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The combined effect of tocopherols, L-ascorbyl palmitate and L-ascorbic acid on the development of warmed-over flavour in cooked, minced turkey

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The combined effect of a natural mixture of tocopherols (extracted from soybean oil), L-ascorbyl palmitate and L-ascorbic acid on oxidation of cooked, minced turkey meat, measured as 2-thiobarbituric acid reactive substances (TBARS) after reheating, was studied for three concentrations of each additive in a total of 19 combinations plus two control batches, each at two different oxygen pressures (21 and 1% O_2), during 9 days of chilled storage (5°C), and compared with an accelerated oxygen-bomb test at 90°C. For initial screening of antioxidative activity, the latter test has been found to be a valuable analytical tool. The effect of the additives from the storage experiment could be measured by two parameters; (i) M, the maximal level of TBARS, and (ii) r, a first-order rate constant for development of TBARS. Tocopherols reduced M most significantly, L-ascorbyl palmitate to a lesser degree, and L-ascorbic acid increased M, in effect acting as a prooxidant. For 21% O_2 packaging, the effect on M of the three additives and their concentrations was multiplicative and could be quantified by a protection factor, $P_{(i\cdot x,j\cdot y,k\cdot z)}$, obtained by multiplication of the relative protection obtained by each additive at the lowest concentration used. In contrast to M, r was reduced more by L-ascorbyl palmitate than by tocopherols, while L-ascorbic acid had only a small effect on r. The combined use of tocopherols and L-ascorbyl palmitate in cooked, minced turkey meat products, optimises oxidative protection as a result of indirect synergism, i.e. tocopherols reduced mainly the maximum level of oxidation, while L-ascorbyl palmitate reduced the rate at which the maximum level of oxidation is approached.

INTRODUCTION

Warmed-over flavour (WOF), the off-flavours developed in reheated cooked meat within a few days of chill storage, often limits consumer acceptance of precooked meat products (Love, 1988). Membranal phospholipids are rather susceptible to oxidation, and membrane damage during grinding or cooking initiates the formation of the secondary lipid oxidation products responsible for the off-flavours characteristic of WOF (Igene & Pearson, 1979). Both synthetic and natural antioxidants have been shown to delay the development of WOF in meat products but, due to pressure from consumers to reduce the amount of synthetic additives in foods, natural antioxidants are attracting increasing attention.

The autoxidation of membranal phospholipids may be delayed by natural antioxidants, but the effect achieved is closely related to the concentration of antioxidants. Depending on the nature of the additive used, a low concentration may either have an antioxidative or a prooxidative effect. L-ascorbic acid has a prooxidative effect in meat products in low concentrations ($\leqslant$200 ppm) (St. Angelo *et al.*, 1988), whereas both L-ascorbyl palmitate and tocopherols show antioxidative effects at the same concentrations (Bruun-Jensen *et al.*, 1994). Thus, the prevention of WOF in meat products, depends to a large extent on choosing the proper additives in the optimal concentrations, and increased protection is often achieved by using combinations of antioxidants.

In an attempt to develop a mathematical model for the interaction between tocopherols and L-ascorbyl palmitate, which are often used in combination as antioxidants, we have studied cooked, minced turkey, which is very susceptible to the development of WOF upon

reheating. A natural mixture of tocopherols (extracted from soybean oil) was combined with L-ascorbyl palmitate in turkey meat balls, together with L-ascorbic acid as a potential prooxidant, in a chill storage experiment at 21% O_2 or 1% O_2 packaging. For both storage conditions, the development of WOF was measured as 2-thiobarbituric acid reactive substances (TBARS). The effect of the additives on the oxidative stability of the product was further characterised by an accelerated O_2 bomb method.

