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Antioxidant activity in meat treated with oregano and sage essential oils

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Abstract

The antioxidant activity of meat treated (homogenized) with oregano and sage essential oils, during meat storage, was determined using the following assays: a thiobarbituric acid (TBA) assay, a diphenylpicrylhydrazyl (DPPH) assay and a crocin assay. Porcine and bovine ground meat samples were divided into three experimental treatments, namely: control (no antioxidant), oregano (oregano essential oil 3% w/w) and sage (sage essential oil 3% w/w). Subsequently, the samples from each treatment were stored at 4 °C, in the raw and cooked (at 85 °C for 30 min) state, and the antioxidant activity was determined after 1, 4, 8 and 12 days of storage. The results showed that the essential oil treatments significantly reduced the oxidation, while the heat treatment and storage time significantly affected the antioxidant activity of the meat. The role of antioxidants appeared to be much more important in cooked meat than raw and the meat proteins greatly affected the antioxidant activity.

Keywords: Antioxidant; Oregano; Sage; Essential oil; TBA; DPPH; Crocin

1. Introduction

Lipid oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavours and changes in the colour of meat (Guillén-Sans & Guzmán-Chozas, 1998). The complex mechanism by which this oxidation takes place, apart from membrane phospholipids, also affects proteins. This may lead to loss of protein solubility, loss of colour and reduced nutritional value. Vitamins are also oxidized during this process and, for this reason, vitamin E (α-tocopherol) is often used as an antioxidant. Vitamins A, β-carotene and ascorbic acid are also susceptible to oxidation. Vitamin oxidation may protect the fatty acids; however, the nutritional value of meat is negatively affected as a result of a general reduction in the availability of vitamins A, D, E and C (Madhavi, Deshpande, & Salunkhe, 1996).

Various antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely used to help meat preservation. However, over the past few years, increasing consumer demand for more natural, "preservative-free" products has led the food industry to consider the incorporation of natural antioxidants in a range of products. The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as "non chemical". In addition, they do not require safety tests before being used. Their drawbacks are that they are more expensive and less effective than are synthetic antioxidants. A quality consistency issue may also arise since antioxidant level and composition found in plants can be affected by the time of harvesting (Badi, Yazdani, Ali, & Nazari, 2004), and the variety (Ložienė, Vaičiūenė, & Venskutonis, 2003).

An assessment of the antioxidant activity of 22 herbs, such as oregano, sage, thyme, cinnamon, basil, black and

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white pepper, incorporated (as liquid extracts) at levels ranging from 0.5% to 2.5% w/w, on homogenized samples of porcine meat, revealed that lipid oxidation was prevented by all the extracts tested (Tanabe, Yoshida, & Tomita, 2002). Various plants that contain lycopene, such as tomato and red pepper, have been shown to have significant antioxidant activity (Sánchez-Escalante, Torrescano, Djenane, Beltrán, & Roncalés, 2003). Tea catechins (Tang, Sheehan, Buckley, Morrissey, & Kerry, 2001) and various carotenoids (Viljanen, Sundberg, Ohshima, & Heinonen, 2002) could also prevent lipid oxidation. Other plants used as antioxidants include *Origanum dictamnus* (Møller, Madsen, Aaltonen, & Skibsted, 1999), *Thymus sipyleus* subsp. *sipyleus* var. *Sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var.*Rosulans* (Tepe et al., 2005), *Origanum onites*, *O. indecredens*, *Salvia officilalis* and *S. fruticosa* (Pizzale, Bortolomeazzi, Vichi, Überegger, & Conte, 2002).

It is possible that animal nutrition can serve as a route to pass antioxidant activity from the diet to the meat. This has been confirmed in experiments conducted in broilers (Kulisic, Radonic, Katalinic, & Milos, 2004; Sárraga & Garcia Regueiro, 1999; Wong, Hashimoto, & Shibamoto, 1995) and turkeys with dietary oregano essential oil and α-tocopheryl acetate included in feed at concentrations ranging from 100 to 200 mg kg^{-1} feed (Botsoglou, Fletouris, Florou-Paneri, Christaki, & Spais, 2003; Botsoglou, Grigoroloulou, Botsoglou, Govaris, & Papageorgiou, 2003).

