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in

Brufau J. (ed.), Tacon A. (ed.).
Feed manufacturing in the Mediterranean region: Recent advances in research and technology

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 37

1999
pages 365-377

Dietary treatment and oxidative stability of broiler meat. Nutritive value, sensory quality and safety

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SUMMARY - The purpose of the study was to determine the effect of some 'natural' antioxidants such as vitamin E and β-carotene on the oxidative stability of poultry meats from chickens which had received different sources of fat (lard, olive oil and sunflower oil) and on some sensorial characteristics. Dietary fat affected the fatty acid composition of intramuscular fat, in the sense that tended to reflect the dietary fat profile. Polyunsaturated fat was more oxidized and more susceptible to oxidation. Monounsaturated fat was similar to saturated fat. There was a reciprocal response between vitamin E and TBARS (an indicator of oxidative status of lipids). β-Carotene showed a prooxidant effect when tissue levels of vitamin E were low, but an antioxidant effect at higher tissue levels of vitamin E. It is possible that certain combinations of β-carotene and vitamin E could result in the same effects as greater levels of vitamin E. Cholesterol oxides increased during cooking. Vitamin E showed a protective effect. Olive oil gave the lowest and sunflower oil the highest levels of cholesterol oxides. The same trends were observed for hexanal formation, the most important volatile. β-Carotene did not have any effect on hexanal or cholesterol oxides. Colour and cooking losses were affected by type of fat and storage, but not by antioxidants. The sensorial evaluation, which was conducted on fresh meat, indicated that firmness of fat was the most important trait influenced by type of fat. The effects of vitamin E and β-carotene were small. β-Carotene tended to increase some rancid traits. These results suggest that supplementation with vitamin E improves the 'safety' of meat, and that the role of β-carotene should be further explored, in order to determine if certain combinations of β-carotene and vitamin E can result in similar antioxidant effects as higher levels of vitamin E. However, vitamin E concentrations should be closely monitored when β-carotene is supplemented, since at low tissue levels of vitamin E, β-carotene may have a prooxidant effect.

Key words: Fat, oxidative stability, poultry meat, vitamin E, β-carotene, sensorial characteristics.

RESUME - *"Traitement alimentaire et stabilité oxydative de la viande de poulet. Valeur nutritionnelle, qualité sensorielle et sécurité". Le propos de cette étude a été de déterminer l'effet de certains antioxydants "naturels" tels que la vitamine E et le β-carotène sur la stabilité oxydative des viandes de poulets ayant reçu différentes sources de gras (saindoux, huile d'olive et huile de tournesol) et sur certaines caractéristiques sensorielles. Le gras alimentaire a affecté la composition en acides gras du gras intramusculaire, dans le sens qu'il a eu tendance à refléter le profil en gras alimentaire. Le gras polyinsaturé était plus oxydé et plus susceptible à l'oxydation. Le gras monoinsaturé était semblable au gras saturé. Il y avait une réponse réciproque entre vitamine E et TBARS (un indicateur de l'état d'oxydation des lipides). Le β-carotène a montré un effet pro-oxydatif lorsque les niveaux de vitamine E dans les tissus étaient faibles, mais un effet anti-oxydatif à des niveaux tissulaires plus élevés de vitamine E. Il est possible que certaines combinaisons de β-carotène et de vitamine E puissent causer les mêmes effets que des niveaux plus élevés de vitamine E. Les oxydes du cholestérol ont augmenté pendant la cuisson. La vitamine E a montré un effet de protection. L'huile d'olive a donné les niveaux les plus faibles d'oxydes du cholestérol, et l'huile de tournesol, les niveaux les plus élevés. On a observé les mêmes tendances pour la formation d'hexanal, l'élément volatil le plus important. Le β-carotène n'a eu aucun effet sur l'hexanal ou les oxydes du cholestérol. Les pertes de couleur et pertes à la cuisson ont été influencées par le type de gras et l'entreposage, mais non par les antioxydants. L'évaluation sensorielle, qui a été menée sur de la viande fraîche, a indiqué que la fermeté du gras était la caractéristique la plus importante qu'influençait le type de gras. Les effets de la vitamine E et du β-carotène ont été faibles. Le β-carotène a eu tendance à accroître certaines caractéristiques de rancissement. Ces résultats suggèrent que la supplémentation avec de la vitamine E améliore la "sécurité" de la viande, et que le rôle du β-carotène devrait faire l'objet d'études ultérieures, afin de déterminer si certaines combinaisons de β-carotène et de vitamine E peuvent avoir les mêmes effets antioxydants que des niveaux plus élevés de vitamine E. Cependant, les concentrations en*

vitamine E devraient être suivies de près lorsqu'il y a supplémentation en β-carotène, car à de faibles niveaux de vitamine E dans les tissus, le β-carotène peut avoir un effet pro-oxydatif.

