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INFLUENCE OF FEED ENRICHED WITH NATURAL ANTIOXIDANTS ON THE OXIDATIVE STABILITY OF FROZEN BROILER MEAT

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Introduction

Meat as well as precooked and restructured meat products have a low oxidative stability, which causes discoloration, drip losses, off-odor and off-flavor development, and the production of potentially toxic compounds (Gray et al., 1996; Morrissey et al., 1998). Freezing slows down oxidation, but it does not stop the process (Kanner, 1994).

To maximize the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate (αTAc), are added to PUFA rich feeds. The beneficial effect of dietary αTAc supplementation on the subsequent stability of lipids in foods from animal origin has been extensively reported for poultry, beef cattle, veal calves and pigs (see e.g. Gray et al., 1996; Jensen et al., 1998). More recently, an increasing number of studies report on the antioxidative properties of plant products and compounds (Halvorsen et al., 2002; Pellegrini et al., 2003; Schwarz et al., 2001). Several studies have already demonstrated that plant antioxidative compounds supplemented to meat post mortem are able to improve the lipid stability of the meat (Govaris et al., 2003; Lau et al., 2003; Nissen et al., 2000). However, only a limited number of studies have been performed investigating the effect of dietary administration of natural antioxidants, other than natural α-tocopherol (αTC), on the oxidative stability of meat or meat products (Botsoglou et al., 2002, 2003; Tang et al., 2000).

Objectives

The aim of this study was to investigate the effect of supplementation of a broilers' diet with several plant extracts, rich in natural antioxidants, on the oxidative stability of meat after 8 months of frozen storage. The effects of individual extracts were tested, as well as combinations of extracts to investigate possible additive or synergistic effects between antioxidants.

Methodology

Animals and experimental design

Two thousand and forty one-day old chickens (Ross 308, cocks) were divided over 60 pens and were fed for 6 weeks a diet containing 4% refined linseed oil (n-3 PUFA enrichment) and one of 20 antioxidants or antioxidant mixtures. Within each phase (3 phases), diets were formulated to an equal protein and energy content. In the premix no synthetic antioxidants were added, but a basal amount of 20 ppm αTAc was present to meet physiological requirements. The experimental antioxidants were mixed in the refined linseed oil prior to the feed manufacturing. Five extracts (natural αTC, rosemary, green tea, grape seed and tomato; Table 1) were supplemented separately at 100 ppm or 200 ppm, making 10 treatments. In addition, αTC, rosemary and green tea were supplemented in equal proportions in all combinations two by two and all three combined at a final concentration of 100 or 200 ppm, making a further 8 treatments. Two control treatments with a mixture of synthetic antioxidants (BHT, BHA, ethoxyquin) and with or without 200 ppm αTAc were also included. All antioxidant extracts were supplemented at 100 or 200 mg total product/kg feed and not as 100 or 200 mg active compounds/kg feed. These 20 antioxidant treatments were replicated in three pens (Table 2).

Sampling

At the moment of slaughter, 5 birds per pen were selected with a live weight close to the average pen weight. After slaughter, the right part of the breast muscle of the 5 selected animals was pooled, minced, vacuum-packed and stored at -18°C. After 8 months, three patties (approximately 100g) were prepared and over-wrapped in an oxygen permeable polyethylene film and placed in an illuminated chill cabinet (illuminance of 1000 lux) at 3°C for 11 days. Samples were analysed for lipid oxidation at day 1, 4 and 11 of display and for protein oxidation at day 11.

Methods

Lipid oxidation was assessed by the TBARS-method (thiobarbituric acid reactive substances) based on Tarladgis et al. (1960) and is expressed as μg malondialdehyde/g tissue.

Oxidative damage to proteins was determined by measuring the decrease in the amount of thiol groups. Thiol groups are expressed as nmol free SH-groups/mg protein (Batifoulier et al., 2002).

Statistical analysis

Data of lipid and protein oxidation were analysed by a linear model including the fixed effects of antioxidant treatment and days of storage. The interaction term was not significant and was therefore not included in the model. TBARS values were log

transformed to account for heterogeneity of variances. To compare single antioxidant treatments and to test for dose effects and possible synergistic action, specific contrasts were defined at a significance level of $P < 0.05$ (e.g. c1 vs. c2; for number of treatments see Table 2). The analyses were performed in S-Plus 6.1.

Results & Discussion

TBARS values significantly increased with time of display ($P<0.05$). After thawing (day 1), the meat samples gave low TBARS values (<0.25 µg malondialdehyde/g meat). These values increased considerably during storage in the illuminated chill cabinet (Table 2). TBARS values on fresh broiler meat samples from the same treatments (reported by Smet et al., 2005) similarly increased during display, but the increase was much more marked for the frozen/thawed samples in this study. So, freezing slows down but does not stop the oxidation process. As lipid-free radicals are soluble in the lipid fraction and more soluble at lower temperatures, it allows them to diffuse to longer distances and to spread the reaction (Kanner, 1994).

Compared to all other antioxidant treatments, the treatment with 200 ppm αTAc resulted in the lowest TBARS values (<0.6 µg malondialdehyde/g meat). Even after 11 days of refrigerated storage, these meat samples still had acceptable values. Also in literature, it has been clearly demonstrated that αTAc supplementation results in good oxidative stability (e.g. Botsoglou et al., 2003; Coetzee & Hoffman, 2001; Lopez-Bote et al., 1998).