Meat processing and storage, prior to consumption can have a significant effect on meat quality (Pokorny, Yanishlieva, & Gordon, 2001). The aim of this work was to assess the antioxidant activity of two popular essential oils (namely oregano and sage) in porcine and bovine meat samples upon storage at 4 °C, in the raw or cooked state, over a 12 day period. It is known that antioxidant activity levels can vary significantly, depending on the detection method used (Pokorny et al., 2001). Therefore, in order to overcome possible methodology limitations in assessing antioxidant activity, this work has employed two different assay methods, namely a thiobarbituric acid (TBA) assay and a diphenylpicrylhydrazyl (DPPH) assay. Additionally, a crocin assay was employed to offer additional information in the experiment as it takes place in an aqueous solution and therefore detects the antioxidant activity of water-soluble substances.

2. Materials and methods

2.1. Meat sample preparation

Essential oils were extracted from oregano (*Origanum vulgare* subsp. *hirtum*) and sage (*Salvia officilalis*) with hydrodistillation from air-dried plants grown in Karditsa in central Greece and their compositions were determined using GC/MS according to Tepe et al. (2005).

Freshly cut porcine and bovine meat samples were obtained from the local market and visible fat was removed. Samples from each type of meat were then divided into three treatment groups and were homogenized with 3% w/w of either oregano essential oil (OREGANO) or sage essential oil (SAGE). Samples in the control (CONTROL) treatment had no essential oils added. Meat samples were then stored at 4 °C for 24 h before assays (see below) for antioxidant activity (i.e. day 1) were performed. Consequently, each sample was split into two 3 g portions of which one remained in the raw state and the other received heat treatment (at 85 °C for 30 min) and is referred herein as "cooked". Both raw and cooked samples were subsequently stored at 4 °C and assayed three more times (i.e. at 4, 8 and 12 days of storage) for antioxidant activity using the three assay methods (see below).

2.2. TBA assay

Oxidation of lipids is assessed by the TBA (thiobarbituric acid) assay which is based on the reaction between TBA and MDA (malondialdehyde) and the production of a coloured pigment, the concentration of which can be calculated by measuring the absorbance at 532 nm. Some of the MDA is formed during the oxidation process; however, most of it is generated by the decomposition of lipid peroxides during the acid-heat treatment of the assay (Guillén-Sans & Guzmán-Chozas, 1998).

For the TBA assay, a modified method of that described by Ruberto and Baratta (1999) was used. In brief, 0.05 g of each sample was mixed with 1 ml d H_2O, 1.5 ml of 20% acidic acid (pH was adjusted to 2 for maximum colouration according to Uchiyama & Mihara (1977)) and 1.5 ml 0.8% (w/w) of TBA in 1.1% (w/w) SDS in a test tube, vortexed and heated to 100 °C for 60 min in a water bath. After cooling, 5 ml butan-1-ol were added and mixed. Samples were then centrifuged at 10 000 rpm for 10 min. The absorbance of the upper layer was determined at 532 nm (band width 0.5 nm). Butan-1-ol was used as blank. No free radical (ABAP) was used to induce oxidation. The results presented in the Tables 1 and 2 are expressed as absorbance. In Figs. 1 and 2, results are expressed as TBA value calculated from the relative absorbance of each sample against that of the control sample on day 1. Higher TBA values indicate a greater accumulation of TBARS (thiobarbituric acid-reactive substances) as a result of increased oxidation.

2.3. DPPH assay

The DPPH assay (2,2′-diphenylpicrylhydrazyl) is based on the reaction where the purple-coloured DPPH (a stable free radical) is reduced to the yellow-coloured diphenylpicrylhydrazine when reacting with the free radicals of the sample (Kirby & Schmidt, 1997).