Mots-clés : *Gras, stabilité oxydative, viande de volaille, vitamine E, β-carotène, caractéristiques sensorielles.*

Introduction

There is growing concern about the relationship between the incidence on certain cardiovascular diseases and dietary composition. In particular, palmitic acid has been associated with an increase of plasma LDL which is considered detrimental and related to an increase with risk of ischemic diseases. Polyunsaturated fatty acids seem to lower plasma LDL, but also plasma HDL which in turn appear to have a protective effect, while monounsaturated fatty acids, and in particular oleic acid, seem to lower plasma LDL without decreasing HDL (Hegsted, 1990).

In addition, the fatty acid composition of meat products seems to be related with their oxidative stability. This is important in terms of the shelf life of meats, and on the possible negative effects of lipid oxidation products which increase the risk of atherosclerosis (Kubow, 1993). In particular, the oxidation of LDL cholesterol appears to contribute to the development of heart disease (Dwyer, 1995).

Objective

The objectives of the study were to determine the effects of 'natural' antioxidants, namely vitamin E and β-carotene, on the oxidative stability of poultry meats -fresh and cooked- fed different sources of fat, lard (saturated), olive oil (monounsaturated) or sunflower oil. Later, the analytical results were compared to the evaluation of a trained panel, in order to establish the importance of certain analytical parameters on sensory characteristics.

Materials and methods

Broiler chickens were fed a single diet throughout the experiment between 1 and 42 days of age. The diet was a typical maize-soy diet supplemented with 6% of each of the fat types (lard, sunflower or olive oil). The basal diet contained 20 mg/kg of supplemental vitamin E plus that of the ingredients which resulted in about 30 mg/kg of vitamin E (Table 1). This diet was supplemented with either 200 mg/kg of vitamin E, or 15 or 50 mg/kg of β-carotene. At 42 days, chickens were processed in a commercial processing plant, and their thighs obtained and stored at -20°C, except for some determination such as colour and sensorial evaluation, which was performed with samples stored in polyethylene bags at 2-4°C within 3 days, or a maximum of 7 days for colour. Storage tests were conducted on samples which had been previously frozen, and thawed at room temperature over 5 h, at 4°C for 7 days under fluorescent light.

Fresh muscle

(i) Fatty acid profile, by gas chromatography of the methyl esters, in the total lipid fraction, and in the phospholipid and free fatty acid fractions.

(ii) Lipid peroxidation. Thiobarbituric reactive substances (TBARS). TBARS was measured immediately after thawing, in cooked meat and after 7 days of chilled storage at 4°C under fluorescent light (12 h).

(iii) Vitamin E. HPLC method and fluorimetric detection.

(iv) Colour was measured with a Minolta Chromameter.

(v) Water holding capacity was measured by different procedures: drip losses, expressible water (centrifugation) and cooking losses.

Table1. Composition of the basal diets

Ingredient	Exp 1	Exp 2	Exp 3
Maize	50.56	50.56	50.56
Soybean meal	39.58	39.58	39.58
Lard	6.00		
Sunflower oil		6.00	
Olive oil, refined			6.00
Calcium carbonate	1.00	1.00	1.00
Dicalcium phosphate	2.00	2.00	2.00
Salt	0.40	0.40	0.40
DL-methionine	0.16	0.16	0.16
Vitamins and minerals[†]	0.40	0.40	0.40

[†]One kg of feed contained: Vitamin A: 12,000 IU; Vitamin D_3: 2,400 IU; *Vitamin E: 20 mg*; Vitamin K_3: 2 mg; Vitamin B_1: 2.0 mg; Vitamin B_2: 5 mg; Vitamin B_6: 3.5 mg; Vitamin B_{12}: 15 µg; Folic acid: 0.6 mg; Biotin: 200 µg; Calcium pantothenate: 15 mg; Nicotinic acid: 30 mg; Mn: 332 mg; Zn: 50 mg; I: 1.19 mg; Fe: 85 mg; Cu: 9 mg; Se: 0.15 mg

Cooked muscle

Cooking of samples was conducted in polyethylene bags. Samples were placed in a water bath at 85°C, until inside temperature reaches 80°C (50 min). Samples were cooked before each determination.

Cholesterol oxides

The method is based on extraction with organic solvents, cleanup with florisil columns, removal of excess cholesterol by TLC and analysis of the following cholesterol oxides: 7α-hydroxycholesterol, 7β-hydroxycholesterol, α-epoxycholesterol, β-epoxycholesterol, 20α-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, Triol and cholesterol by GLC and PTV injection. Detection is by FID. Identification was verified using GC-MS. Overall recoveries were above 90, and repeatability presented RSD below 10%. This method minimizes degradation of cholesterol oxides and artifact formation during the analytical process.

Volatiles

Determination was performed as follows: the samples was heated at 90°C in a closed glass container. The sample was then left to cool at room temperature and after 15 min the container was opened and connected to nitrogen stream, by which the volatiles were collected which were trapped in a cartridge of graphited charcoal. This was then placed on a microwave energy desorber (Retorik, Switzerland) which is connected directly to a capillary column (5% phenylmethylsilicone, 40 m x 180 µm, 0.4 µm). Detection was by mass spectrometry. This is intended to simulate the conditions of the sensorial analysis, in order to compare results.