For the other antioxidant treatments, some dose effects were observed. Grape seed and tomato showed lower TBARS values at 200 vs. 100 ppm (c5 – c10; c7 – c12; $P<0.05$), while αTC and rosemary showed no difference between the two doses (c3 – c8; c4 – c9; $P>0.05$). In contrast, TBARS values were much higher for the 200 ppm compared to the 100 ppm green tea treatment (c6 – c11; $P<0.05$). For the combined extracts, some trends for an antioxidative dose effect were observed (TBARS100ppm>TBARS200ppm), however this was not significant. These findings confirm the work of Lau et al. (2003) for grape seed. However for green tea, Tang et al. (2000) observed a clear antioxidative dose-response effect, which is in contrast with our results. The different effects between our study and the one of Tang et al. (2000) could be due to a different content of catechins present in the green tea extracts.

Possible synergistic effects were investigated by making combinations vs. single antioxidant additions. However, few synergistic effects were revealed because of the high variation between different pen repetitions. Nevertheless, the combination of αTC and green tea at 200 ppm yielded significantly ($P<0.05$) lower TBARS values compared to 200 ppm αTC or 200 ppm green tea alone, which may suggest a synergistic effect between αTC and green tea at this dose. Further, the combination of rosemary and green tea at 100 and 200 ppm resulted in significantly higher TBARS values than 100 ppm rosemary or green tea alone. This suggests that the combination of both extracts gave a lower oxidative stability than the single addition. Few data are available about possible synergistic effects of natural extracts on the oxidative stability of meat. Basmacioglu et al. (2004) reported a synergistic effect for oregano and rosemary essential oils in broilers at a dose of 300 ppm. Also a synergistic action between oregano oil and αTAc (200 ppm)

resulted in a higher oxidative stability than 200 ppm αTAc alone in turkeys (Botsoglou et al., 2003; Papageorgiou et al., 2003).

As oxidation is a very general process, also proteins, DNA, pigments and carbohydrates are affected (Kanner, 1994). Therefore, oxidative stability should not only be evaluated by lipid oxidation (Dotan et al., 2004). In this experiment, protein oxidation was also measured by the amount of thiol groups disappearing during storage. However, the thiol content at day 11 (Table 2) did not differ much between dietary treatments and did also not differ from our results on the fresh broiler meat (Smet et al., 2005), suggesting no effect on protein oxidation.

Conclusions

During frozen storage, the oxidative stability of broiler meat decreased, which resulted in higher TBARS values compared to fresh meat evaluated in a previous study. Yet, for protein oxidation no differences were revealed compared to the fresh meat. The addition of different antioxidants to the feed influenced lipid stability. The highest oxidative stability of meat was obtained with 200 ppm α-tocopheryl acetate. Grape seed and tomato showed an antioxidative dose-response effect. In contrast, the addition of green tea resulted in a pro-oxidative dose-response effect. However, the combined use of α-tocopherol and green tea resulted in a net antioxidative effect.

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Tables and Figures

Table 1: Specifications of the antioxidant extracts (supplied by Nutri-AD International, Belgium)

Extract	Specification
Synth antioxidant	BHT/ethoxyquin/BHA
α-tocopheryl acetate	500 IU/g
α-tocopherol	700 mg/g mixed tocopherols with 80-150 mg α-tocopherol, 10-30 mg β-tocopherol, 350-450 mg γ-tocopherol, 140-200 mg δ-tocopherol
Rosemary	3% carnosic acid, 0.1% carnosol, 0.03% Me-carnosate
Green tea	Cafein 4% to 8%, EGCG > 14,5%, epicatechins > 2.4%, catechins (as epicatechins) > 60%, catechins (as EGCG) 23.25% to 26.75%
Grape seed	89% polyphenols, containing 3.1% catechins; 11.2% oligomeric procyanidins
Tomato	No information available

Table 2: Mean values for lipid oxidation (µg malondialdehyde/g meat) and protein oxidation (nmol free SH groups/mg protein) of the breast muscle stored in a chill cabinet during 1, 4 or 11 days (after 8 months of frozen storage)

Antioxidant treatment		Lipid oxidation				Protein oxidation	
		Day 1	Day 4	Day 11	SEM	Day 11	SEM
1	Synthetic	0.16	0.45	1.09	0.17	70.7	2.39
2	Synthetic + 200 ppm αTAc	0.04	0.31	0.51	0.16	76.6	3.67
3	100 ppm α-tocopherol (αTC)	0.19	0.38	1.42	0.34	71.7	2.07
8	200 ppm α-tocopherol	0.20	0.53	1.65	0.39	85.5	11.1
4	100 ppm rosemary (ros)	0.12	0.80	2.44	0.50	76.9	1.77
9	200 ppm rosemary	0.20	0.45	1.14	0.17	70.9	3.00
5	100 ppm grape seed	0.20	2.02	4.32	0.81	77.0	5.38
10	200 ppm grape seed	0.11	0.49	1.06	0.16	79.7	2.38
6	100 ppm green tea	0.20	0.48	1.02	0.15	67.8	8.38
11	200 ppm green tea	0.23	1.39	4.51	0.66	68.5	0.80
7	100 ppm tomato	0.22	2.23	5.90	0.86	61.7	8.88
12	200 ppm tomato	0.19	0.61	2.96	0.47	75.3	0.84
13	αTC + ros (100 ppm)	0.17	0.33	2.24	0.37	70.6	3.35
16	αTC + ros (200 ppm)	0.18	0.30	1.31	0.24	76.0	2.77
14	αTC + tea (100 ppm)	0.18	0.30	1.23	0.28	77.6	2.39
17	αTC + tea (200 ppm)	0.14	0.34	1.01	0.15	73.0	4.32
15	Ros + tea (100 ppm)	0.22	1.54	4.32	0.89	67.4	9.19
18	Ros + tea (200 ppm)	0.22	0.78	3.06	0.67	72.8	0.20
19	αTC + ros + tea (100 ppm)	0.24	0.53	1.88	0.30	86.0	5.76
20	αTC + ros + tea (200 ppm)	0.23	0.47	2.31	0.46	69.3	0.29