MATERIALS AND METHODS

Product, packaging and storage

Fresh, deboned turkey thighs (mainly *Musculus iliotibialis*), were obtained from Harboe Farm A/S, Skælskør, Denmark, within 4 h of slaughter. All skin, connective tissue and subcutaneous fat were removed from the thighs (then weighing approximately 500 g each) before they were vacuum-packed and stored at 5°C until the following day. The thighs were cut into pieces (each weighing approximately 50 g) and mixed in order to obtain homogeneous batches. Analysis of the initial content of vitamin E in the turkey meat (by HPLC) showed only a very low α-tocopherol content (around 0.5 μg α-tocopherol/g turkey meat). 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%) and black pepper (0.02%) were added. Additives were added in concentrations according to Table 1, using GRINDOX™ 1032 with 20% natural tocopherols (extracted from soybean oil containing 1.4% α-, 12.8% γ- and 5.8% δ-tocopherol, as analysed by HPLC), GRINDOX™ ascorbyl palmitate fine with a purity minimum of 98%, and sodium ascorbate, granular (SFK, Hvidovre, Denmark). GRINDOX™ 1032, especially developed for use in products to which additives cannot be added via a lipid phase, contains emulsifiers as carriers to obtain the optimal dispersion. GRINDOX™ 1032 was added to the minced turkey meat via a predispersion, as described previously (Bruun-Jensen *et al.*, 1994). To eliminate the effect of the emulsifiers included in the GRINDOX™ 1032 formulation, emulsifiers were added to L-ascorbyl palmitate and sodium ascorbate to match the level in GRINDOX™ 1032. L-ascorbic acid was added as sodium ascorbate in accordance with the common practice in the meat industry. A small-scale experiment showed that the pH value in the meat balls did not change significantly due to the addition of sodium ascorbate in the concentrations used in the experiment. The additive formulations (including emulsifiers, salt and pepper) were added to the minced turkey meat in a fixed quantity of water (200 g per 1000 g total turkey meat) and mixed in a Stephanmixer UMC-5 (Hamlen, Germany) at 600 rpm for 1.5 min.

Each of the additives was applied in 0, 1, 2 or 3 units, the concentration of the units being different for different additives in order to obtain both antioxidative and prooxidative effects; one unit of tocopherols was 50 mg/kg meat, one unit of L-ascorbyl palmitate was 100 mg/kg meat, and one unit of L-ascorbic acid was 42.5 mg/kg meat. Thus, tocopherols and L-ascorbyl palmitate/L-ascorbic acid units were defined in the ratio of 1:2 based on molar concentrations. Moreover, the additives were added in several combinations with either two or three additives together. Two control batches were made, one batch without any additives and one

Table 1. Induction Periods for accelerated oxidation in an O_2 atmosphere at 90° C for cooked, minced turkey meat with tocopherols, L-ascorbyl palmitate, and L-ascorbic acid added

Units[a]	Natural tocopherols[b] (ppm)	Ascorbyl palmitate (ppm)	Ascorbic acid (ppm)	Induction Periods[c] Mean IP ± STD (number of samples)(h)
0,0,0	0	0	0	0.0 (3)
0,0,0[d]	0	0	0	0.0 (3)
3,0,0	150	0	0	40.9 ± 1.2 (2)
0,3,0	0	300	0	8.1 ± 0.1 (2)
0,0,3	0	0	127.5	4.2 (1)
2,1,0	100	100	0	45.6 ± 2.0 (2)
2,0,1	100	0	42.5	45.8 ± 1.3 (2)
1,2,0	50	200	0	30.6 ± 1.6 (2)
0,2,1	0	200	42.5	7.4 ± 0.6 (2)
1,0,2	50	0	85	29.4 ± 0.9 (2)
0,1,2	0	100	85	8.9 ± 0.4 (2)
1,1,1	50	100	42.5	28.8 ± 0.1 (2)
2,0,0	100	0	0	42.8 ± 0.7 (2)
0,2,0	0	200	0	9.5 ± 2.8 (2)
0,0,2	0	0	85	0.0 (2)
1,1,0	50	100	0	33.7 ± 0.5 (2)
1,0,1	50	0	42.5	38.4 ± 0.2 (2)
0,1,1	0	100	42.5	7.9 ± 0.6 (3)
1,0,0	50	0	0	45.2 ± 5.7 (2)
0,1,0	0	100	0	9.5 ± 0.4 (2)
0,0,1	0	0	42.5	0.0 (3)

[a] 'Coded units' for tocopherols, L-ascorbyl palmitate, L-ascorbic acid.[b] Natural mixture from soybean oil, containing 1.4% α-, 12.8% γ-, and 5.8% δ-tocopherol.[c] Determined in OXIPRESS™ O_2 bomb at 90°C with an initial O_2 pressure of 5 kg/cm^2 at ambient temperature.
[d] Control batch with emulsifiers added (cf. text).

batch with only emulsifiers added. All additive formulations were obtained from Grindsted Products, Danisco A/S, Brabrand, Denmark.