Sensorial evaluation

A sensorial evaluation of the 9 treatments corresponding the first set of experiments has been conducted with 6 expert panelists. The design of the experiment was a randomized incomplete block design in 16 sessions. Each day, 3 treatments were compared in addition to a negative control

containing no added far nor any additive (pro- or antioxidant). The descriptive profile included 25 attributes of which 14 were on the cooked product (broiled in the oven at 170°C for 25 min to achieve internal temperature of 80°C) and 11 on fresh meat, one day after slaughter. Samples were stored at 4°C and vacuum sealed. The order of presentation of samples was balanced for each member of the panel.

The following traits were determined:

(i) Fresh meat

- Colour: of meat, of skin, of fat (yellowness).
- Odors: bitter/acid, rancid, sulphur, others (to be defined).

(ii) Cooked meat

- Odors: grass, sulphur, liver, toasted, rancid, meat broth, cooked vegetables, cardboard, others.
- Flavour: same as odors plus astringent, metallic, chemical, sweet, bitter, others.
- Texture: juiciness, oiliness, fibrousness.

Results

Effect of dietary acid profile on lipid composition, meat quality and oxidative stability

Dietary fat affected the fatty acid composition of intramuscular fat, in the sense that tended to reflect the dietary profile. This was quite apparent in the total lipid fraction (Table 2). Lard resulted in the greatest amount of saturated fatty acids, sunflower in polyunsaturates and olive oil in monounsaturates, although the amount of monounsaturates was relatively important in lard. Polyunsaturates were similar in lard and olive oil. In the phospholipid fraction, there were more monounsaturated fatty acids in the lard than in the olive oil chickens, while in the free fatty acids, both fats gave a similar profile. This suggests that the different fractions give a different response to the dietary profile. However, it is clear that sunflower resulted in a considerable increase in the proportion of polyunsaturated in all fractions mostly at the expense of monounsaturated fatty acids, which could be responsible for the greater oxidation observed in several parameters (see below).

Table 2. Fatty acid profile of intramuscular fat

	Total			Phospholipids			Free fatty acids		
	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive
Saturated	34.8	29.5	27.6	34.4	35.4	32.2	25.5	22.5	27.4
Monounsaturated	38.6	29.6	45.1	22.3	11.3	17.2	36.5	20.3	36.6
Polyunsaturated	26.6	40.9	27.3	37.9	44.0	39.6	37.2	55.2	34.1
w-3	1.4	0.7	1.2	3.5	2.6	3.6	2.8	0.3	1.3
w-6	25.2	40.1	26.0	34.4	41.4	36.0	34.4	54.9	32.8
Polyun/saturat	0.77	1.39	0.99	1.10	1.24	1.23	1.46	2.45	1.24
w-6/w-3	18.54	56.00	21.78	9.83	15.92	10.00	12.29	183.0	25.23

Triglycerides were also determined (not shown). Type of fat had a strong influence on the proportions of triglycerides with 50 and 54 CN. Thus, lard presented proportions of PPO, PPL, and PPS greater than OOO, SSO, SOO, OOL and OLL, while chickens fed the vegetable oils presented the opposite situation.

TBARS was measured immediately after thawing, in cooked meat and after 7 days of chilled storage at 4°C under fluorescent light (12 h). Iron-induced TBARS was also measured (not shown) Results do not always parallel those of the non-induced measurements, specially for sunflower and olive oil after 7 days of storage.

Vitamin E was very efficiently deposited in intramuscular fat, when compared to other species. As an example, work from other members of the Dietox project indicated that muscle levels in pigs or rabbits fed diets supplemented with 200 mg/kg of vitamin E were around 6 mg/kg of tissue, while in turkeys the concentration in sartorius muscle was similar to that in chickens. In beef, supplementation with 2,150 IU/head/day resulted in levels of 4 to 8 mg/kg of meat, depending on the muscle (den Hertog-Meischke *et al.,* 1997). This would suggest that poultry meat is a very good vehicle for vitamin E administration via food.

TBA and vitamin E showed a reciprocal response (see Fig. 1). Thus, as vitamin E increased, TBARS decreased in a curvilinear fashion. The shape of the curves for lard and olive oil for fresh and cooked meat was similar as shown by the A and B parameters which define the equations (Table 3) and the points were the x value of the first derivative equals -1. This parameter represents the level at which, for a marginal increase of vitamin E, corresponds an identical decrease (in the units used) of TBARS. However, for 7 days of chilled storage values tended to be lower for olive oil. Sunflower shows greater values of the A and B parameters and x for y' = -1, suggesting that sunflower shows a greater susceptibility to oxidation. This was confirmed in the iron induced TBARS (not shown) in which vitamin E had a protective effect, but much smaller than that of the lard and olive oil chickens.

