Oxidative stability of chicken meat with natural and synthetic feed antioxidants

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1. Introduction

Lipid oxidation in meat products is an important issue on nutritional quality, both loss of polyunsaturated fatty acids (PUFA) and production of cholesterol oxidized products are examples of the interest for maintaining the proper stability. An improvement in oxidative stability can be achieved by modifications in the lipid composition of muscle cells membranes (Barroeta and Cortinas, 2002). Higher levels of PUFA in the diet increase the PUFA/saturated fatty acid (SFA) balance in the carcass (Grau et al., 2001). The PUFA are healthier than SFA, but also are more prone to oxidation and can act further as pro-oxidants. Some authors have studied the addition of different natural and/or synthetic antioxidants to the animals' diet (poultry, turkey, pigs) and their influence on the shelf-life (Bou et al., 2005; Carreras, 2005).

Natural antioxidants are present in plant parts (e.g. bark, seeds and/or fruits); tocopherols (vitamin E) and ascorbic acid (vitamin C) are among the most important ones. While the first one represents an essential nutrient (it must be consumed in the diet), the latter is biosynthesized by poultry. Vitamin E present in the membrane acts as a second barrier, by preventing the propagation of the lipoperoxidative chain. Numerous studies have reported the positive effect of vitamin E enriched diets on the susceptibility to lipoperoxidation of plasma and tissues such as muscle and adipose tissue.

Synthetic antioxidants have been widely used as food preservatives, because of their effectiveness and relatively low cost. The most used antioxidants are those derived from phenolic structures, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and dodecyl, propyl and octyl gallate. Ethoxyquin (ETOX) is another synthetic antioxidant with a non-phenolic structure mainly used in animal feeds. All of them have an admissible daily ingest (ADI). Synergistic effect on meat stability has been described between dietary ethoxyquin and vitamin E (Bartov et al., 1981).

Other authors (Botsoglou et al., 2002; López-Bote et al., 1998) investigated the effect of adding rosemary and sage extracts and vitamin E to the broiler diet on the meat, and found a significant decrease in oxidation levels of the white muscle. Hexanal is the most important warmed off flavour (WOF) compound; solid phase microextraction (SPME) is a solvent-free technique that allows quantifying it rapidly. Besides, there are some studies that properly correlate TBARS and hexanal. Both analyses are a very reliable measure of oxidation (Beltran et al., 2003).

The aim of this study was to evaluate three feed antioxidants on the oxidative stability of chicken meat under different storage conditions. Two concentrations of ethoxyquin were assessed, as well as the potential synergistic effect in combination with vitamin E. The mixture of *Rosmarinus officinalis* extract with vitamin E was also studied.

2. Materials and methods

2.1 Sample preparation

Forty eight 1-day old male broilers (Ross 308) chickens were obtained from a commercial hatchery and randomly allocated to 12 cages (4 birds per cage), fed a common diet till 21d and from 22 to 42 d diets with different concentrations of antioxidants. The control chickens (T1, without any antioxidant) were compared with supplementations of 150 and

75 mg/kg of ethoxyquin (T2 and T3 respectively), 75 mg/kg of ethoxyquin with addition of 50 and 100 mg/kg of vitamin E (T4 and T5 respectively), and finally the combination of 50 mg/kg of *R. officinalis* extract with 50 mg/kg of vitamin E (T6). Chickens were slaughtered and breast and thighs separated, packed in Ziploc plastic bags and frozen at -80ºC and kept in darkness until the analysis.

2.2 Experimental conditions

Four different conditions were applied to raw samples (breast or thigh) to favour oxidation in different levels: uncooked and cooked meat, addition of NaCl, storage temperature and light conditions. Samples within the bags were thawed at 4ºC for 24h before analysis and minced with a household mincer and introduced into a Sterilin plastic glass. A 5% NaCl (w/w) solution was added when necessary to the minced sample and mixed homogeneously. The cooked meat samples were heated during 3 min in a microwave oven at 900 W. After that samples were stored under the conditions described for each treatment: storage at room temperature (20ºC) or refrigeration temperature (4ºC) and darkness storage (in completely absence of light within aluminium foil protected glasses) or light storage (commercial fluorescent light tube). Samples were analyzed at different days depending on the conditions applied. Each treatment was repeated twice.

2.3 Analysis

Uncooked and cooked meat were analysed by two methods in order to determinate the secondary lipid oxidation. The 3rd derivate TBAR's-test was used to quantify the amount of MDA in meat (expressed in µg of malondialdehyde/g meat) following the extraction method of Botsoglou et al. (1994) and the modifications done by Beltran et al. (2003). The SPME method was applied to determinate the nonanal and hexanal levels according to Beltran et al. (2003). Two replicates of each sample were analyzed.