Small meat balls (approximately 2 g each) were made through a 15 mm plate on an Esna ball machine (Nørre Åby, Denmark) and cooked in 90°C water for 3 min, then cooled on precooled aluminium trays in one layer in air for 10 min, and then packed using a Röschermatic VM-19/S packaging machine (Röscherwerke Osnabrück, Germany) for packaging at initially 21% O_2 (79% N_2) or a VacuMIT Model P10/20 GAS packaging machine (Duggendorf, Germany) for packaging at initially 1% O_2 (99% N_2). High barrier foil with a very low O_2 transmission rate (< 2 $cm^3/m^2/24h/bar$) was used. The packages were stored in a dark storage room at 5° ±0.5°C for up to 9 days. The fat content in the cooked turkey meat balls was determined to be approximately 1%.

Chemical analyses

The O_2 content was analysed during storage in each package prior to further chemical analysis using a Systech Gasspace 2 gas analyser (Oxfordshire, UK) and hypodermic needles. Samples were replaced by new samples whenever the O_2 content indicated leaks in the package.

2-Thiobarbituric acid reactive substances (TBARS) were determined by the distillation method according to Tarladgis *et al.* (1960) with minor modifications. While still packed, the meat balls were reheated in 90°C water for 20 min before analysis. All samples were analysed in duplicate (6–8 meat balls per sample), and results were expressed as "equivalent μmoles malondialdehyde per kilogram meat" according to a standard curve in the concentration range of 1–20 μM tetraethoxypropane (Merck, Darmstadt, Germany).

An OXIPRESS™ O_2 bomb (MicroLab, Århus, Denmark) was used to determine the Induction Periods, i.e. the time during which the meat balls were resistant to oxidation. All 21 batches were tested in duplicate (40 g ± 1 g/sample) at 90°C, and the induction periods were expressed in hours. The initial O_2 partial pressure was 5 kg/cm^2 at ambient temperature. Prior to analysis, the meat balls were stored at −70°C.

Statistical analysis of TBARS values

For the statistical analysis, a two-compartment model was used. The development of TBARS as a function of time, t, could be described by the expression

$$\text{TBARS} = c + M(1 - \exp(-rt)) \qquad (1)$$

where the parameter c is the starting level for the TBARS values prior to storage ($t = 0$); c is a measure of the oxidation during mincing of the meat and cooking of the meat balls with the additives used. The parameter M is approximately the maximum level of TBARS values since c is small. The parameter r is a rate constant for the development of TBARS values during storage.

The statistical analysis focuses on the effect of the additives on the parameters M and r. Thus, for each of the packaging conditions, the parameters M and r were modelled as functions of the three additives involving linear effects, general main effects, and interactions. The significance of each of these terms was determined by an approximate F-test in the resulting non-linear model for the entire experiment. The variance was stabilised by a power transformation found by the Box–Cox method.

RESULTS

Lipid oxidation in the turkey meat balls during storage at 5°C for 9 days was quantified after reheating of the product by determination of TBARS. Moreover, Induction Periods (IPs) were determined for meat balls which had not been stored at 5°C or reheated, at 90°C in an O_2 atmosphere.

Effect of additives

The development of TBARS in reheated turkey meat balls during storage at 5°C was adequately described by the first-order model of equation (1) as may be seen in Fig. 1. The two parameters, M and r, i.e. the maximum level of TBARS and the first-order rate constant for the process, respectively, were used to quantify the combined effect of the three additives and to describe the interaction between the additives. It should be noted that the secondary oxidation products measured as TBARS were transformed into other products in slower processes, and that lower TBARS values than the estimated M values were observed for longer storage time for the products with minimum oxidative resistance.