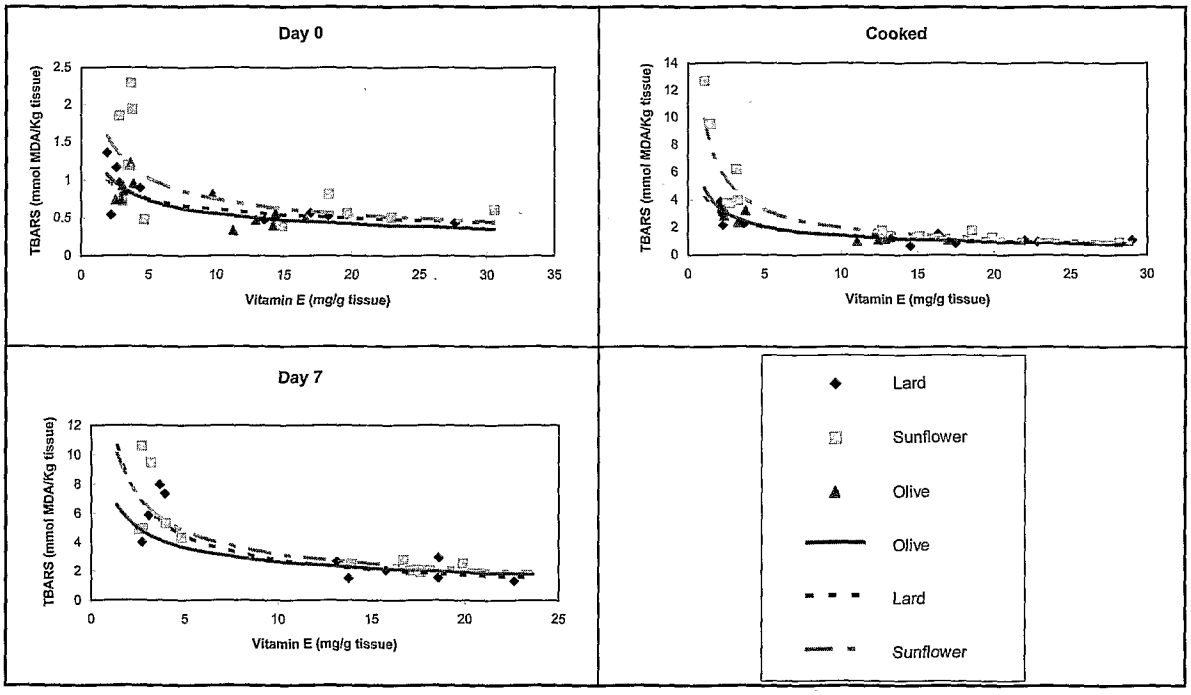

Fig. 1. TBA and vitamin E.

Results of β-carotene were rather complex. In the first experiment with lard, β-carotene was used at a concentration of 50 mg/kg, and showed a pro-oxidant effect (Table 4). In subsequent experiments it was lowered to 15 mg/kg. In the sunflower chickens, β-carotene showed an antioxidant effect both at 15 and 50 mg/kg, except for day 7. In olive oil, the effect was in general protective, in cooked meat at the low concentration, but not at 50 mg/kg, or in 7 days of chilled storage. The apparent erratical behaviour of β-carotene is more clear when the concentrations of vitamin E in muscle are examined (Table 5). Thus, in lard and olive oil, β-carotene reduced the vitamin E levels, specially at 50 mg/kg, while it had no effect in sunflower. It must be noted that sunflower oil contains a considerable amount of vitamin E, for which reason the control of the sunflower diets contained about twice as much

vitamin E as the lard and olive oil diets. When the results of β-carotene are examined in conjunction with those of tissue vitamin E, they suggest that β-carotene has an antioxidant effect when vitamin E concentrations are above a certain level. However, when the vitamin E concentration decreases (possibly as a result of competition during absorption) β-carotene shows no effect, and even in some cases a pro-oxidant effect. This pro-oxidant effect may be indirect, due to the reduction of vitamin E, or direct.

Table 3. Parameters of the equations

	Equation $y = A\,x^{-B}$		R^2	y' = -1 when x = ...
Lard				
Fresh	A = 1.19	B = 0.29	0.60	0.44
Cooked	A = 4.41	B = 0.49	0.81	1.68
Stored	A = 13.28	B = 0.68	0.75	3.71
Sunflower				
Fresh	A = 2.06	B = 0.46	0.45	0.96
Cooked	A = 10.55	B = 0.72	0.92	3.25
Stored	A = 12.28	B = 0.58	0.78	3.47
Olive				
Fresh	A = 1.39	B = 0.40	0.58	0.65
Cooked	A = 5.09	B = 0.56	0.89	1.95
Stored	A = 7.60	B = 0.46	0.74	2.35

Table 4. TBARS (µmol MDA/kg of tissue) in muscle

Treatment	TBARS								
	Day 0			Cooked			Day 7		
	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive
Control	0.94^{bc}	1.41^{a}	0.91^{a}	2.96^{a}	6.64^{a}	2.97^{a}	6.29^{b}	6.59^{b}	4.61^{b}
Vit. E 200	0.51^{c}	0.56^{b}	0.52^{b}	1.03^{b}	1.41^{b}	1.21^{c}	2.01^{c}	2.37^{c}	2.43^{c}
β-car 15	1.18^{ab}	0.99^{ab}	1.10^{a}	3.94^{a}	3.52^{b}	2.13^{b}	9.72^{b}	9.18^{b}	3.57^{bc}
β-car 50	1.54^{a}	1.24^{a}	1.10^{a}	3.68^{a}	3.64^{b}	2.97^{a}	22.24^{a}	17.57^{a}	10.46^{a}
SE	0.18	0.19	0.12	0.50	0.78	0.22	2.42	2.13	1.41

a,b,c: Means within a column followed by different superscripts are significantly different $P<0.05$

