialdehyde/g of meat.

Measurement of the Volatile Components. Determination of the volatile components was carried out on three control and three supplemented samples of both meat parts. Samples were analyzed after thawing and 1 day of storage at 4 °C.

Aroma concentrates were prepared by using a Likens-Nickerson (SDE) apparatus; 25 g of thawed and mixed meat (kitchen blender) was submitted to simultaneous steam distillation−extraction with 60 mL of dichloromethane as extraction solvent. The extract was concentrated to a final volume of 0.5 mL; 1 μL of the resultant aroma concentrate was injected into the gas chromatograph.

A Hewlett-Packard Model HP5890 gas chromatograph equipped with a methyl silicone column (50 m × 0.21 mm i.d.) was used to separate the volatiles. The gas chromatograph was coupled to a HP 5971A mass spectrometer, which allowed identification of the volatile components. Analysis was carried out by using helium as carrier gas, with the column temperature maintained initially at 40°C for 5 min and then programmed from 40 to 250 °C at a rate of 5 °C/min where it was held for 13 min. The injector and transfer lines were respectively at 200 and 250 °C. The ionization voltage applied was 70 eV.

Semiquantitative analysis of the different components identified in the aroma concentrates was carried out by spiking the dichloromethane with tetradecane as internal standard (10 μg). The volatile composition was calculated by relating the peak areas to the peak area of tetradecane as internal standard. Results are expressed as μg/g of meat.

RESULTS AND DISCUSSION

The average α-tocopherol levels in breast muscle (n = 8 samples) of chickens was influenced by diet and increased from 1.25 ± 0.19 to 8.57 ± 1.15 μg/g of tissue and in leg tissue from 2.02 ± 0.33 to 12.89 ± 1.82 μg/g of tissue. It was shown that compared to the control samples all supplemented samples had higher α-tocopherol levels.

Variations in tocopherol levels were found between the tissue types. This study showed about 1.6 times more α-tocopherol in leg muscle than in breast muscle. These differences apparently result from variations in the vascular network between muscle tissues. Because chicken legs have a more highly developed vascular system than breast tissues, tocopherol is deposited to a greater degree in leg tissues than in breast tissues. (Sheldon, 1984).

Figure 1 presents the mean values (n = 72 replications for off-odor and off-flavor, respectively) and the 90% confidence limits of the sensory evaluations for the control and the supplemented samples, respectively. The sensory evaluations of breast and leg meat carried out after 4, 5, and 6 days of storage at 4 °C were treated together. Although off-odor scores were higher for control samples than for supplemented samples, the difference was not significant. A significant difference at the 90% level was observed for off-flavor scores. It may be concluded that the supplemented samples had a fresher flavor compared to the control samples.

The TBA test is a frequently used method for objective measurement of lipid oxidation. Determination of the TBA values was carried out as a function of forced oxidation of thawed leg and breast meat. Eight control and eight supplemented samples were examined, and the mean TBA values are presented in Figure 2 as a function of forced oxidation time. Results indicated that homogenates of muscle from chickens, fed a basal diet, were more susceptible to induced oxidation than muscle homogenates from chickens fed a diet with a supplemented level of vitamin E. The TBA values of supplemented samples after 120 min of incubation were the same as before incubation, indicating low susceptibility to oxidation. Results clearly demonstrated that dietary treatment had a major impact on the oxidative stability of the broiler meat (leg and breast), the meat of the broilers fed the α-tocopherol-supplemented diet being more stable compared to the control meat.

Results in breast muscle and leg muscle were evaluated separately. Athough their α-tocopherol level was