From the parameter M it is seen that tocopherols and L-ascorbyl palmitate had antioxidative effects in the reheated turkey meat balls, while L-ascorbic acid acted as a prooxidant (Fig. 1). For meat balls stored in atmospheric packages, M was reduced by each unit of tocopherols to 80% (with a 95% confidence interval of 76–84%) of the control values, and by each unit of L-ascorbyl palmitate to 91% (85–97%). Each unit of L-ascorbic acid increased the maximum TBARS values to 114% (110–119%) of the control value. For meat balls stored in 1% O_2 packages, M was reduced by each unit of the natural tocopherols to 85% (79–92%) of the control values, and by each unit of L-ascorbyl palmitate to 89% (79–100%). Each unit of L-ascorbic acid increased the maximum TBARS values to 106% (100–113%) of the control value, thus the prooxidative effect of L-ascorbic acid was greater at ambient atmosphere than at reduced O_2 pressure. For each O_2 pressure, an analysis of the dependence of M on the additive combination showed that a multiplicative model provided a promising quantitative description of the interaction;

$$P_{(i\cdot x,j\cdot y,k\cdot z)} = \left(\frac{M(x)}{M_{\text{control}}}\right)^i \left(\frac{M(y)}{M_{\text{control}}}\right)^j \left(\frac{M(z)}{M_{\text{control}}}\right)^k \qquad (2)$$

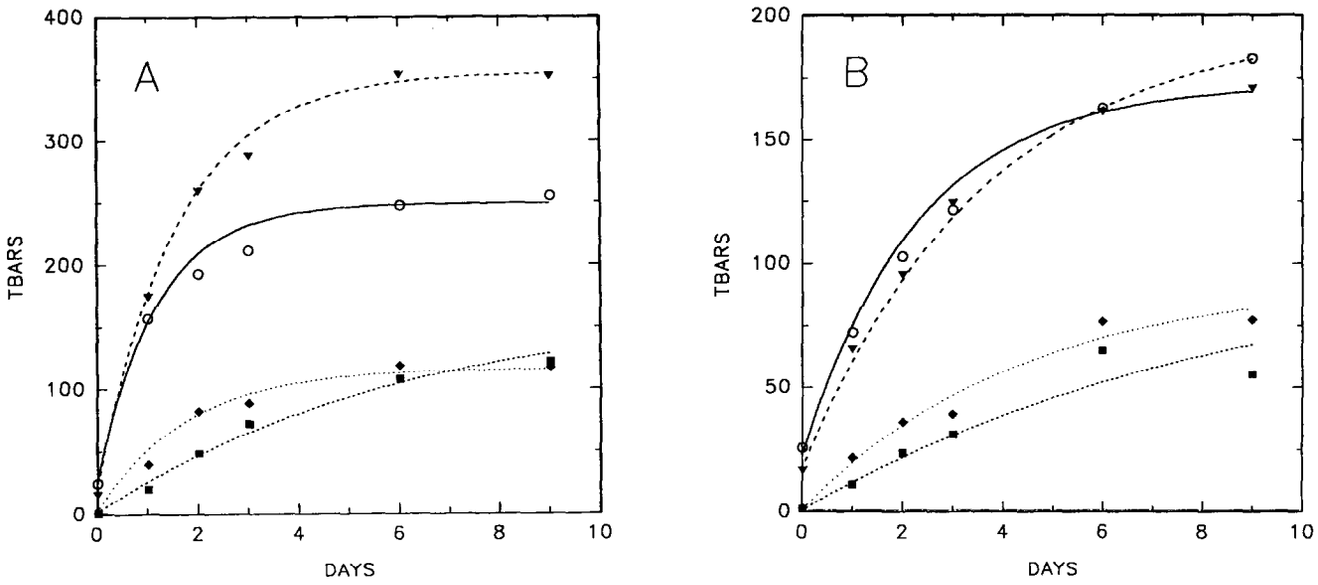

Fig. 1. Lipid oxidation measured as TBARS values (μmol malondialdehyde per kg meat) during storage at 5°C of turkey meat balls each added 3 units of tocopherols (◆), L-ascorbyl palmitate (■), and L-ascorbic acid (▼) in (A) 21% O_2, and (B) 1% O_2 packaging. For comparison, results from meat balls without additives (○) are shown. Curves were calculated according to the model $TBARS = c + M(1 - \exp(-rt))$.