During 7 day chilled storage, β-carotene shows a pro-oxidant effect in all diets, which could be caused by destruction of vitamin E during the regeneration of β-carotene radicals developed during oxidation (Palozza and Krinsky, 1992). Table 6 shows the actual and expected TBARS values as calculated from the equations from Table 3. At day 0, TBARS are low, and there is good agreement between predicted and measured values, suggesting little effect of β-carotene, except for lard and β-carotene at 50 ppm, which suggests a slight prooxidant effect. In cooked meat, measured TBARS in all β-carotene groups are lower than expected, suggesting an antioxidant effect of β-carotene. In contrast, chilled storage during 7 days shows the same pattern for all fats, in the sense that predicted TBARS for β-carotene at 50 ppm are always lower than the measured TBARS, suggesting that β-carotene has a pro-oxidant effect *per se*. This suggests that in order to obtain an antioxidant effect

of β-carotene, tissue vitamin E must reach a threshold level, although this should be confirmed. It is not possible to predict what would occur if tissue levels of vitamin E were greater, in the presence of β-carotene at 50 ppm.

Table 5. Vitamin E in muscle (µg/g tissue)

Treatment	Vitamin E								
	Day 0			Cooked			Day 7		
	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive	Lard	Sunfl.	Olive
Control	2.81^{b}	3.56^{b}	3.20^{b}	2.48^{b}	2.37^{b}	2.79^{b}	2.93^{b}	3.34^{b}	3.19^{b}
Vit. E 200	19.20^{a}	20.11^{a}	13.17^{a}	20.36^{a}	17.89^{a}	13.13^{a}	17.07^{a}	16.76^{a}	11.75^{a}
β-car 15	2.48^{b}	4.18^{b}	3.49^{b}	1.45^{c}	2.53^{b}	2.16^{bc}	1.79^{c}	2.60^{bc}	2.60^{b}
β-car 50	1.53^{c}	3.60^{b}	2.15^{c}	1.21^{c}	1.90^{b}	2.00^{c}	1.33^{c}	1.53^{c}	1.90^{c}
SE	0.92	1.27	0.57	1.16	1.16	0.50	0.80	0.60	0.30

a,b,c: Means within a column followed by different superscripts are significantly different P<0.05

Table 6. TBARS predicted by equations showed in Table 3

Treatment	β-car 15								
	Lard			Sunflower			Olive		
	Vit. E measur.	TBARS measur.	TBARS predic.	Vit. E measur.	TBARS measur.	TBARS predic.	Vit. E measur.	TBARS measur.	TBARS predic.
Day 0	2.48	1.18	0.91	4.18	0.99	1.07	3.49	1.10	0.84
Cooked	1.45	3.94	3.68	2.53	3.52**	5.39**	2.16	2.13**	3.31**
Day 7	1.79	9.72	8.95	2.60	9.18*	7.03*	2.60	3.57**	4.91**
	β-car 50								
	Lard			Sunflower			Olive		
	Vit. E measur.	TBARS measur.	TBARS predic.	Vit. E measur.	TBARS measur.	TBARS predic.	Vit. E measur.	TBARS measur.	TBARS predic.
Day 0	1.53	1.54*	1.05*	3.60	1.24	1.15	2.15	1.10	1.02
Cooked	1.21	3.68	4.02	1.90	3.64**	6.63**	2.00	2.97	3.45
Day 7	1.33	22.24*	10.95*	1.53	17.57*	9.58*	1.90	10.46*	5.67*

*β-carotene acts as a pro-oxidant *per se*
**β-carotene acts as an antioxidant *per se*

The antioxidant effect of β-carotene is obtained at very low dietary level (15 mg/kg). Apparently there is no further response at greater concentrations, suggesting that a small concentration in tissue is sufficient. It has not been possible to detect β-carotene in tissue, but the conversion to retinol is unlikely, since retinol was not detected in muscle (not shown). These results also suggest that vitamin E and β-carotene may have a synergistic effect, and that it could be possible to find combinations of

both antioxidants that would have similar effects to those obtained with a higher level of vitamin E. This is supported by some observations *in vitro* (Palozza and Krinsky, 1992) However, results of chilled storage at 7 days, indicate that this should be confirmed.

