ELSEVIER

PII: S0308-8146(96)00125-2

Food Chemistry, Vol. 56, No. 4, pp. 347–354, 1996

0308-8146/96 $15.00 + .00

RAPID COMMUNICATION

The antioxidative activity of RRR-α-tocopherol vs RRR-δ-tocopherol in combination with ascorbyl palmitate in cooked, minced turkey

Lone Bruun-Jensen,[a] Ib M. Skovgaard[b] & Leif H. Skibsted[a]*

[a]*Department of Dairy and Food Science, Section of Food Chemistry, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark*
[b]*Department of Mathematics and Physics, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*

(Received 3 November 1995; revised version received and accepted 10 January 1996)

RRR-α-tocopherol and RRR-δ-tocopherol (100 or 200 ppm) had similar and significantly better antioxidative effects compared to ascorbyl palmitate (200 ppm) in turkey meat balls during storage at 5°C for up to 9 days, as determined by measurement of thiobarbituric acid reactive substances (TBARS) and by static head space detection of hexanal. The antioxidative effect of α-tocopherol at 100 ppm was, measured as a reduction of head space hexanal, significantly enhanced by ascorbyl palmitate (200 ppm), resulting in a further 50% reduction compared to δ-tocopherol. At the 200-ppm level, only the antioxidative activity of α-tocopherol was enhanced significantly by ascorbyl palmitate (200 ppm), in contrast to the effect on the antioxidative activity of δ-tocopherol (200 ppm). The antioxidative activity obtained by α-tocopherol at the 100-ppm level in combination with ascorbyl palmitate (200 ppm) was similar to the effect obtained by α-tocopherol at the 200-ppm level in combination with ascorbyl palmitate (200 ppm) measured as head space hexanal. Ascorbyl palmitate was in all storage experiments depleted in the product at a rate which appeared to be independent of the presence of tocopherols. Of the tocopherol homologues, α-tocopherol was most affected by the presence of ascorbyl palmitate, and the concentration of α-tocopherol was found to decrease during storage in the absence of ascorbyl palmitate, in contrast to when ascorbyl palmitate was present. A similar effect of ascorbyl palmitate was less evident for δ-tocopherol and for γ-tocopherol in a natural mixture of the tocopherol homologues added to turkey meat balls. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In order to prevent oxidative deterioration in food lipid vitamin E, a mixture of α-, β-, γ- and δ-tocopherol has been widely used as a chain-breaking antioxidant. As tocopherols may be extracted from natural sources, they have received increasing interest as natural alternatives to synthetic phenolic antioxidants like BHT and BHA. While α-tocopherol has been found to be the most efficient of the four tocopherol homologues *in vivo* (Burton & Ingold, 1981; Burton *et al.*, 1983), the antioxidative activity *in vitro* is a matter of continuing discussion, and results obtained in simple model systems have often been extrapolated uncritically to the more complex matrices found in food products, neglecting factors such as proper incorporation of the antioxidant, and decomposition during processing and heat treatment of the product. Moreover, conflicting results obtained for the tocopherol homologues in model systems and in a variety of food products may also be due to the different analytical methods used for assessment of lipid oxidation.

Processed meat is one important type of product for which testing of antioxidative activity should rely on storage experiments rather than on model experiments. Inhibition of warmed-over flavour (WOF), the rapidly developed off-flavour of reheated cooked meat (Love, 1988), may be used for direct evaluation of antioxidants or may be replaced by analytical methods. For the tocopherol homologues, such studies are few (Mielche & Bertelsen, 1994), and the purpose of the present

*To whom correspondence should be addressed.

Table 1. Combinations of antioxidants added to cooked turkey meat balls

Experiment	Batch	Antioxidants	Concentrations[a](ppm)
I	1	RRR-α-tocopherol	100
	2	RRR-δ-tocopherol	100
	3	Ascorbyl palmitate	200
	4	RRR-α-tocopherol	100
		Ascorbyl palmitate	200
	5	RRR-δ-tocopherol	100
		Ascorbyl palmitte	200
	6	None	
II	1	RRR-α-tocopherol	200
	2	RRR-δ-tocopherol	200
	3	Ascorbyl palmitate	200
	4	RRR-α-tocopherol	200
		Ascorbyl palmitate	200
	5	RRR-δ-tocopherol	200
		Ascorbyl palmitate	200
	6	None	
III	1	Natural tocopherol mixture	50
	2	Ascorbyl palmitate	200
	3	Natural tocopherol mixture	50
		Ascorbyl palmitate	200
	4	None	

[a]Concentrations are based on the weight of meat.

study was to compare the antioxidative activity of α-tocopherol with δ-tocopherol in a cooked, minced turkey meat product. A combination of two analytical methods (determination of thiobarbituric acid reactive substances and head space hexanal) is used, and the interaction between the tocopherols and another antioxidant, ascorbyl palmitate, is further studied in the product during chill storage.