where $M_{control}$ is the maximum level of TBARS for meat balls without additives. $M(x)$, $M(y)$ and $M(z)$ are the maximum TBARS values for meat balls with each of the additives at lowest level. i, j and k have the values 0, 1, 2, or 3 and are indices of the level of each of the three additives. $P_{(i\cdot x,j\cdot y,k\cdot z)}$, the protection factor relative to control (equals 1.00) for each of the 19 combinations of additives at each of the two O_2 levels is found in Table 2 together with M_{calc}, the estimated values according to the model (equation 1), and M_{obs}, the maximum TBARS value observed during storage (as a 95% confidence interval).

For 21% O_2 packaging, the model provides an adequate description as three-quarters of the estimated M_{calc} values lie in the 95% confidence interval. For 1% O_2 packaging, only one-quarter of the estimated M_{calc} values are in the 95% confidence interval. Generally, the estimated M values which lie outside the 95% confidence interval are slightly overestimated. This is due to the fact that, in the model (equation 1), M is an estimate

Table 2. Comparison between M_{calc}, estimated maximum TBARS values for reheated turkey meat balls with different combinations of tocopherols, L-ascorbyl palmitate, and L-ascorbic acid added, and M_{obs}, observed maximal TBARS values during 5°C storage in 21% O_2 or 1% O_2 packaging. The estimated TBARS values are based on a protection factor, $P_{(i\cdot x,j\cdot y,k\cdot z)}$, calculated according to a multiplicative model[a]

Atmospheric packaging (21% O_2/79% N_2)[b]										
Batch	(0,0,0)	(3,0,0)	(0,3,0)	(0,0,3)	(2,1,0)	(2,0,1)	(1,2,0)	(0,2,1)	(1,0,2)	(0,1,2)
$P_{(i\cdot x,j\cdot y,k\cdot z)}$	1.00	0.51	0.75	1.48	0.58	0.73	0.66	0.94	1.04	1.18
M_{calc}	230	118	173	341	134	168	152	217	240	272
M_{obs}	188-273	93-141	97-146	284-389	87-133	140-204	110-164	123-181	176-250	168-239
Batch	(1,1,1)	(2,0,0)	(0,2,0)	(0,0,2)	(1,1,0)	(1,0,1)	(0,1,1)	(1,0,0)	(0,1,0)	(0,0,1)
$P_{(i\cdot x,j\cdot y,k\cdot z)}$	0.83	0.64	0.83	1.30	0.73	0.91	1.04	0.80	0.91	1.14
M_{calc}	191	147	191	300	168	210	240	184	210	263
M_{obs}	140-203	124-182	137-198	251-346	111-165	158-227	141-204	142-206	160-229	230-328
1% O_2 packaging (1% O_2/99% N_2)[b]										
Batch	(0,0,0)	(3,0,0)	(0,3,0)	(0,0,3)	(2,1,0)	(2,0,1)	(1,2,0)	(0,2,1)	(1,0,2)	(0,1,2)
$P_{(i\cdot x,j\cdot y,k\cdot z)}$	1.00	0.61	0.70	1.19	0.64	0.77	0.67	0.84	0.96	1.00
M_{calc}	157	96	110	187	101	121	105	132	151	157
M_{obs}	123-191	58-94	40-68	122-186	52-86	63-101	40-68	48-79	74-117	36-62
Batch	(1,1,1)	(2,0,0)	(0,2,0)	(0,0,2)	(1,1,0)	(1,0,1)	(0,1,1)	(1,0,0)	(0,1,0)	(0,0,1)
$P_{(i\cdot x,j\cdot y,k\cdot z)}$	0.80	0.72	0.79	1.12	0.76	0.90	0.94	0.85	0.89	1.06
M_{calc}	126	113	124	176	119	141	148	134	140	167
M_{obs}	38-65	79-123	53-87	137-203	69-109	86-132	57-92	80-124	95-144	113-181