Colour

Colour measurements were influenced by dietary fat and by time of storage, while antioxidant treatment did not show any clear effect (not presented). *L* and *b* values were slightly greater with lard and olive than with sunflower (Table 7). It must be mentioned that during the sensorial evaluation, chickens fed sunflower were perceived as showing less yellow colour than those receiving lard or olive oil (Table 13). Saturation (SI) and Hue (not shown) were greater for lard and olive oil than for sunflower oil. Time of storage tended to decrease *L*, and increased *a*, while *b* increased during the first three days and then decreased after 6 days. The increase in *a* (redness) was not caused by an increase in the amount of oxymyoglobin or metmyoglobin (not shown), however the amount of total myoglobin measured increased with time. Since it unlikely that there is myoglobin synthesis during storage, a possible explanation is that proteolysis postmortem makes myoglobin more readily extractable during the analytical process, and at the same time the effects of myoglobin on redness become more apparent as a result of this proteolytic process.

Table 7. Colour measurements

Day	L			a			b		
	Lard	Sunf.	Olive	Lard	Sunf.	Olive	Lard	Sunf.	Olive
1	56.33	53.88	56.96	2.35	2.37	2.33	4.10	3.11	4.38
3	55.98	54.45	57.01	2.67	2.74	2.83	5.24	4.30	5.59
6	54.96	53.09	55.36	2.98	3.74	3.65	5.50	4.03	5.30
7	53.84	52.46	54.55	3.81	3.97	3.88	4.88	3.70	4.92

Cooking losses

Water holding capacity was measured by different procedures: drip losses, expressible water (centrifugation) and cooking losses. The fist two measurements (not shown) did not show any effect of type of fat or antioxidant. It must be mentioned that this losses are very small, specially as compared to other species like pork. Cooking losses were affected by type of fat (Table 8), olive oil causing greater losses than either lard or sunflower oil. The reason for this difference is not clear.

Table 8. Cooking losses in fresh muscle

Fat	Percent water loss (%)				
	Control	Vit. E 200	β-car 15	β-car 50	Mean
Lard	4.66	4.71	4.52	4.90	4.69^{b}
Sunflower oil	5.42	5.39	4.10	-	4.88^{ab}
Olive oil	5.23	6.07	5.07	-	5.47^{a}
Mean	4.97	5.39	4.56	4.90	

a,b: Mean values with different small letters are significantly different (P<0.05)

Cholesterol oxides

Vitamin E showed a protective effect on the formation of cholesterol oxides (Table 9). The oxides detected were: 7α-hydroxycholesterol, 7β-hydroxycholesterol, α-epoxycholesterol, β-epoxycholesterol, 20α-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, Triol and cholesterol. In fresh muscle, the concentrations were very small, below 1 µg/g, but increased during cooking up to tenfold. Dietary fat influenced the amount present. Olive oil gave the lowest and sunflower the highest concentrations. β-Carotene did not show significant effects.

Table 9. Cholesterol oxides

	Fresh			Cooked		
	Control	Vitamin E	β-carotene	Control	Vitamin E	β-carotene
Lard						
ox†	0.39	0.17	0.39	3.69	0.97	2.27
chol††	60.99	64.25	70.83	92.05	91.94	93.27
ox/chol (%)	0.06	0.03	0.06	0.4	0.11	0.24
Sunflower						
ox†	0.48	0.22	0.26	4.3	2.9	5.06
chol††	56.67	53.86	58.52	79.16	86.51	86.98
ox/chol (%)	0.09	0.04	0.04	0.54	0.34	0.58
Olive						
ox†	0.17	0.05	0.33	1.81	1.18	2.21
chol††	51.1	49.1	49.96	77.48	79.6	74.01
ox/chol (%)	0.03	0.01	0.07	0.23	0.15	0.3

†Expressed in µg per g of muscle
††Expressed in mg/100g of muscle

Volatile profile

The main volatiles detected were: 2-methylpentane, 3-methyl pentane, hexane, octane, styrene, α-methylstyrene, 1-octene, 1-dodecene, dodecane, tridecane, tetradecane, p-xylene, 1,2-dimethylbencene, pentanal, hexanal, octanal, nonanal, decanal, benzaldehyde, phenol, benzylalcohol, 2-butoxyethanol, 1-butanol 1-octen-3-ol and 1-dodecanol (Table 10). The concentration of hexanal, the major aldehyde, was greater in the chickens receiving sunflower oil (Table 11). Vitamin E decreased by 30 to 50% its concentration in all dietary fats except for sunflower which only decreased by 20%. β-Carotene did not reduce the concentration of hexanal. In the case of sunflower and lard, results parallel those of TBA, but not in the case of olive oil, in which β-carotene reduced TBA both in cooked meat (Table 4), and in the iron induced TBA (not shown).

Sensorial evaluation

There were no significant fat x antioxidant interactions, indicating that effects were independent. The traits which showed significant differences are shown in Table 12 and in Table 13. As can be seen, there were some significant differences, but rather small in magnitude, indicating that most of the effects were marginal. The only important difference was is a pronounced effect on the tactile firmness of the fat. Birds fed lard showed a more firm fat in all cases. There was no clear effect of the antioxidant on the sensorial characteristics associated with this trait, except for the fact that chickens receiving β-carotene tended to show more rancid traits than the other treatments. This would be in agreement with the results of the hexanal measurements, in which β-carotene increased hexanal, except in sunflower oil. In any case, this effect was not very pronounced.