MATERIALS AND METHODS

Product, packaging and storage

Fresh, deboned turkey thighs (mainly *Musculus iliotibialis*) were obtained from a local butcher the day before each experiment. All skin, connective tissue and subcutaneous fat were removed as the thighs were cut in pieces determined by the anatomical structure of the meat and mixed in order to obtain homogeneous batches. Subsequently, the meat was packed in vacuum and stored at 5°C overnight. Analysis of the initial content of vitamin E in the turkey meat (by HPLC as described below) showed only very low levels of tocopherols (0.8 $\mu g\,g^{-1}$ turkey meat of α-tocopherol and less than 0.1 $\mu g\,g^{-1}$ turkey meat of β-, γ- and δ-tocopherol). The homogeneous batches were ground through a 3 mm plate in a mincing machine Model A 90693 B (Bankeryds Maskine AB, Bankeryd, Sweden), and salt (1.5%, w/w) and black pepper (0.02%, w/w) were added. Three similar experiments were carried out with different additives and concentrations of additives added as outlined in Table 1. Commercially available antioxidant emulsions were used, and analysed by HPLC prior to addition as described below (cf. Table 2): GRINDOX™ TS-G 376 with 2R,4′R,8′R-α-tocopherol (20.3% α-, <0.1% β-, 0.4% γ- and <0.1% δ-tocopherol); GRINDOX™ TS-G 377 with 2R,4′R,8′R-δ-tocopherol (0.2% α-, <0.1% β-, 0.8% γ- and 19.4% δ-tocopherol); GRINDOX™ 1032 with 20% natural tocopherols (as extracted from soybean oil, actual content see Table 3); and GRINDOX™ ascorbyl palmitate

Table 2. Antioxidant concentration (ppm) in cooked turkey meat balls with added RRR-α-tocopherol or RRR-δ-tocopherol or ascorbyl palmitate, or added RRR-α-tocopherol or RRR-δ-tocopherol together with ascorbyl palmitate[a]

Time (days)	α-Tocopherol	δ-Tocopherol	Ascorbyl palmitate	α-Tocopherol	Ascorbyl palmitate	δ-Tocopherol	Ascorbyl palmitate
(Added)	200	200	200	200	200	200	200
0	99	95	35	99	39	98	46
1	100	103	21	113	21	92	36
2	99	105	16	96	16	95	25
6	80	92	4	125	3	97	7
9	n.m.[b]	108	<3	114	<3	93	3

[a]Experiment II of Table 1, lipid oxidation analysis may be found in Fig. 1.
[b]Not measured.

Table 3. Antioxidant concentrations (ppm) in cooked turkey meat balls with added mixture of natural tocopherols alone or together with ascorbyl palmitate[a]

Time (days)	α-Tocopherol	ß-Tocopherol	γ-Tocopherol	δ-Tocopherol	Ascorbyl palmitate
(Added)	n.m.[b]	n.m.[b]	n.m.[b]	n.m.[b]	0
0	4.2	0.6	23	9.5	
1	0.8	0.4	16	7.8	
2	0.1	0.4	13	8.0	
6	0.7	0.5	12	7.5	
(Added)	n.m.[b]	n.m.[b]	n.m.[b]	n.m.[b]	200
0	4.1	0.5	19	7.4	57
1	2.5	0.4	15	6.2	28
2	3.2	0.5	17	7.3	19
6	2.4	0.3	13	5.5	14

[a]Experiment III of Table 1, batch 1 and batch 3.
[b]Not measured.

fine with a minimum purity of 98%. The GRINDOX™ mixtures containing tocopherols were added to emulsifiers as carriers to obtain water-dispersible formulations. The antioxidants were added to the minced turkey meat via a pre-dispersion, as described previously (Bruun-Jensen *et al.*, 1994) in a fixed quantity of water containing salt and pepper (200 g dispersion per 1 kg total turkey meat) and mixed in a Stephanmixer UMC-5 (Hamlen, Germany) at 600 rpm for 1.5 min. A control batch without any antioxidants added was prepared using the same recipe but with water added instead of antioxidant emulsion. All antioxidant formulations were obtained from Danisco Ingredients (Brabrand, Denmark).

Small meat balls (approximately 2 g each) were made by grinding the meat through a 15 mm plate on an Esna ball machine (Nørre Åby, Denmark) and cooked in 90°C water for 3 min, subsequently cooled on pre-cooled aluminium trays in one layer in air for 10 min, and then packed using a Röschermatic VM-19/S packaging machine (Röscherwerke Osnabrück, Germany) and high barrier foil with a very low O_2 transmission rate (< 2 cm^3/m^2/24 h/atm). The packages were stored in a dark storage room at 5 ± 0.5°C for up to 9 days.