[a] For concentrations of additives in different batches, see Table 1.[b] TBARS values in μmol malondialdehyde per kilogram meat.

of the maximum TBARS values obtainable, i.e. if the meat balls have not reached the maximum level of oxidation either because of reduced O_2 availability in the package or discontinued storage, the estimated M value will show a slightly higher value than the actual TBARS value. In meat balls packed in 21% O_2 atmosphere, oxidation is far more advanced than in meat balls packed in 1% O_2 atmosphere, which easily explains the better agreement between estimated M values and the actual TBARS values for meat balls stored in 21% O_2 packages than for meat balls stored in 1% O_2 packages.

The rate at which the TBARS values approach the maximum level M was reduced by all three additives and greatly affected by the O_2 availability in the packages. In particular, the addition of tocopherols or L-ascorbyl palmitate to the meat balls reduced the rates strongly as shown in Table 3 for the rate constant r, whereas L-ascorbic acid had only minor effects on this rate constant. The addition of tocopherols reduced r to approximately two-thirds and one-half of the control level in 21% O_2 and 1% O_2 packages, respectively. The reduction of r due to tocopherols was independent of the amount added, whereas the somewhat larger effect of L-ascorbyl palmitate on r showed a significant concentration effect. The reductions of r due to L-ascorbyl palmitate were larger than reductions due to tocopherols at the three levels investigated. One unit of L-ascorbyl palmitate reduced r to approximately one-third and one-quarter of the control level in 21% O_2 and 1% O_2 packages, respectively. The reductions of the rate constant (r) due to L-ascorbyl palmitate were not enhanced by adding tocopherols, whereas the addition of L-ascorbic acid to the meat balls with added L-ascorbyl palmitate enhanced the reduction of the rate constant r (data not shown).

The difference in the rates between L-ascorbyl palmitate and tocopherols is most manifest for meat balls stored in 21% O_2 packages as may be seen in Fig. 1, where the TBARS values for meat balls with added L-ascorbyl palmitate show a slower approach towards the maximum level of TBARS values (M) than meat balls with added tocopherols. L-ascorbic acid was found to have both a prooxidative and an antioxidative effect when added to the turkey meat balls. The prooxidative effect was reflected by the increasing maximum level of TBARS, whereas the antioxidative effect was reflected by the reduction of the rate, at which TBARS approached the maximum level. But since the reduction in the rate was rather small, the total effect of adding L-ascorbic acid was mainly prooxidative. For 21% O_2 packaging, the net effect was prooxidative throughout the whole storage period, whereas in 1% O_2 packages the antioxidative effect outweighed the prooxidative effect in the first 5–6 days of the storage period. In conclusion, the prooxidative effect of L-ascorbic acid in cooked, minced turkey becomes more pronounced when more O_2 is available.

The control batch, with emulsifiers added showed a minor reduction in TBARS, as the TBARS values at the end of the storage period for the control batch with only emulsifiers added were 8% lower than for the control batch without any additives (data not shown).

Table 3. Estimates for the rate constant, r, at which the TBARS values of turkey meat balls with or without addition of one, two or three units of tocopherols, L-ascorbyl palmitate, or L-ascorbic acid approach the maximum level M for meat balls packed in 21% O_2 or 1% O_2 packaging

Additives	21% O_2 packaging (h^{-1})	1% O_2 packaging (h^{-1})
Without additives	0.85	0.43
One unit of natural tocopherols	0.57	0.23
Two units of natural tocopherols	0.57	0.23
Three units of natural tocopherols	0.57	0.23
One unit of L-ascorbyl palmitate	0.37	0.14
Two units of L-ascorbyl palmitate	0.27	0.13
Three units of L-ascorbyl palmitate	0.16	0.11
One unit of L-ascorbic acid	0.78	0.38
Two units of L-ascorbic acid	0.70	0.32
Three units of L-ascorbic acid	0.63	0.27