Table 10. Main components of the volatile profile

Compound	Lard	Sunflower	Olive oil
Pentane-2methyl	++	++	-
Pentane-3methyl	++	++	-
Hexane	+++	++	++
1-hexene	-	-	++
1-octene	-	-	++
1-dodecene	++	+	++
Dodecane	++	+	+++
Tridecane	++	++	++
1-tetradecene	+	+	++
Tetradecane	++	++	++
1,2-dimethylbenzene	-	-	+
Ethylbenzene	-	-	+
Pentanal	+	+	-
Hexanal	+++	+++	+
Heptanal	+	+	-
Octanal	+	+	-
Nonanal	++	++	+
Decanal	+	+	-
Benzaldehyde	-	-	++
Styrene	+	+	+
1-octen-3-ol	+/-	+	-

Table 11. Hexanal concentrations (ng/g)

	Control	Vitamin E	β-carotene	
			15	50
Lard	318.4	144.7		623.6
Sunflower oil	742.3	609.6	618.2	
Olive oil	146.4	85.0	416.9	

Table 12. Effect of antioxidant treatments on sensorial characteristics

	Control	Vitamin E	β-carotene 15 mg/kg
Rancid odor	0.86^{ab}	0.61^{b}	1.03^{a}
Rancid flavour	0.04^{b}	0.00^{b}	0.21^{a}
Peanut flavour	1.25^{b}	1.12^{b}	1.49^{a}
Initial juiciness	5.10^{ab}	4.89^{b}	5.34^{a}
Tenderness	4.78^{b}	5.05^{ab}	5.28^{a}
Fibrosity	2.09^{a}	1.99^{ab}	1.66^{b}
Colour uniformity	5.00^{a}	4.57^{b}	4.38^{b}
Adhesion to teeth	2.56^{a}	2.61^{b}	3.07^{b}
Raw chicken	4.90^{b}	5.32^{ab}	5.42^{a}

a,b: Means followed by different letters are significantly different ($P<0.05$)

Table 13. Effect of dietary fat on sensorial characteristics†

	Lard	Sunflower oil	Olive oil
Yellow colour	4.26^{ab}	3.78^{b}	4.27^{a}
Dark colour	4.99^{a}	4.62^{ab}	4.41^{b}
Hardness of fat	4.74^{a}	2.76^{b}	3.18^{b}

†Scores from 0 to 10
a,b: Means followed by different letters are significantly different (P<0.05)

Conclusions and recommendations

Results of this study suggest that type of fat and supplementation with antioxidants has several effects on meat characteristics:

(i) Dietary fat affected the fatty acid composition of intramuscular fat, in the sense that tended to reflect the dietary profile. This was quite apparent in the total lipid fraction. Sunflower resulted in a considerable increase in the proportion of polyunsaturated in all fractions mostly at the expense of monounsaturated fatty acids, which could be responsible for the greater oxidation observed in several parameters.

(ii) Polyunsaturated fat supplementation results in deposition of intramuscular fat which is more oxidized, and more susceptible of oxidation. Monounsaturated fat shows a pattern similar to saturated fat.

(iii) TBA and vitamin E showed a reciprocal response. The shape of the curves for lard and olive oil was similar. Sunflower shows a greater susceptibility to oxidation.

(iv) When the results of β-carotene are examined in conjunction with those of tissue vitamin E, they suggest that β-carotene has an antioxidant effect when vitamin E concentrations are above a certain level. However, when the vitamin E concentration decreases (possibly as a result of competition during absorption) β-carotene shows no effect, and even in some cases a pro-oxidant effect. This pro-oxidant effect may be indirect, due to the reduction of vitamin E, or direct in chilled storage during 7 days. This suggests that in order to obtain an antioxidant effect of β-carotene, tissue vitamin E must reach a threshold level. The effect of β-carotene is obtained at very low dietary level (15 mg/kg). Apparently there is no further response at greater concentrations, suggesting that a small concentration in tissue is sufficient. These results also suggest that vitamin E and β-carotene may have a synergistic effect, and that it could be possible to find combinations of both antioxidants that would have similar effects to those obtained with a higher level of vitamin E. Result of chilled storage during 7 days indicate some destruction of vitamin E in the presence of β-carotene, which emphasizes the need for a greater level of vitamin E in order to obtain an antioxidant effect of β-carotene, although this should be confirmed. In fact, β-carotene shows a pro-oxidant effect *per se* under these conditions. Further studies are needed to determine if β-carotene can act as an antioxidant when tissue levels of vitamin E are higher.

(v) Vitamin E showed a protective effect on the formation of cholesterol oxides. In fresh muscle, the concentrations were very small, below 1 μg/g, but increased during cooking up to tenfold. Dietary fat influenced the amount present. Olive oil gave the lowest and sunflower the highest concentrations. β-Carotene did not show significant effects.

(vi) The concentration of hexanal, the major aldehyde, was greater in the chickens receiving sunflower oil. Vitamin E decreased by 30 to 50% its concentration in all dietary fats except for sunflower which only decreased by 20%. β-Carotene did not reduce the concentration of hexanal.