Chemical analysis

All chemicals used were of analytical grade.

2-Thiobarbituric acid reactive substances (TBARS)
2-Thiobarbituric acid reactive substances were determined by the extraction method according to Vyncke (1975). Results were expressed as equivalent μmol of malondialdehyde per kg meat according to a standard curve in the concentration range of 1–14 μM tetraethoxypropane (Merck, Darmstadt, Germany). TBARS were measured on turkey meat balls from two packages from each batch on days 0, 1, 2, 3, 6 and 9, and all values reported are thus the mean of the two determinations.

Hexanal content
The hexanal content was determined by a GC static head space method according to Shahidi & Pegg (1994) with minor modifications. Two grammes of homogenized turkey meat ball samples were placed in 5 ml glass vials sealed with Teflon-lined septa. Prior to injection, the vials were thermostatted on a heating block (90°C) for 45 min. For the GC separation, a Chrompack CP 9001 chromatograph (Middelburg, The Netherlands) was used: injection, 1 ml manually by syringe; split 1:7; column, fused silica CPSIL 88 (25 m; 0.32 mm ID, 0.2-μm film, Chrompack, Middelburg, The Netherlands); carrier gas, helium, 10 ml/min; temperature programming, 50°C (5 min) to 115°C (1 min) at 10°C/min followed by a cleaning step, 200°C (10 min); injector temperature, 180°C; FID temperature, 230°C. 2-Heptanone was used as the internal standard in an approximate concentration of 100 ppm w/w. Hexanal and 2-heptanone were identified by comparing retention times of volatiles from meat samples with retention times of standards. The hexanal content of the samples was calculated as the peak area relative to the area of the internal standard peak and expressed in mg hexanal per kg meat product. Development of hexanal was measured on two packages of turkey meat balls from each batch on days 0, 1, 2, 3, 6 and 9, and all values reported are thus the means of two determinations.

Recovery of antioxidants
For determination of the concentration of the tocopherols in the turkey meat balls, the lipid phase was extracted from each batch of the turkey meat product and subsequently analysed by HPLC on days 0, 1, 2, 6 and 9, as described below. Only one extraction was made per batch per day and injected twice on the HPLC, and the results reported are means of two such injections of a single extraction. Fifty grammes of minced turkey meat balls were mixed with 30 ml of water and 80 ml concentrated HCl. The mixture was homogenized by stirring with Teflon-coated magnetic stirring bars, and then heated at 100°C for 5 min. The mixture was left to cool and subsequently 100 ml ethanol, 200 ml diethyl ether and 200 ml petroleum ether (b.p. 60–80°C) were added. The mixture was stirred for 2 min and protected from light. The phases were left to separate under N_2, and the upper phase was transferred to a round-bottomed glass flask. The extraction procedure was then repeated on the lower phase with 150 ml

diethyl ether and 150 ml petroleum ether. The combined extracts were transferred to a rotary evaporator, and solvents were evaporated using a water bath (40°C). The residual, i.e. the lipid phase, was transferred to a 50 ml volumetric flask and diluted to volume with heptane, the resulting solution was degassed and constantly flushed with N_2 to exclude O_2. The solution was filtered through a 0.45-μm filter prior to injection on a Nucleosil Si 50 column (5 μm, 250×4.6 mm ID, Macherey-Nagel, Düren, Germany) fitted to a Waters Model 6000 Pump (Milford, Mississippi, USA) with a Hewlett-Packard Model 1050 Autosampler (Geneva, Switzerland), a Merck-Hitachi Model F 1000 Fluorescence Spectrophotometer (excitation at $\lambda = 290$ nm, and emission at $\lambda = 330$ nm) (Darmstadt, Germany) and a Shimadzu C-R5A Chromatopac integrator (Kyoto, Japan). Mobile phase was 0.005% 2-propanol in heptane with a flow rate of 2 ml/min, and the injection volume was 10 μl.

For determination of the concentration of ascorbyl palmitate in the turkey meat product, an ethanolic extract of homogenized turkey meat product was analysed by HPLC. Five grammes of minced turkey meat product from each batch was sonicated for 15 min with 10 ml of ethanol added to 0.3% ascorbic acid for stability reasons. The ethanolic extract was filtered through a 0.45-μm filter prior to injection on a Spherisorb ODS2 S5 column (120×4.6 mm ID, Phase Separations, Queensferry, UK) fitted to a Hewlett-Packard HP 1090 system with an HP 85B Personal Computer, a HP Thinkjet and an HP 9153B Harddisk. Quantification was done by UV detection at $\lambda = 244$ nm. The mobile phase was 82.5% CH_3OH and 17.5% 20 mM NaH_2PO_4 (pH = 2.15) with a flow rate of 1.0 ml/min and the injection volume was 20 μl. Standard addition experiments showed a recovery of 66 ± 5% on the overall sample preparation of ascorbyl palmitate.