Measurements with the DPPH assay were taken using a method used by Tepe et al. (2005). 0.05 g of the samples and 5 ml of 0.004% DPPH in methanol were added to a test

Table 1
Antioxidant activity levels in porcine raw and cooked meat samples upon storage as determined by the TBA and DPPH assays

Parameters	Treatment				Sampling time				
	CONTROL	OREGANO	SAGE	Sig.	Day 1	Day 4	Day 8	Day 12	Sig.
Raw samples									
TBA	$0.12^{a} \pm 0.01$	$0.02^{b} \pm 0.01$	$0.03^{b} \pm 0.01$	**	$0.054^{a} \pm 0.001$	$0.038^{b} \pm 0.003$	$0.070^{c} \pm 0.001$	$0.062^{a-c} \pm 0.016$	**
DPPH	$0.99^{a} \pm 0.01$	$0.36^{b} \pm 0.01$	$0.97^{a} \pm 0.01$	***	$0.813^{a} \pm 0.009$	$0.814^{a} \pm 0.006$	$0.777^{a} \pm 0.019$	$0.702^{b} \pm 0.011$	**
Cooked samples									
TBA	$0.36^{a} \pm 0.02$	$0.02^{b} \pm 0.02$	$0.05^{c} \pm 0.02$	**	$0.055^{a} \pm 0.001$	$0.094^{b} \pm 0.009$	$0.201^{c} \pm 0.020$	$0.224^{c} \pm 0.039$	**
DPPH	$1.05^{a} \pm 0.01$	$0.28^{b} \pm 0.01$	$0.97^{c} \pm 0.01$	***	$0.813^{a} \pm 0.009$	$0.755^{b} \pm 0.014$	$0.733^{b} \pm 0.013$	$0.756^{b} \pm 0.008$	**

Data shown for each parameter represent means ± respective standard errors. Within each treatment or within each storage time, means with different superscripts (a–c) in each row differ significantly ($^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$ and $^{***}P \leqslant 0.001$).

Table 2
Antioxidant activity levels in bovine raw and cooked meat samples upon storage as determined by the TBA and DPPH assays

Parameters	Treatment				Sampling time				
	CONTROL	OREGANO	SAGE	Sig.	Day 1	Day 4	Day 8	Day 12	Sig.
Raw samples									
TBA	$0.07^{a} \pm 0.01$	$0.02^{b} \pm 0.01$	$0.03^{b} \pm 0.01$	**	$0.030^{a} \pm 0.003$	$0.028^{a} \pm 0.003$	$0.052^{b} \pm 0.003$	$0.053^{b} \pm 0.005$	**
DPPH	$1.03^{a} \pm 0.01$	$0.36^{b} \pm 0.01$	$0.96^{c} \pm 0.01$	***	$0.875^{a} \pm 0.015$	$0.791^{b} \pm 0.009$	$0.748^{c} \pm 0.017$	$0.728^{b,c} \pm 0.040$	***
Cooked samples									
TBA	$0.13^{a} \pm 0.01$	$0.03^{b} \pm 0.01$	$0.05^{c} \pm 0.01$	***	$0.031^{a} \pm 0.003$	$0.057^{b} \pm 0.003$	$0.073^{c} \pm 0.003$	$0.108^{d} \pm 0.006$	***
DPPH	$1.06^{a} \pm 0.03$	$0.28^{b} \pm 0.03$	$0.95^{a} \pm 0.03$	***	$0.875^{a} \pm 0.015$	$0.719^{b} \pm 0.008$	$0.690^{b,c} \pm 0.044$	$0.760^{c} \pm 0.006$	***

Data shown for each parameter represent means ± respective standard errors. Within each treatment or within each storage time, means with different superscripts (a–c) in each row differ significantly ($^{*}P \leqslant 0.05$, $^{*}P \leqslant 0.01$ and $^{***}P \leqslant 0.001$).