(vii) Colour measurements were influenced by dietary fat and by time of storage. *L* and *b* values were slightly greater with lard and olive than with sunflower. It must be mentioned that during the

sensorial evaluation, chickens fed sunflower were perceived as showing less yellow colour than those receiving lard or olive oil. Saturation (SI) and Hue (not shown) were greater for lard and olive oil than for sunflower oil. Time of storage tended to decrease *L*, and increased *a*, while *b* increased during the first three days and then decreased after 6 days. The increase in *a* (redness) was not caused by an increase in the amount of oxymyoglobin or metmyoglobin (not shown), however the amount of total myoglobin measured increased with time. Since it unlikely that there is myoglobin synthesis during storage, a possible explanation is that proteolysis postmortem makes myoglobin more readily extractable during the analytical process, and at the same time the effects of myoglobin on redness become more apparent as a result of this proteolytic process.

(viii) The sensorial evaluation was conducted on fresh meat, differently from most of the other parameters of the study which were conducted on frozen meat. In general results indicate that there is more effect of the type of supplemental fat (lard, sunflower oil, or olive oil) than of the antioxidant added. There is a pronounced effect on the tactile firmness of the fat. Birds fed lard showed a more firm fat in all cases. There was no apparent effect of the antioxidant treatments on rancidity, nor in the sensorial characteristics associated with this trait, except for β-carotene which caused an increase in some rancid traits, in agreement with the observations for hexanal in the volatile profile. In any case, these results would suggest that the incorporation of antioxidants into the diet of broiler chickens does not cause any marked effect on the sensorial quality of meat, in the conditions of the assay.

(ix) TBARS during chilled storage does not give the same pattern of response as iron-induced TBARS, as shown by the results of β-carotene. Therefore, iron-induced TBARS may not be a good predictor of oxidative status during storage.

(x) Cooking losses were affected by type of fat, olive oil causing greater losses than either lard or sunflower oil.

Recommendations

From the conclusions listed above, there are some recommendations which concern the industry and the consumer:

(i) The most apparent effect that the consumer detects from a sensorial point of view when dietary fat is modified is the consistency of fat. If meat is consumed fresh during the first three days, differences caused by type of fat or antioxidants are very small and hardly detected. Cooking losses seem to be affected by type of fat, monounsaturates causing greater losses. Colour is also affected, sunflower resulting in less yellow colour, both as measured instrumentally or by a trained panel.

(ii) There are other indicators of the oxidative status, that at least in frozen meat -which can be considered a stressor- indicate that type of fat and antioxidants have some effects which may be important from the point of view of the 'safety' for the consumer, in particular with some risk factors related to oxidative status. Thus, TBARS as a model for oxidation, and cholesterol oxides, which have been implied in the development of atherosclerosis are affected by dietary fat and antioxidants.

(iii) With regard to antioxidant, vitamin E gave an overall protective effect on several parameters. β-Carotene shows rather complex effects which can be summarized suggesting that it shows an antioxidant effect as measured by TBARS, but only when the concentration of vitamin E is above some threshold level. The effect is at low doses (15 mg/kg) and if vitamin E in the diet is low, competition for absorption may reduce the amount of vitamin E in tissues, resulting in an overall pro-oxidant effect. It is possible that some combinations of vitamin E and β-carotene could have a similar antioxidant effect to that obtained with greater concentrations of vitamin E, which at the moment result too expensive to be used in practice. However, caution must be taken if β-carotene is used. If vitamin E levels are too low, it can exert a pro-oxidant effect, either by competition for absorption, or during storage, because vitamin E is used in the regeneration of β-carotene radicals. This should be further explored to confirm that in chilled storage, β-carotene and higher tissue levels of vitamin E have an antioxidant effect.

(iv) Iron-induced TBARS does not parallel normal TBARS during chilled storage, as shown by the results of β-carotene. Therefore, if the oxidative status during storage is to be monitored, iron-induced TBARS may not be an appropriate test.

(v) Poultry meat can be also a source of 'natural' antioxidants for humans, when supplemented in the diet of broilers, since they show a high efficiency of deposition, greater than that found in pigs or turkeys.

(vi) From these considerations, it can be said that supplementation with antioxidants could be desirable to improve the 'safety' of poultry meat with regard to the oxidative stability and as a source of antioxidants, since poultry deposits vitamin E more efficiently than other species. Vitamin E is a very good antioxidant, but it would be interesting to investigate if certain combinations of vitamin E and β-carotene can achieve similar results to those of high concentrations of vitamin E, which at the moment are considered too costly to be adopted in practice. However, β-carotene should be used in combination with vitamin E, since it can exert some pro-oxidant effects, specially during chilled storage.

(vii) With regard to supplementation of β-carotene in humans, this study suggest that β-carotene alone may have adverse effects on oxidative status, and that vitamin E levels should be closely monitored when β-carotene is supplemented. In contrast, it is possible that combinations of vitamin E and β-carotene in appropriate amounts could produce the same antioxidant effects as higher levels of vitamin E.

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