Statistical analysis

The conclusions presented were based on the two chemical analyses measured on turkey meat product from two packages from each batch on days 0, 1, 2, 3, 6 and 9. For recovery of antioxidants, only a single package of turkey meat product of selected batches was measured per day. The concentrations of the respective antioxidants were determined on days 0, 1, 2, 6 and 9.

To investigate the effects of experimental factors (storage time and presence of antioxidants), the logarithm of the response variables (log hexanal content and log TBARS) were analysed by analyses of variance. The factors included in the analyses were amount of ascorbyl palmitate, amount of tocopherol, tocopherol homologue (α- or δ-), storage time and block (batch number). Non-significant higher-order interactions as well as slightly significant, but small, effects were removed from the model before conclusions on the more substantial effects were drawn. Inspection of significant treatment effects was done by comparison of adjusted means ('least-squares means' in the procedure GLM in SAS) (SAS Institute, 1990).

RESULTS

Evaluation of the relative antioxidative activity of α-tocopherol and δ-tocopherol, and their interaction with ascorbyl palmitate in cooked turkey meat balls, was based on a determination of lipid oxidation products by two analytical methods during chill storage, and by determination of the concentration of added antioxidants during storage. The experiment was divided into three blocks (cf. Table 1). The antioxidative activity of a low and a high concentration of the two tocopherol homologues (100 or 200 ppm) was evaluated in two separated blocks. In a third block, the antioxidative activity of a mixture of natural tocopherols (50 ppm) was evaluated. The interaction with ascorbyl palmitate (200 ppm) was investigated in all three blocks.

Effect of antioxidants

Lipid oxidation in cooked turkey meat balls during storage was quantified by determination of TBARS and of hexanal. Both the TBARS values and the hexanal head space concentration were significantly reduced by adding either α- or δ-tocopherol individual ($P < 0.0001$). Without ascorbyl palmitate added, the antioxidative activity was found to be the same for the two tocopherol homologues, and ascorbyl palmitate alone was less efficient than each of the tocopherol homologues.

Ascorbyl palmitate interacted with both α- and δ-tocopherol in inhibition of lipid oxidation ($P < 0.0001$). In combination with ascorbyl palmitate, inhibition of lipid oxidation, measured as reduction of TBARS values and hexanal in head space, was enhanced for both α- and δ-tocopherols added in low concentration (100 ppm), as may be seen from Fig. 1. Lipid oxidation was most efficiently inhibited by addition of α-tocopherol in combination with ascorbyl palmitate and, based on adjusted means, it was found that, in combination with ascorbyl palmitate, α-tocopherol gave a further 50% reduction of hexanal in head space compared to δ-tocopherol.

As for the low concentration of α- and δ-tocopherol (100 ppm), the reduction of lipid oxidation by α- and δ-tocopherol added individually was not different to the high concentration (200 ppm). The effect of added ascorbyl palmitate was less pronounced at the high concentration compared to the low concentration. Combination of ascorbyl palmitate with α-tocopherol gave a further reduction in lipid oxidation of approximately 44% compared to δ-tocopherol. The experimental TBARS values and hexanal in head space concentrations from the storage experiment for cooked turkey meat balls with antioxidants added in high concentrations may be found in Fig. 2.

For the cooked, minced turkey meat product investigated, the most efficient inhibition of lipid oxidation, measured both as TBARS and as head space hexanal, was found for α-tocopherol in combination with ascorbyl palmitate. However, the antioxidative activity obtained by α-tocopherol at the 100-ppm level in