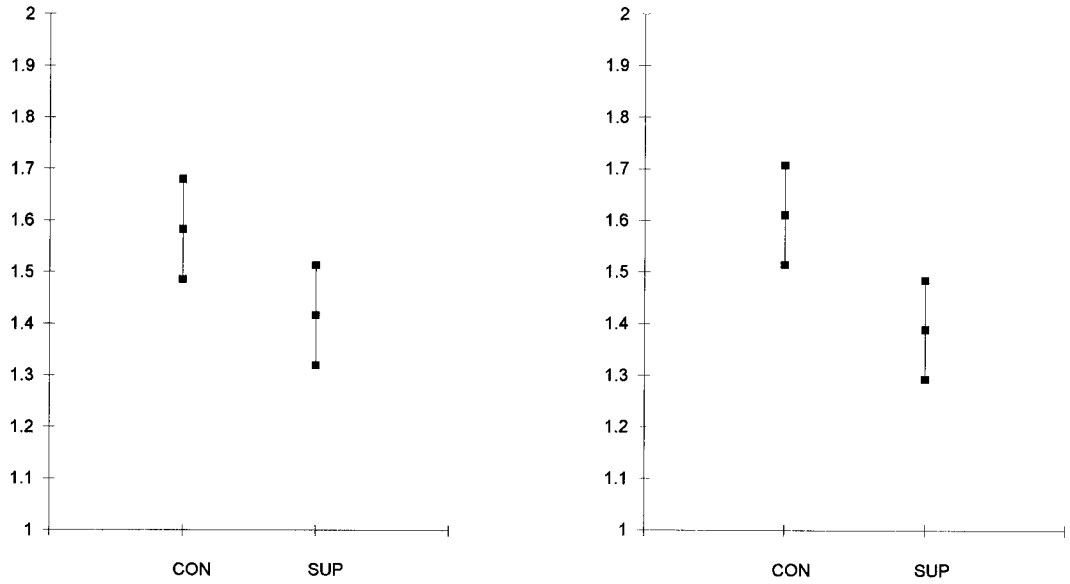

Figure 1. Means and 90% confidence limits for off-odor (left) and off-flavor (right) evaluation of control and supplemented samples.

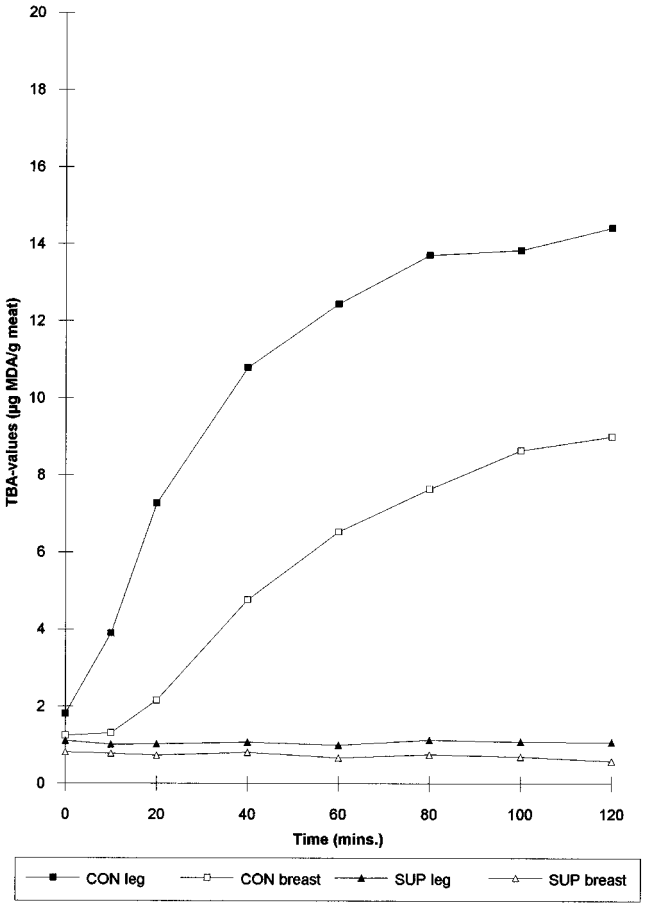

Figure 2. Mean induced TBA values (n = 8 samples) for control and supplemented samples as a function of incubation time.

somewhat higher, oxidative changes were much more extensive in the muscle tissue of the control dark meat (legs) compared to the muscle tissue of the control white meat (breast). These results confirm the findings of Harel and Kanner (1985), who reported a higher rate of lipid peroxidation in microsomes from dark muscle tissue of broilers compared to white muscle microsomes. Asghar et al. (1989) found that oxidative changes were much more extensive in the subcellular membranes isolated from dark meat compared to membranes from white meat. These investigators claimed that the higher total lipid contents in the dark meat may contribute to the higher TBA values for these membranes.