Induction periods determinded by OXIPRESS™ O_2 bomb

The Induction Periods (IPs) were determined from pressure decrease in the OXIPRESS™ O_2 bomb as a result of O_2 consumption. The calculation was based on the extrapolation procedure which is based on a comparison with the uninhibited oxidation rate for the product in question. Meat balls without tocopherols added all had very low IPs, whereas meat balls with only one unit of tocopherols added had IPs between 40 and 45 h, the same IPs as those of meat balls with two or three units of tocopherols added. The IPs of meat balls with added tocopherols in combination with either one or two units of L-ascorbyl palmitate or L-ascorbic acid did not have any significant increase in IP compared with meat balls with only tocopherols added. One unit of tocopherols was sufficient to ensure IPs of 40 h or more. Compared with the low level of additives, i.e. meat balls with one unit of the additive, tocopherols had a high effect ($IP = 45.2 \pm 5.8$), L-ascorbyl palmitate had a low effect ($IP = 9.5 \pm 0.4$), whereas L-ascorbic acid had no effect ($IP = 0.0$). L-ascorbic acid only had an effect when added at the high concentration, i.e. with three units added, and L-ascorbyl palmitate had a much lower effect than tocopherols at all levels. None of the control batches had any resistance to oxidation under these conditions, i.e. the oxidation rate was uninhibited from the start of the trial.

DISCUSSION

Both the antioxidative and the prooxidative effects of tocopherols, L-ascorbyl palmitate and L-ascorbic acid have been studied in several model systems (Cort, 1974, 1982; Löliger, 1989; Thomas, 1992; Frankel *et al.*, 1994).

Evidence of antioxidative synergism between tocopherols and L-ascorbyl palmitate or L-ascorbic acid has been presented in some of these model systems. However, inconsistencies between the results obtained in different model systems along with the complexity of meat products, makes it questionable to transfer results obtained in simplified model systems to meat products. For meat products, packaging conditions and in particular oxygen availability should also be considered, and experiments with the products concerned should be conducted prior to practical use of antioxidant mixtures. For tocopherols and L-ascorbyl palmitate, a synergistic effect was observed in turkey meat balls, most noticeably for atmospheric packaging and towards the end of the storage period (Bruun-Jensen *et al.*, 1994). However, a more detailed understanding of the synergistic effect was not obtained in our previous study of cooked, minced turkey meat, which is highly labile in terms of oxidation.

The mathematical model used to describe the progression of oxidation in the present study for each of the separate storage experiments provides two parameters for a discussion of the interaction between tocopherols and L-ascorbyl palmitate or L-ascorbic acid. M is the maximum level of oxidation measured as TBARS, and r is the rate constant for a first-order process at which oxidation approaches the maximum level. The model is based on TBARS values, since the literature provides high correlations between sensory scores and TBARS values in turkey meat (Mielche, 1994; Nolan *et al.*, 1989; Wu & Sheldon, 1988). The statistical analysis revealed different modes of action for tocopherols and L-ascorbyl palmitate; the addition of tocopherols resulted primarily in an antioxidative effect by lowering the maximum level of TBARS, M, whereas addition of L-ascorbyl palmitate mainly resulted in an antioxidative effect by lowering the rate constant, r, by which TBARS approach the maximum level. These two types of effect are illustrated in Fig. 1, where the tocopherol curve ascends faster than the L-ascorbyl palmitate curve, though the curves from the two additives seem to end at approximately the same level of TBARS values after 9 days of storage for the same type of packaging. The meat balls with added tocopherols or L-ascorbyl palmitate; (i) had a lower TBARS level on day 0 after production, i.e. mincing and cooking, than the control meat balls, and (ii) never reached the same high level of TBARS values as the control meat balls. Thus, the antioxidative effect of the additives is seen not only during storage, but also in the fresh product. It is assumed that the additives not only delay oxidation during storage, but also have some effect during the production of the meat balls, as the TBARS values for meat balls with tocopherols or L-ascorbyl palmitate never reach the same maximum level as those without antioxidants or with L-ascorbic acid added. A possible, although somewhat speculative explanation is that the precursor for the TBARS is developed in the production of the meat balls, and that tocopherols and L-ascorbyl palmitate partly inhibit this development in marked contrast to L-ascorbic acid.