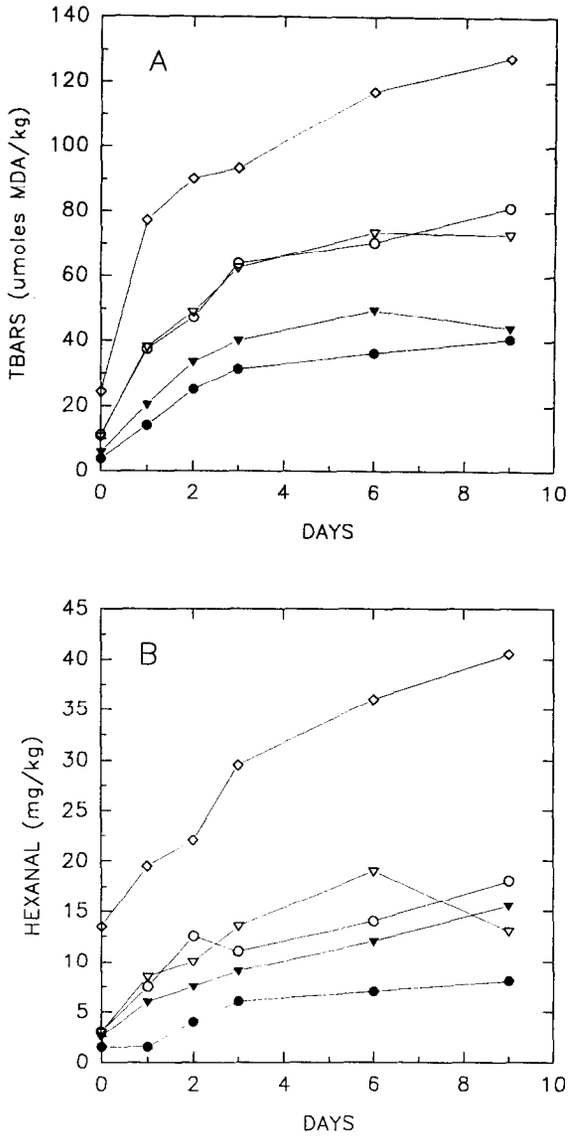

Fig. 1. The effect of RRR-α-tocopherol and RRR-δ-tocopherol added individually or in combination with ascorbyl palmitate (AP) to cooked turkey meat balls based on measurement of (A) TBARS and (B) hexanal in head space. 100 ppm α-tocopherol (○), 100 ppm δ-tocopherol (▽), 100 ppm α-tocopherol + 200 ppm AP (●), 100 ppm δ-tocopherol + 200 ppm AP (▼), and control batch without antioxidants added (◇)

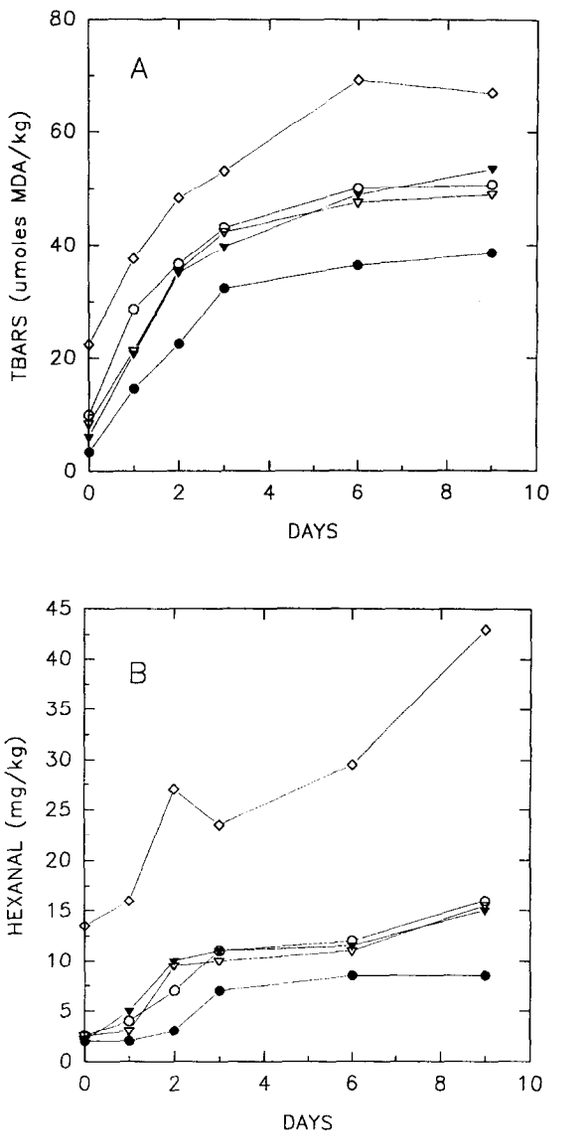

Fig. 2. The effect of RRR-α-tocopherol and RRR-δ-tocopherol added individually or in combination with ascorbyl palmitate (AP) to cooked turkey meat balls based on measurement of (A) TBARS and (B) hexanal in head space. 200 ppm α-tocopherol (○), 200 ppm δ-tocopherol (▽), 200 ppm α-tocopherol + 200 ppm AP (●), 200 ppm δ-tocopherol + 200 ppm AP (▼), and control batch without antioxidants added (◇).

combination with ascorbyl palmitate (200 ppm) was similar to the effect obtained by α-tocopherol at the 200 ppm level in combination with ascorbyl palmitate (200 ppm) measured as head space hexanal. The inhibition of ascorbyl palmitate added individually to the turkey meat product on lipid oxidation was significant ($P < 0.0001$), but smaller than the effect obtained by either α- or δ-tocopherol. Based on adjusted means, ascorbyl palmitate was found to reduce lipid oxidation, measured as head space hexanal, to 80% of the lipid oxidation in the control batch with no antioxidants added, while α- or δ-tocopherol added individually in low concentration reduced head space hexanal to approximately 40%. In comparison, α- or δ-tocopherol added individually in high concentration reduced lipid oxidation to approximately 30%.