Sklan et al. (1983) mentioned that the phospholipid concentration (and so the polyunsaturated fatty acid content) in the dark meat is higher (8 mg of phospholipids/g of meat compared to 6 mg/g of meat in the breast). Also Lin (1988) claimed that higher total lipid content may contribute to the higher TBARS for the membranes from the dark meat. Another factor may also be the faster rate of lipolysis in thigh muscle. Free fatty acids appear to be oxidized at a faster rate than esterified acyl fatty acids (Sklan et al., 1983).

Typical gas chromatograms of the control and the supplemented chicken meat aroma concentrates, obtained by Likens−Nickerson extraction, are given in Figures 3 (leg meat) and 4 (breast meat). Peak numbering is similar for all chromatograms, and the identified compounds together with their retention times are presented in Table 1. Figures 3 and 4 show fewer constituents in the aroma concentrates of supplemented samples compared to the aroma concentrates of control samples. This is in accordance with the suppression of lipid oxidation due to the higher level of vitamin E in the supplemented samples, and it objectively demonstrates the anti-oxidant effect of vitamin E in retarding lipid oxidation.

Although the gas chromatograms of the control and the supplemented samples show similar components, there is an important difference in concentration. By referring the peak intensities of the different components to the intensity of tetradecane as internal standard, a detailed semiquantitative composition could be calculated (Table 2). The aldehydes in control and supplemented samples for leg and breast meat have been classified as low molecular weight saturated and unsaturated aldehydes and as higher aldehydes. Also the concentrations of 3-hydroxy-2-butanone, benzaldehyde, and 2-pentylfuran and of two alcohols, 1-octen-3-ol and benzyl alcohol, have been determined semiquantitatively.

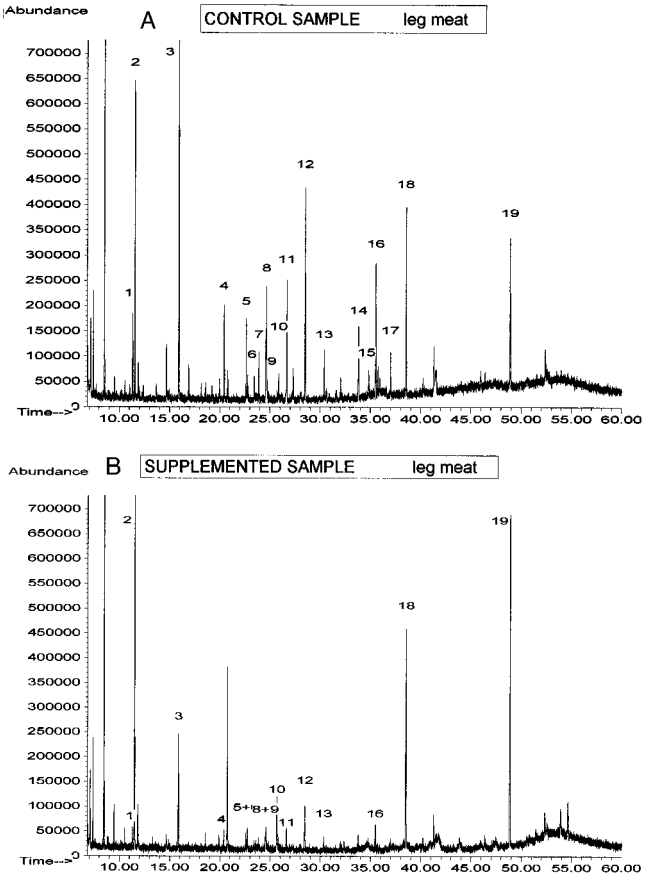

Figure 3. Typical gas chromatograms of a leg meat aroma concentrate of a control sample (A) and a supplemented sample (B). Peak numbering is in accordance with Table 1.