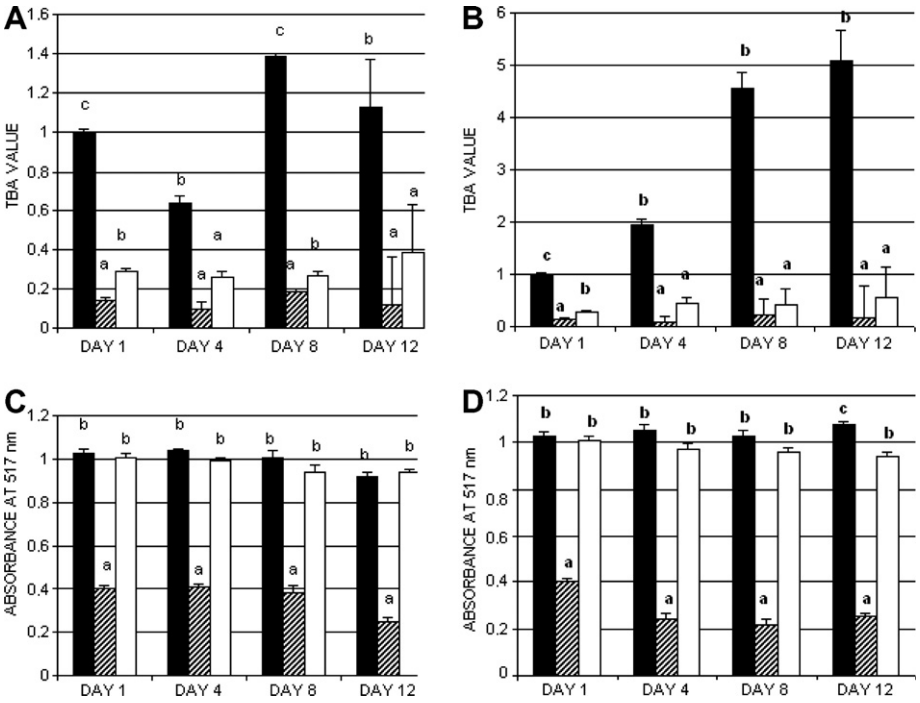

Fig. 1. Profiles of antioxidant activity level in porcine meat samples upon storage as determined by the TBA (A: raw samples, B: cooked samples) and DPPH (C: raw samples, D: cooked samples) assays (CONTROL: ■, OREGANO: ▨, SAGE: □). TBA values were calculated from the relative absorbance of each sample against that of the control sample on day 1. Data represent means ± respective standard errors. Bars with different superscripts (a–c) within the same sampling day differ significantly ($P \leqslant 0.05$).

tube. The samples were kept at room temperature for 30 min with constant mixing. Absorbance measurements were then taken at 517 nm, using methanol as a blank. Measurements were expressed as absorbance. Decreasing absorbance levels indicated increasing antioxidant activity of the essential oils.

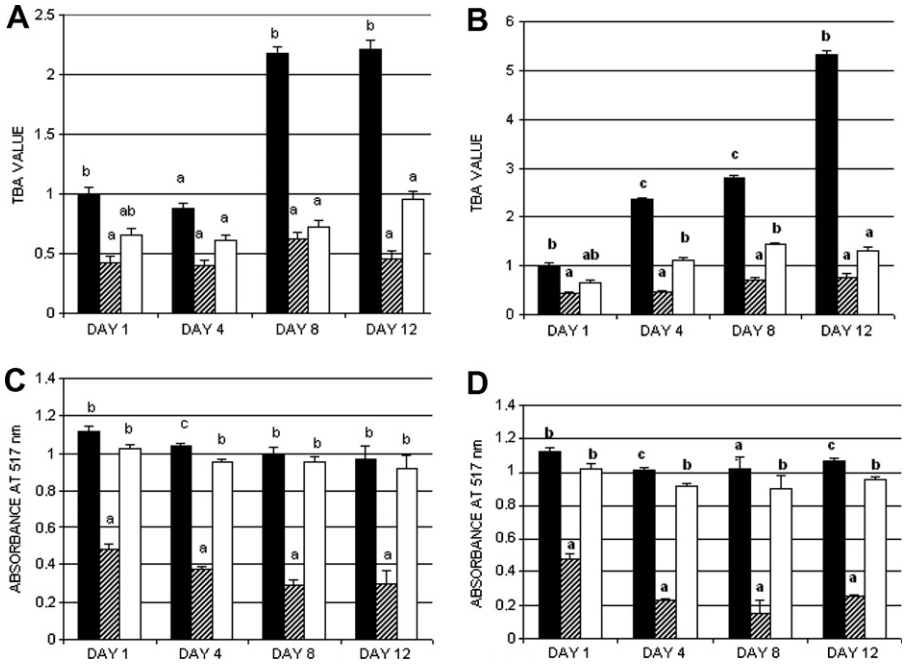

Fig. 2. Profiles of antioxidant activity level in bovine meat samples upon storage as determined by the TBA (A: raw samples, B: cooked samples) and DPPH (C: raw samples, D: cooked samples) assays (CONTROL: ■, OREGANO: ▨, SAGE: □). TBA values were calculated from the relative absorbance of each sample against that of the control sample on day 1. Data represent means ± respective standard errors. Bars with different superscripts (a–c) within the same sampling day differ significantly ($P \leqslant 0.05$).