Addition of the mixture of natural tocopherols (50 ppm) to the cooked turkey meat balls was likewise found to reduce lipid oxidation. Based on adjusted means, TBARS values were reduced by the mixture of natural tocopherols to 58% of the values obtained in the control batch with no antioxidants added. This reduction was similar to the reduction in TBARS values obtained by addition of 100 ppm of either α- or δ-tocopherol. In combination with ascorbyl palmitate (200 ppm), the TBARS values were reduced to 36% of the values obtained in the control batch by the mixture of natural tocopherols (data not shown).

Fate of antioxidants in the meat product

The concentration of each of the added antioxidants in cooked turkey meat balls was followed during the chill storage and the results from Experiments II and III of Table 1 are reported in Tables 2 and 3, respectively. Comparable results were obtained in Experiment I. The concentration of each of the antioxidants was, prior to storage (day 0), significantly lower than the added amount, indicating: (i) that during the processing, including heating of the product, antioxidants were consumed; and/or (ii) that the recovery during extraction was incomplete. The ensuing discussion will accordingly focus on changes relative to day 0 rather than on absolute concentrations. Ascorbyl palmitate was, in all storage experiments, depleted in the product at a rate which appeared to be independent of the presence of tocopherols. Of the tocopherol homologues, α-tocopherol was most affected by the presence of ascorbyl palmitate. The concentration of α-tocopherol was found not to decrease during storage, when ascorbyl palmitate was present, in contrast to when α-tocopherol alone was added. This effect of ascorbyl palmitate was less evident for δ-tocopherol and for γ-tocopherol.

DISCUSSION

Early investigations of lipid oxidation and its inhibition by antioxidants used mainly bulk lipid systems such as oils and lard. Results obtained by Chipault (1962) in this type of system at 100°C seemed to indicate that α-tocopherol had a lower antioxidative effect *in vitro* than other natural antioxidants and also than synthetic phenolic compounds. This apparent difference between the antioxidative effect of α-tocopherol under different types of conditions, i.e. that α-tocopherol is the most potent antioxidant *in vivo* and the least effective *in vitro*, has been accepted as standard knowledge in textbooks (Belitz & Grosch, 1987). However, more recent investigations in both homogeneous and heterogeneous model systems have clearly shown that α-tocopherol is also the most effective chain-breaking phenolic antioxidant *in vitro* (Burton *et al.*, 1980; Burton & Ingold, 1981). Moreover, both calculation of electron distribution in the tocopherol homologues in relation to hydrogen abstraction and results from kinetic investigations confirm that, in food systems, α-tocopherol is expected to be a better antioxidant than the other tocopherol homologues (Burton & Ingold, 1981; Mukai *et al.*, 1986; Niki *et al.*, 1986*b*; Pryor *et al.*, 1988).

For meat and meat products, both an antioxidative effect (Benedict *et al.*, 1975; Whang *et al.*, 1986; Shahidi & Rubin, 1987) and a pro-oxidative effect have been reported for α-tocopherol (St. Angelo *et al.*, 1988; Decker & Crum, 1993). Likewise, both an antioxidative (Roozen, 1987; Bruun-Jensen *et al.*, 1994, 1995) and a pro-oxidative activity of ascorbyl palmitate has been demonstrated in different products (St. Angelo *et al.*, 1988). The balance between pro- and antioxidative effects seems to depend on the concentration of additive and also on the processing of the meat and the storage conditions of the product including access to O_2. Moreover, different analytical methods used to follow lipid oxidation during storage of meat products may refer to different stages of oxidation. Evaluation of the antioxidative activity of the tocopherols and ascorbyl palmitate individually and in combination in a given product should, accordingly, include storage experiments with different concentrations of the antioxidants and use of a combination of analytical methods.

In the present study, the measurement of lipid oxidation in cooked turkey meat balls during storage was based on two analytical methods in combination with a study of the recovery of the respective antioxidants added to the product. The study of the recovery of the added antioxidants should provide an evaluation of any synergistic effect in the actual product. TBARS values and head space hexanal were selected as analytical methods because both TBARS (Mielche, 1995; Nolan *et al.*, 1989; Wu & Sheldon, 1988) and head space hexanal have been found to correlate with sensory scores for warmed-over flavour (Spanier *et al.*, 1992). α-Tocopherol was found to provide the best protection for the product of the antioxidants tested. At both concentration levels used for α- and δ-tocopherol, the two homologues yield comparable protection when measured both as TBARS and head space hexanal. An increase from 100 to 200 ppm only slightly increased the protection for any of the two tocopherols, indicating that 200 ppm is above a linear dose–response region for these antioxidants. Moreover, a combination of ascorbyl palmitate with the tocopherols gave different results for the two homologues. For the high concentration of the tocopherols, ascorbyl palmitate enhanced only the effect of α-tocopherol as evident from both the head space hexanal and the TBARS. This observation is of interest in relation to the different reactivities of the two tocopherol homologues.