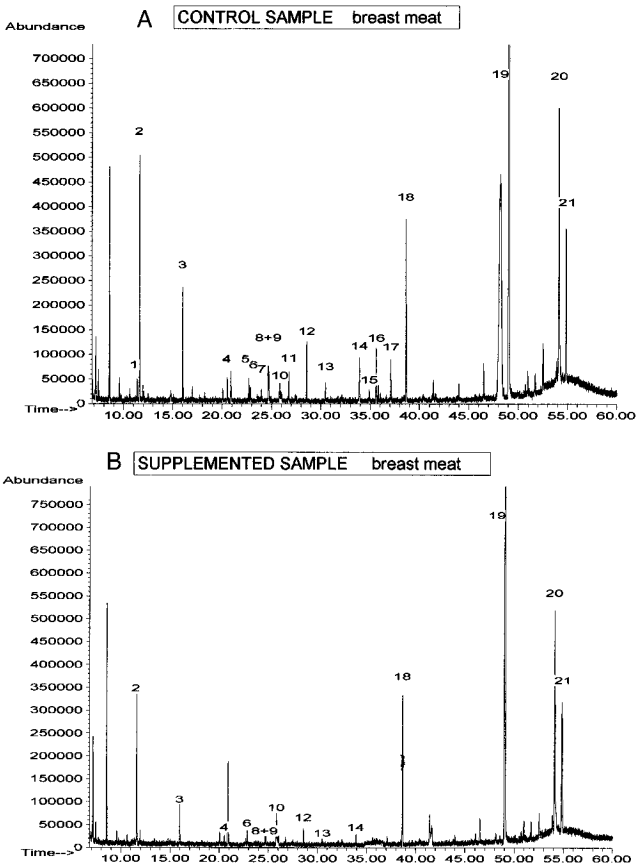

Figure 4. Typical gas chromatograms of a breast meat aroma concentrate of a control sample (A) and a supplemented sample (B). Peak numbering is in accordance with Table 1.

Table 1. Identified Compounds in the Aroma Concentrates of Leg and Breast Meat of Control and Supplemented Samples

peak no.	compound	retention time (min)
1	pentanal	11.3
2	3-hydroxy-2-butanone	11.5
3	hexanal	15.9
4	heptanal	20.5
5	2-heptenal	22.6
6	benzaldehyde	22.7
7	1-octen-3-ol	24.0
8	2-pentylfuran	24.6
9	octanal	24.7
10	benzyl alcohol	25.8
11	2-octenal	26.8
12	nonanal	28.6
13	2-nonenal	30.5
14	2-decenal	33.9
15	*cis*,*trans*-2,4-decadienal	34.9
16	*trans*,*trans*-2,4-decadienal	35.6
17	2-undecenal	37.1
18	tetradecane (internal standard)	38.6
19	hexadecanal	49.0
20	9-octadecenal	53.9
21	octadecanal	54.6

Of the components identified in the present work, aldehydes were found to be present in major quantities in both control and supplemented samples. They are formed due to the oxidation of unsaturated lipids, and especially the lower saturated and unsaturated aldehydes should have a significant contribution to rancidity development during storage of chicken meat (Shahidi, 1986). The most frequently reported aldehydes in meat are C5−C9 *n*-alkanals such as pentanal, hexanal, heptanal, octanal, and nonanal. In the case of saturated aldehydes, the control samples of the leg meat contained the highest concentration, while the supplemented samples of the breast meat contained the lowest amount of saturated aldehydes. This was in accordance with what was found for the determination of the TBA values (Figure 2). In the leg and breast meat of the control samples, there was respectively a 5- and a 3-fold increase in the concentration of the volatile saturated aldehydes, of which hexanal and nonanal were the main representatives, compared to the vitamin E samples (Table 2).

The saturated aldehydes are usually accompanied by the corresponding 2-alkenals. The unsaturated aldehydes detected in chicken meat were 2-heptenal, 2-octenal, 2-nonenal, 2-decenal, and 2-undecenal, all found in appreciable levels in the control samples, while in the supplemented samples they were present in traces or absent. The increase in volatile unsaturated aldehydes in the control meat compared to the supplemented meat was more important. These unsaturated aldehydes and especially the isomeric decadienals have even lower threshold values (Devos et al., 1990) than the saturated aldehydes and should have a considerable contribution to rancidity development. Also in cooked and stored chicken, Dimick and MacNeil (1970) found that unsaturated aldehydes were good indicators of off-flavor development.

Both saturated and unsaturated aldehydes should be considered as the most important contributors to the oxidative rancidity of the meat. Especially hexanal (peak 3), nonanal (peak 12), and 2,4-decadienal (peaks 15 and 16) should have an important impact due to their high concentration and very low threshold value.

