

# Effect of four natural antioxidants on automatically deboned broiler meat

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Abbreviated title: Natural antioxidants on ADM

## Summary

Many antioxidants barriers are defeated after chicken slaughtering. It is well known that of lipid oxidation of broiler meat causes a fast deterioration in its nutritional and sensory parameters (De Winne and Dirink, 1996). Automatically deboned meat (ADM) is very susceptible to suffer lipid oxidation. The use of synthetic antioxidant has been questioned.

The aim of our study was to evaluate the protective effect of applying 4 natural antioxidants and one synthetic antioxidant on ADM. We evaluated the degree of lipid oxidation of ADM through TBARS and peroxides analysis. In addition, microbiological quality of ADM was assessed by aerobic mesophilic bacteria counting.

Basal and induced lipid oxidation levels in general were lower as a result of the application of antioxidants when compared to the control sample. Synthetic antioxidant produced lower lipid oxidation levels; yet, this result was similar to the outcome obtained by the application of two natural antioxidants (oregano and quillay). Regarding microbiological analysis, the different antioxidants used did not reduce bacterial counts.

We concluded that the application of natural antioxidants was successful in delaying the lipid oxidation.

## Introduction

Trough time, food and specially meats suffer different processes which end up with their deterioration (De Winne and Dirink, 1996). The oxidation of lipids is one of these processes. In fact, the lipid oxidation is considered as one of the main ways meat deteriorates (Valenzuela and Nieto, 1996). Broiler meat is particularly susceptible to lipid oxidation due to the composition of its lipids (Grau *et al.*, 2001). One way to delay lipid oxidation is applying antioxidants (AOX) to broiler feed (Fellenberg and Speisky, 2006) and another way is applying the antioxidants directly to the meat (Fellenberg *et al.*, 2007). Nevertheless, synthetic antioxidants are questioned due to the possibility that at high doses some of these agents may be exert carcinogenic and/or mutagenic effects (Kahl and Kaplus, 1993), and thus, one way to protect the broiler meat of lipid oxidation is applying natural antioxidants. However, there is little information about the application of natural antioxidants directly to the meat. Fellenberg *et al.*, (2007a-2007b) applied quillay (*Quillaja saponaria*) extract and wine extract into the marinade of broiler meat diminishing the extent of lipid oxidation when compared to the control treatment without antioxidants. Also, research by Mielnik *et al.* (2008) concluded that the application of rosemary, sage or thyme decoction in the marinade of turkey meat reduced lipid oxidation and inhibited development of rancid off-flavours in stored meat. Gokoglu *et al.* (2009) evaluated the antioxidant protection of pomegranate sauce on anchovy meat, finding that this sauce diminished the lipid oxidation. Automatically deboned meat (ADM) is obtained through a process that uses high pressure at environmental temperature and in the presence of atmospheric oxygen. These conditions and a high content of fat in the extracted material increase the susceptibility of ADM to undergo lipid oxidation.

In a previous study we evaluated the effect of a polyphenol extract of quillay applied in the marinade of broiler meat obtaining good results (Fellenberg *et al.*, 2007a). The aim of this study was to evaluate the effect of four natural antioxidants and a synthetic one on lipid oxidation of ADM.

## Materials and Methods

The study was performed in a local slaughtering and processing plant. Antioxidants were applied to the poultry meat before entering the deboning machine. After deboning, a sample was taken and stored aerobically at 6°C for 0, 1, 2, 3, 4, 5, 6 and 7 days. After the appropriate time of refrigerated storage, samples were frozen at -80°C until analysis. The natural antioxidants that were evaluated were: quillay extract (QLPerm), citric extract (CE), oregano extract (OE) and rosemary extract (RE) at two concentrations each. The first concentration used was as recommended by the manufacturer (recommended dose-RD). The second concentration used was an equivalent in antioxidant activity estimated in the laboratory (equivalent dose-ED) by FRAP method (Benzie and Strain, 1996). Concentrations used are in Table 1. The synthetic antioxidant used as positive control was tBHQ (SAOX). The concentrations were determined as mentioned before. The control treatment was ADM without application of any antioxidant.

Table 1: Antioxidant concentrations

	Antioxidant (ppm/kg fat)				
	SAOX	CE	RE	QLPerm	OE
RD	1,000	500	1,000	1,000	400
ED	1,000	1,644	3,589	3,423	233

RD: Concentration recommended by manufacturer, ED: Concentration equivalent in FRAP units.