Based on ESR spectroscopy of a chicken fat liquid fraction, α-tocopherol has been demonstrated to be the tocopherol homologue consumed first, as the α-tocopherol reacted most rapidly with free lipid radicals compared to β-, γ-, and δ-tocopherol (Lambelet & Löliger, 1984), in agreement with the relative stabilities of the different tocopheroxyl radicals. This observation has been rationalized on the basis of a high resonance stability of the α-tocopheroxyl radical compared to the δ-tocopheroxyl radical (Burton *et al.*, 1985). In relation to our observations, this would imply that α-tocopherol, as the most reactive of the tocopherols, is consumed faster and also acts as a better antioxidant. The recovery studies (Tables 2 and 3) are important in this context, as they show that α-tocopherol is protected during storage to a larger degree in the presence of ascorbyl palmitate than δ-tocopherol, both in meat with only one tocopherol homologue added (Table 2) and in the meat with a natural mixture of tocopherols added

(Table 3). We feel, accordingly, that it is safe to conclude that, in the present turkey meat product, α-tocopherol is the better antioxidant, in contrast to what has been concluded from studies of O_2 consumption and antioxidant recovery in homogeneous and heterogeneous model systems (Niki *et al.*, 1986*a,b*). A possible explanation for this difference is that O_2 may be depleted inside the product during storage and the antioxidant properties of α-tocopherol, at least to a certain degree, depending on the O_2 level.

The processing of the meat product, including heat treatment, is an important factor in determining the actual concentration of the antioxidants at the beginning of the storage period. In the present study, the initial concentrations of tocopherols and ascorbyl palmitate in turkey meat were found to be significantly lower than calculated from the amount of tocopherols added, and the consumption of antioxidants is believed to yield a protection during processing, as can be seen from the lower starting values for both hexanal and TBARS compared to products with no antioxidants added.

In homogeneous model systems, a regeneration of α-tocopherol at the expense of ascorbic acid (Packer *et al.*, 1979) or ascorbyl palmitate (Lambelet *et al.*, 1985) has been demonstrated quantitatively by direct detection of the respective antioxidant radicals. For the present meat product such a regeneration of α-tocopherol could not be confirmed quantitatively as the measured concentration of α-tocopherol during storage was found to be relatively independent of the presence of ascorbyl palmitate. However, in meat products, the interaction between tocopherols and ascorbyl palmitate is likely to be different from that demonstrated in model systems. In a previous study based on the same turkey meat product (Bruun-Jensen *et al.*, 1995), the combined use of tocopherols and ascorbyl palmitate was found to optimize the oxidative protection as a result of a so-called indirect synergism. The indirect synergism appeared from an effect of tocopherols on the maximum level of TBARS, while ascorbyl palmitate affected the rate at which the maximum level of TBARS was reached. In meat products with tocopherols and ascorbyl palmitate added, the combined effect of the antioxidants may thus be concluded to be the result of a shielding of (especially) α-tocopherol by ascorbyl palmitate rather than a direct regeneration.

ACKNOWLEDGEMENTS

The experimental part of the study has been carried out at Danisco Ingredients, Brabrand, Denmark, and is part of a co-operation project between the KVL Centre for Food Research and Danisco Ingredients, sponsored by the Danish Ministry of Education and Research, the Danish Ministry of Agriculture, and the Danish Ministry of Industry as part of the FØTEK Programme. The assistance given by Product Manager Jens Birk Lauridsen, Senior Application Manager Lars Høegh, and all involved co-workers in the laboratories at Danisco Ingredients is gratefully acknowledged.

REFERENCES

Benedict, R., Strange, E. D. & Swift, C. E. (1975). Effect of lipid autoxidation on the stability of meat during storage. *J. Agric. Food Chem.*, **23**, 167–173.

Bruun-Jensen, L., Skovgaard, I. M., Madsen, E. A., Skibsted, L. H. & Bertelsen, G. (1996). The combined effect of tocopherols, L-ascorbyl palmitate, and L-ascorbic acid on the development of warmed-over flavour in cooked, minced turkey. *Food Chem.*, **55**(1), 41–47.

Bruun-Jensen, L., Skovgaard, I. M., Skibsted, L. H. & Bertelsen, G. (1994). Antioxidant synergism between tocopherols and ascorbyl palmitate in cooked, minced turkey. *Z. Lebensm. Unters. Forsch.*, **199**, 210–213.

Burton, G. W., Doba, T., Gabe, E. J., Hughes, L., Lee, F. L., Prasad, L. & Ingold, K. U. (1985). Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *J. Am. Chem. Soc.*, **107**, 7053–7065.

Burton, G. W. & Ingold, K. U. (1981). Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *J. Am. Chem. Soc.*, **103**, 6472–6477.