Oxidative deterioration is most significant for 21% O_2 storage. In these storage conditions, the maximum TBARS level M could be described for the combinations of additives and the three concentration levels for each additive by a relative protection factor, $P_{(i\cdot x,j\cdot y,k\cdot z)}$, which could be calculated by multiplication of the protective factors determined for each unit of additive. It should be noted that the model is also capable of describing the prooxidative effect of L-ascorbic acid. However, the lack of direct synergism is a surprising result. It has been argued that tocopherols and L-ascorbic acid are dissolved in the lipid and water phases, respectively, whilst L-ascorbyl palmitate is placed at the interface between the two phases. Thus, the physical distance from tocopherols to L-ascorbyl palmitate is shorter than to L-ascorbic acid, i.e. the regeneration of tocopherols should be favoured by L-ascorbyl palmitate. For the present product, this effect could not be confirmed. In contrast to tocopherols, L-ascorbyl palmitate efficiently reduced the rate constant r, and the effect was dependent on concentration. Based on these findings, the interaction between tocopherols and L-ascorbyl palmitate is better characterised as an indirect synergism, since tocopherols affect the maximum level of TBARS, and L-ascorbyl palmitate affects the rate. We have no ready explanation for this difference, but are currently investigating the interaction between tocopheroxyl radicals and L-ascorbic acid derivatives using transient absorption laser flash spectroscopy. Better dispersion of L-ascorbyl palmitate than L-ascorbic acid in the meat product tested may explain the more pronounced antioxidative effect of the former additive, as the two additives were used in equimolar concentrations. Thus, based on the concentration levels of the additives used in the present study, the combined use of tocopherols and L-ascorbyl palmitate rather than L-ascorbic acid is strongly recommended, since the different modes of action combine to provide indirect synergism, which clearly prolongs the shelf-life of this oxidation-labile product.

Parallel to the storage study, the antioxidative efficiency of the three additives in the turkey meat was evaluated using an OXIPRESS™ O_2 bomb, cf. Table 1. In this accelerated test, turkey meat balls without antioxidants had no resistance to oxidation, and L-ascorbic acid yielded little if any protection. Tocopherols provided a substantial protection, while L-ascorbyl palmitate provided less protection, and for both of the additives which acted as antioxidants, the effect was almost independent of concentration. The accelerated test thus yielded information related to M, the maximum TBARS level, rather than to r, the rate constant. Moreover, it should be evident from the result, that tocopherols are most important in determining IPs, as may be seen from a comparison of IPs for the series of combinations (2,0,0), (2,0,1) and (2,1,0), and for the series of combinations (1,0,0), (1,2,0), (1,0,2), (1,1,1), (1,1,0), (1,0,1). However, the use of an OXIPRESS™ O_2 bomb to estimate the antioxidative efficiency of tocopherols confirms the impression that the antioxidative

efficiency of tocopherols increases with increased temperature due to changes in the mechanism of participation of tocopherols and tocopheroxyl radicals in the oxidation reactions. In general, tocopherols tend to have a better antioxidative effect when tested with accelerated methods (Marinova & Yanishlieva, 1992). However, considering the overall agreement between the results observed in the accelerated test with the OXIPRESS^{TM} O_2 bomb and the storage experiment, it is concluded that the OXIPRESS^{TM} O_2 bomb is a valuable tool for initial screening of antioxidative activity.

Turkey meat is rather susceptible to oxidation and the development of WOF in reheated products. In the present study, oxidative deterioration has been described by a rate parameter and a parameter for maximal oxidation. It has been shown that the combined use of L-ascorbyl palmitate, which most significantly influences the rate parameter, and tocopherols, which most significantly influence the parameter for maximum oxidation, delays the development of WOF as a result of indirect synergism.

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