### **Analytical techniques:**

The antioxidant capacity of the AOX compounds was estimated with the FRAP method (Benzie and Strain, 1996). Antioxidant capacity was determined using a FeSO<sub>4</sub> (1-30 µM) standard curve. Lipid oxidation was estimated with TBARS method (Fellenberg *et al.*, 2008) and Peroxide index (Mehlenbacher, 1979). We performed three different TBARS analysis. The first one was basal lipid oxidation which indicates the extent of the lipid oxidation directly in the sample. The second and third analysis evaluated the susceptibility of the sample to suffer lipid oxidation under prooxidant conditions (incubation at 37°C and incubation at 37°C with Fe application). The aerobic mesophilic count was estimated with Petrifilm technic 3M (St. Paul, Minn., USA).

Statistical analysis: The experimental model was a completely randomized design with repeated measures in time. The covariance structure selected was unstructured of experimental units was 3 per treatment, with 8 measurements in time each. Polynomials equations were used to study the response through time. Adjust means were compared through Tuckey test. The data was analyzed using SAS software (version 9.1) with the MIXED procedure.

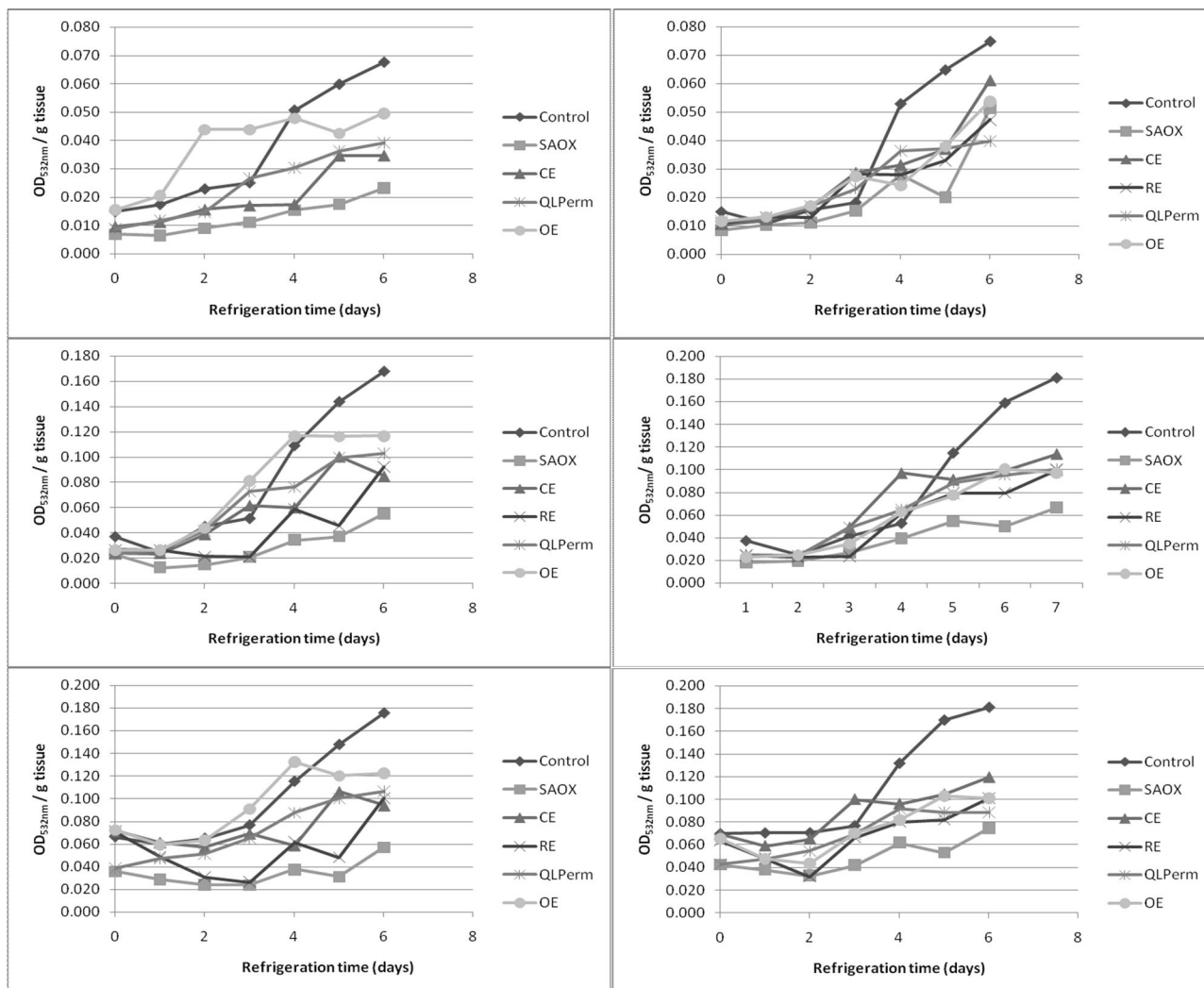
## Results and discussion

**TBARS content and lipid oxidation** (Figure 1): Lipid oxidation showed a linear response against refrigeration time ( $p < 0.0008$ ). In basal and induced by temperature lipid oxidation, TBARS were similar in SAOX and in natural AOX treatments at both concentrations.

At the recommended dose, basal TBARS was not different among SAOX, EC, RE and QLPerm, and the Control was higher than all the antioxidants treatments except OE (SAOX  $p = 0.0019$ ; CE  $p = 0.0143$ ; RE  $p = 0.0110$ ; QLPerm  $p = 0.0297$ ). Comparing temperature-induced TBARS the Control was not different to CE, QLPerm and OE, and in temperature plus Fe-induced TBARS the Control was not different to CE and OE.