Burton, G. W., Joyce, A. & Ingold, K. U. (1983). Is vitamin E the only lipid-soluble chain-breaking antioxidant in human blood plasma and erythrocyte membranes?. *Arch. Biochem. Biophys.*, **221**(1), 281–290.

Burton, G. W., Le Page, Y., Gabe, E. J. & Ingold, K. U. (1980). Antioxidant activity of vitamin E and related phenols. Importance of stereoelectronic factors. *J. Am. Chem. Soc.*, **102**, 7791–7792.

Belitz, H. D. & Grosch, W. (eds) (1987). *Food Chemistry*. Springer, Berlin.

Chipault, J. R. (1962). Antioxidants for use in foods. In *Autoxidation and antioxidants*, Vol. 2, ed. W. O. Lundberg. Interscience, New York, pp. 477–542.

Decker, E. A. & Crum, A. D. (1993). Antioxidative activity of carnosine in cooked ground pork. *Meat Sci.*, **34**, 245–253.

Lambelet, P. & Löliger, J. (1984). The fate of antioxidant radicals during lipid autoxidation. I. The tocopheroxyl radicals. *Chem. Phys. Lipids*, **35**, 185–198.

Lambelet, P., Saucy, F. & Löliger, J. (1985). Chemical evidence for interactions between vitamins E and C. *Experientia*, **41**, 1384–1388.

Love, J. (1988). Sensory analysis of warmed-over flavor in meat. *Food Technol.*, **42**, 140–143.

Mielche, M. M. (1995). Development of warmed-over flavour in ground turkey, chicken and pork meat during chill storage. A model of the effects of heating temperature and storage time. *Z. Lebensm. Unters. Forsch.*, **200**, 186–189.

Mielche, M. M. & Bertelsen, G. (1994). Approaches to the prevention of warmed-over flavour. *Trends Food Sci. Technol.*, **5**, 322–327.

Mukai, K., Watanabe, Y., Uemoto, Y. & Ishizu, K. (1986). Stopped-flow investigation of antioxidative activity of tocopherols. *Bull. Chem. Soc. Jpn*, **59**, 3113–3116.

Niki, E., Takahashi, M. & Komuro, E. (1986*a*). Antioxidant activity of vitamin E in liposomal membranes. *Chem. Lett.*, **9**, 1573–6.

Niki, E., Tsuchiya, J., Yoshikawa, Y., Yamamoto, Y. & Kamiya, Y. (1986*b*). Oxidation of lipids. XIII. Antioxidative activities of α-, β-, γ-, and δ-tocopherols. *Bull. Chem. Soc. Jpn.*, **59**, 497–501

Nolan, N. L., Bowers, J. A. & Korpf, D. H. (1989). Lipid oxidation and sensory analysis of cooked pork and turkey stored under modified atmospheres. *J. Food Sci.*, **54**, 846–849.

Packer, J. E., Slater, T. F. & Willson, R. L. (1979). Direct observation of the free radical interaction between vitamin E and vitamin C. *Nature*, **278**, 737–738.

Pryor, W. A., Strickland, T. & Church, D. F. (1988). Comparison of the efficiency of several natural and synthetic antioxidants in aqueous sodium dodecyl sulfate micelle solutions. *J. Am. Chem. Soc.*, **110**, 2224–2229.

Roozen, J. P. (1987). Effects of types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavor. *Food. Chem.*, **24**, 167–185.

SAS Institute Inc. (1990). *SAS/STAT Users' Guide, Version 6*, 4th edn. Cary, North Carolina, USA.

Shahidi, F. & Pegg, R. B. (1994). Hexanal as an indicator of meat flavor deterioration. *J. Food Lipids*, **1**, 177–186.

Shahidi, F. & Rubin, L. J. (1987). Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. *Food Chem.*, **23**, 151–157.

Spanier, A. M., St. Angelo, A. J. & Shaffer, G. P. (1992). Correlation of sensory, instrumental and chemical attributes of beef as influenced by meat structure and oxygen exclusion. *J. Food Sci.*, **57**, 10–15.

St. Angelo, A. J., Vercellotti, J. R., Dupuy, H. P. & Spanier, A. M. (1988). Assessment of beef flavor quality: A multidisciplinary approach. *Food Technol.*, **42**(6), 133–138.

Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombrus* L.). *Fette Seifen Anstrichm.*, **77**, 239–240.

Whang, K., Aberle, E. D., Judge, M. D. & Peng, I. C. (1986). Antioxidative activity of α-tocopherol in cooked and uncooked ground pork. *Meat Sci.*, **17**, 235–249.

Wu, T. C. & Sheldon, B. W. (1988). Flavor components and factors associated with the development of off-flavors in cooked turkey rolls. *J. Food Sci.*, **53**, 49–54.