2.4 Statistical analysis

Data was analyzed according the experimental design using the GLM procedure of SAS System for Windows V8.02 (2001). Data is presented as LSMeans; when the model reached significance, (significance was declared at $P \leq 0.05$) differences between treatments were established by LSD test.

3. Results

There was a significant decrease in MDA in uncooked breast meat, from the addition of any antioxidant in the feed (Table 1). Although interactions between meat treatment factors were also significant, it can be stated that 150 mg/kg of ethoxyquin (T2) in feed was more effective than 75 mg/kg (T3); addition of 50 mg/kg of vitamin E to 75 mg/kg of ethoxyquin (T4) was more effective than 150 mg of ethoxyquin alone (T2); and the combination of 50 mg/kg of rosmarinus extract with 50 mg/kg of vitamin E (T6) was not significantly different from 75 mg/kg of ethoxyquin with 50 mg/kg of vitamin E (T6). The protection conferred by vitamin E was higher than the effect from the other antioxidants. Storage at room temperature, addition of salt and storage time, all had a significant effect increasing the MDA detected in uncooked breast meat; this increase was lower for feeds with added vitamin E (and this explains the significant interactions between factors).

Levels of MDA in cooked thigh meat were significantly decreased only when 150 mg/kg of ethoxyquin or vitamin E was added to the feeds, although interactions between meat treatment factors were also significant, and not as consistent as in raw breast meat. Almost all uncooked samples, with the exception of the most favourable storage conditions, were spoiled at the end of the experiment (10 days). A prooxidant effect of room temperature storage, salt addition, light and cooking was observed. Finally, neither hexanal nor nonanal was detected in raw samples, only for cooked thigh samples very low levels of hexanal were detected.

Table1. TBARS values (ng MDA/g of meat) for all feeding treatments and storage conditions performed in breast and thigh meat chicken.

Treatment	Day	Uncooked Breast					UncookedThigh	Cooked Thigh	
		Storage at 4 ºC			Storage at 20 ºC		Storage at 4 ºC		
		Dark	Light	Light + Salt	Light	Light + Salt	Dark	Dark	Dark + Salt
T1	1	13,51	16,47	28,33	116,31	189,38	1,52	829,87	1321,76
	3	17,00	18,47	29,43	139,86	184,89	8,41	1081,17	1315,18
	6	18,37	24,43	30,69	169,63	220,84	12,08	1164,60	1614,81
	10	24,47	n.a.	n.a.	n.a.	n.a.	n.a.	1356,73	1972,06
T2	1	11,42	10,79	8,58	92,55	150,70	0,53	733,29	1017,12
	3	11,66	11,27	9,16	98,78	130,57	4,85	1045,00	1192,93
	6	11,21	13,25	11,23	114,47	149,06	7,49	1276,63	1454,80
	10	13,93	n.a.	n.a.	n.a.	n.a.	n.a.	1373,73	1660,24
T3	1	10,53	11,42	6,11	128,32	208,95	0,88	903,00	1384,23
	3	12,30	13,12	8,14	144,98	191,64	12,52	1118,11	1273,25
	6	13,26	16,42	12,07	146,87	191,47	10,02	1279,63	1570,27
	10	16,99	n.a.	n.a.	n.a.	n.a.	n.a.	1385,27	1719,84
T4	1	0,20	5,90	4,53	19,23	31,32	0,86	561,02	773,06
	3	2,57	6,34	5,30	17,85	23,60	8,30	737,20	837,42
	6	5,87	7,88	6,34	20,03	26,05	8,53	923,30	1117,99
	10	6,37	n.a.	n.a.	n.a.	n.a.	n.a.	996,58	1162,47
T5	1	0,83	0,38	2,72	10,11	16,47	0,76	425,71	519,18
	3	1,16	0,95	4,00	12,98	17,15	2,65	570,60	935,89
	6	1,75	2,69	5,93	15,44	20,04	3,44	706,05	1278,51
	10	2,27	n.a.	n.a.	n.a.	n.a.	n.a.	754,31	1119,59
T6	1	0,31	2,01	2,79	12,96	21,10	0,72	522,05	491,15
	3	2,57	2,49	7,18	25,06	33,12	4,37	741,13	947,71
	6	5,56	3,90	14,99	26,50	34,59	13,45	890,59	1393,22
	10	6,39	n.a.	n.a.	n.a.	n.a.	n.a.	1003,20	1382,76

T1 = no antioxidant added, control chickens
T2 = 150 mg/kg of ethoxyquin
T3 = 75 mg/kg of ethoxyquin
T4 = 75 mg/kg of ethoxyquin and 50 mg/kg of vitamin E
T5 = 75 mg/kg of ethoxyquin and 100 mg/kg of vitamin E
T6 = 50 mg/kg of *R. officinalis* extract with 50 mg/kg of vitamin E
n. a. = not analyzed

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