At equivalent dose, basal and temperature-induced TBARS were not different among treatments. In temperature plus Fe-induced TBARS, the Control had higher TBARS except when compared to CE (SAOX  $p = 0.0014$ ; RE  $p = 0.0121$ ; QLPerm  $p = 0.0143$  and OE  $p = 0.0241$ ). These results are in good agreement with the results published by Fellenberg *et al.* (2007a; 2007b), Mielnik *et al.* (2008) and Gokoglu *et al.* (2009). All these authors demonstrated that the application of natural antioxidants delayed lipid oxidation in different meats. Additionally, in this study we demonstrated that effectiveness of natural antioxidant compared with synthetic antioxidant was similar.

Figure 1: Extend of lipoperoxidation measured as TBARS content. 1A: Basal lipoperoxidation at recommended dose. 1B: Induced lipoperoxidation by incubation at 37°C at recommended dose. 1C: Induced lipoperoxidation by incubation at 37°C and Fe application at recommended dose. 1D: Basal lipoperoxidation at equivalent dose. 1E: Induced lipoperoxidation by incubation at 37°C at equivalent dose. 1F: Induced lipoperoxidation by incubation at 37°C and Fe application at equivalent dose.

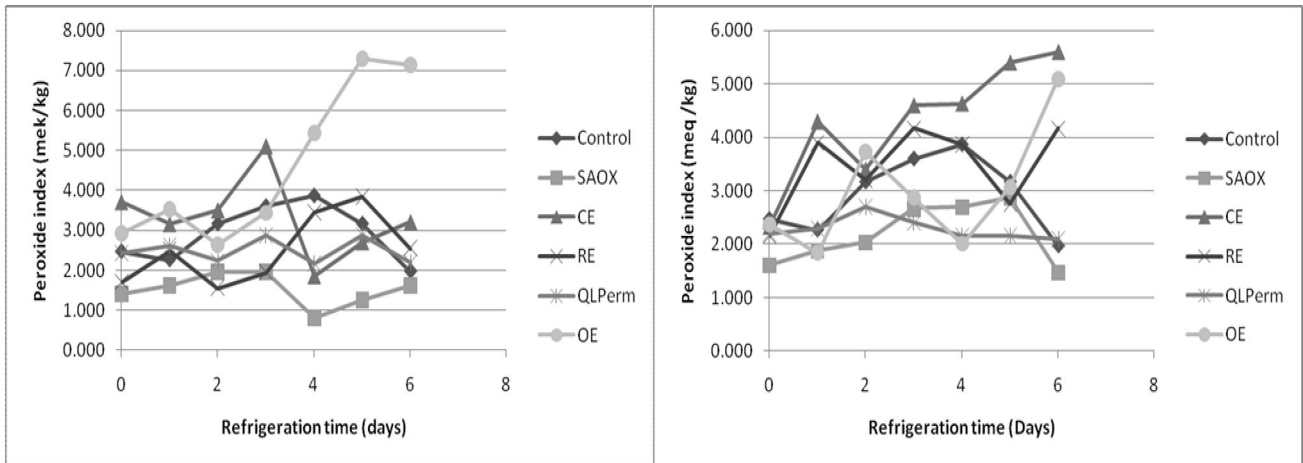


**Peroxide Index:** This index is also an indication of lipid oxidation. The main difference with TBARS is that TBARS are formed after peroxides by degradation of the peroxidized lipids. Thus, it is possible to observe a low peroxide index with a high TBARS content, because peroxides are reduced during the formation of TBARS.