The higher aldehydes such as hexadecanal (peak 19), 9-octadecenal (peak 20), and octadecanal (peak 21) were

Table 2. Mean Concentrations of the Volatile Components (n = 3 Samples, Expressed as μg/g of Meat) in Leg and Breast Meat for Control and Supplemented Samples

	leg				breast			
	control		vitamin E		control		vitamin E	
	concn	SD	concn	SD	concn	SD	concn	SD
saturated aldehydes								
pentanal	0.145	0.022	0.032	0.006	0.069	0.030	<0.010	
hexanal	0.959	0.273	0.176	0.019	0.376	0.159	0.102	0.028
heptanal	0.174	0.029	0.035	0.002	0.092	0.037	0.051	0.008
octanal	0.198	0.016	0.043	0.003	0.108	0.048	0.042	0.007
nonanal	0.431	0.038	0.091	0.004	0.216	0.070	0.067	0.014
SUM	1.907		0.376		0.860		0.262	
unsaturated aldehydes								
2-heptenal	0.151	0.025	0.034	0.008	0.069	0.011	<0.010	
2-octenal	0.305	0.010	0.038	0.005	0.113	0.025	<0.010	
2-nonenal	0.115	0.014	0.023	0.007	0.076	0.005	<0.010	
2-decenal	0.214	0.026	0.030	0.001	0.146	0.018	<0.010	
cis,*trans*-2,4-decadienal	0.111	0.036	<0.010		0.057	0.005	<0.010	
trans,*trans*-2,4-decadienal	0.525	0.167	0.037	0.032	0.141	0.046	<0.010	
2-undecenal	0.154	0.047	0.024	0.003	0.125	0.007	<0.010	
SUM	1.574		0.185		0.725		0.000	
higher aldehydes								
hexadecanal	0.438	0.098	0.581	0.134	8.453	3.896	10.357	1.727
9-octadecenal	0.124	0.014	0.061	0.031	1.622	1.444	1.428	0.575
octadecanal	0.180	0.018	0.080	0.029	1.063	1.122	1.267	0.724
SUM	0.742		0.722		11.137		13.051	
ketones								
3-OH-2-butanone	0.554	0.063	0.595	0.178	0.751	0.206	0.527	0.106
alcohols								
1-octen-3-ol	0.116	0.059	0.038	0.016	0.092	0.030	0.014	0.002
benzyl alcohol	0.028	0.006	0.101	0.023	0.191	0.012	0.226	0.094
aromatic aldehydes								
benzaldehyde	0.064	0.012	0.038	0.014	0.080	0.024	0.071	0.013
furans								
2-pentylfuran	0.141	0.052	0.039	0.010	0.072	0.017	0.035	0.002

present in both samples, but these components were considerably higher in breast meat than in leg meat. Because of the low volatility of these high molecular weight compounds, they are considered to have no contribution to off-odor and off-flavor development in meat. Also the difference in higher aldehyde content between the control and supplemented samples was low.

The concentrations of 3-hydroxy-2-butanone (peak 2), a ketone with a buttery odor, and benzaldehyde (peak 6) are not clearly different in control compared to supplemented meat. Otherwise, 1-octen-3-ol (peak 7) and 2-pentylfuran (peak 8) are clearly associated with oxidation and occured more importantly in both leg and breast meat of the control compared to the supplemented samples. The control samples of the leg meat contained the highest concentration, while the supplemented sample of the breast contained the lowest amount. 1-Octen-3-ol is an oxidation product of arachidonic acid and other polyunsaturated fatty acids. It has been frequently reported as a major component of meat volatiles (Mottram, 1991) and has a mushroom-like odor. 2-Pentylfuran is an oxidation product of linoleic acid, but it may also be formed from 2,4-decadienal (Ho et al., 1994). It has a low odor threshold and is reported to have beany, grassy odor characteristics (Maarse, 1991).

CONCLUSIONS

As a conclusion we may state that feeding high levels of α-tocopheryl acetate to chickens was clearly reflected in the α-tocopherol tissue content. Sensory analysis has shown that high vitamin E levels had a beneficial effect on the time-related flavor characteristics by delaying off-flavor formation. This was confirmed by objective measurement of oxidation by determination of TBA values as a function of forced oxidation time and by determination of the volatile off-flavor compounds by gas chromatography−mass spectrometry.

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