At both concentrations of antioxidants, peroxide index was not different between Control and SAOX, CE, RE and QLPerm (Figure 2). Oregano extract had the highest peroxide index when the manufacturer-recommended

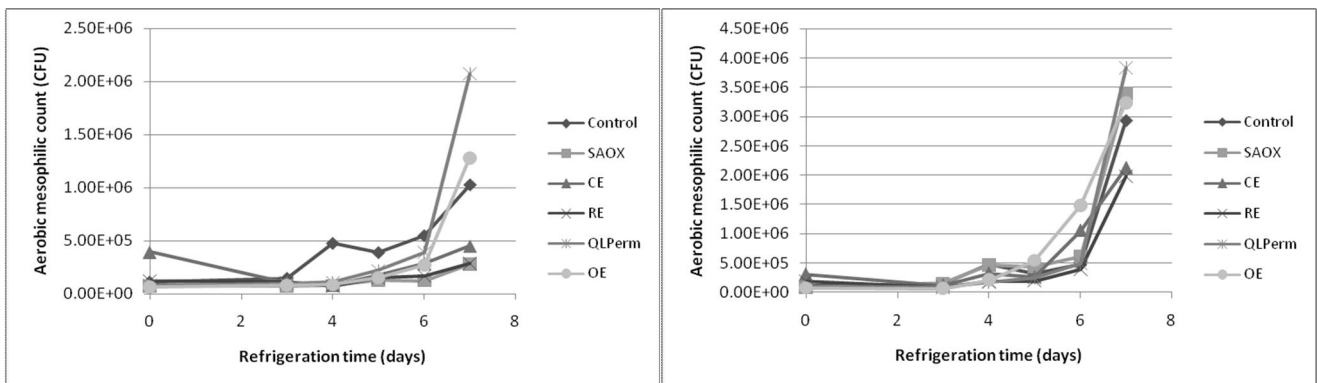
concentration was used. Probably the concentration recommended by the manufacturer is low compared with other AOX because in ED it was not difference between Control and OE.

Figure 2: Extend of lipoperoxidation measured as peroxide index (meq/kg). 2A: Peroxide index at recommended dose. 2B: Peroxide index at equivalent dose.



**Microbiological analysis:** The aerobic mesophilic count (AMC) is a common measure in the meat industry to evaluate the safety of the products. Some authors have observed that different kinds of plant extract can have a bacteriostatic or bactericide effect (Lis-Balchin *et al.*, 1998; Hao *et al.*, 1998; Dickens and Ingram 2001). In our study, all the antioxidant treatments (synthetic and natural) at the recommended concentration had no effect on AMC ( $p=0.36$ ). The same was observed when used the equivalent dose ( $p=0.97$ ) (Figure 3). These results differ with the results reported by Fellenberg *et al.* (2007a) where the quillay extract demonstrated a bactericide or bacteriostatic effect when compared with a control without antioxidant. More research is necessary to better evaluate the contribution of natural antioxidants to the microbiological preservation of meat.

Figure 3: Aerobic Mesophilic Count (AMC) in ADM. 3A: AMC at recommended dose. 3B: AMC at equivalent dose.



Due to its characteristics, ADM is highly susceptible to undergo lipid oxidation and addition of antioxidants may be needed to improve preservation. As expected, application of natural antioxidants reduced lipid oxidation on ADM. One important finding of our research was the similar response in SAOX and most of the natural AOX evaluated. The principal advantage of the natural antioxidant is that consumers perceived these additives as safe because of their origin. Even though their natural origin is not a guarantee of safety, plant extracts (oregano, sage, rosemary, etc) have been in popular use for long time without posing a threat for the people that consume them. That is not the case of synthetic antioxidants. Then, use of natural antioxidants has a great potential as a way to reduce the development of rancidity in stored poultry meat.

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