2.4. Crocin assay

Crocins are the water-soluble carotenoids found in the red stigmas of *Crocus sativus* L. (Saffron). The crocin assay is based on the oxidation of a crocin solution (causing a loss of colour) by a free radical (such as α,α-azodiisobutyramidine dihydrochloride, ABAP) and the simultaneous prevention of the oxidation with the presence of an antioxidant (Bors, Michel, & Saran, 1984).

Crocin was isolated from Saffron by water/methanol extraction after repeated extraction with ethyl ether to eliminate possible interfering substances, as described by Lussignoli, Fraccaroli, Andrioli, Brocco, and Bellavite (1999). The crocin solution was divided into 1.5 ml aliquots and stored at −18 °C away from light prior to use.

Measurements were performed using a modified method of that described by Lussignoli et al. (1999). Briefly, 0.05 g of the samples, 1 ml of distilled water and 100 μl crocin solution were placed in a 1.5 ml microcentrifuge tube and left to stand for 10 min. Following this, 100 μl of 5 mg/ml ABAP in 10 mM phosphate buffer, pH 7.4 were added and the tubes were brought to 45 °C for 30 min in a water bath. The samples were centrifuged at 8000 rpm for 5 min before measuring the absorbance at 450 nm. Distilled water was used as a blank in the spectrophotometer. Measurements were expressed as absorbance. Higher absorbance levels indicated higher antioxidant activity from water-soluble substances in the samples.

2.5. Statistical analysis

Measurements on each meat sample were performed in duplicate. For each assay method (i.e. TBA, DPPH and crocin) and meat type (i.e. porcine and bovine), experimental data were analyzed using the general linear model (GLM) repeated measures analysis of variance (ANOVA) procedure in order to assess the effects of treatments (i.e. CONTROL, OREGANO, SAGE) and storage time (i.e. days 1, 4, 8 and 12) using the SPSS for Windows statistical package programme, version 8.0.0 (SPSS Inc., Chicago, IL). Statistically significant effects were further analyzed and means were compared using Duncan's multiple range test. Statistical significance was determined at $P \leqslant 0.05$.

3. Results

3.1. Chemical composition of the essential oils

The compositions of the essential oils used in this work were determined using GC/MS (data not shown). It was shown for the oregano essential oil that 20 substances account for 99.4% of its composition. From these the most abundant were: thymol 60.9%, *p*-cymene 10.5%, γ-terpinene 7.6% and carvacrol 5.8%. For the sage essential oil, 37 substances were found to account for 97.2% of its composition. The most abundant were: eucalyptol 49.4%, camphor 8.5% and α-pinene 5.4%.

3.2. Samples stored in the raw state at 4 °C

Experimental data obtained with the TBA assay are presented in Tables 1 and 2 as absorbance (ABS) values and in Figs. 1A and 2A as TBA values. Overall, both porcine and bovine meat samples in the CONTROL treatment had significantly ($P \leqslant 0.05$) higher levels of oxidation (i.e. higher ABS values) than had the corresponding ones in the OREGANO and SAGE treatments. In addition, it was shown that storage time had a significant effect on meat oxidation, higher level being noted after 8 days in both meat types (Tables 1 and 2).

Data generated using the DPPH assay are presented in Tables 1 and 2 and in Figs. 1C and 2C. Overall, porcine and bovine meat samples in the OREGANO treatment displayed a significantly higher antioxidant activity (i.e. lower absorbance) than did the SAGE and CONTROL treatments. The SAGE treatment showed a significantly higher antioxidant activity than the CONTROL in bovine but not in porcine meat. Overall, antioxidant activity of all samples decreased significantly with storage time from day 1 to day 12.

According to the crocin assay, the CONTROL treatment showed an overall significantly higher antioxidant activity (i.e. higher ABS values) than did the OREGANO and SAGE treatments. Interestingly, overall antioxidant activity was shown to increase with storage time up to day 12 (Table 3). Porcine meat in the treatments OREGANO and SAGE showed higher antioxidant activity than did the CONTROL until day 4, but subsequently this picture was completely reversed. A similar picture was seen for bovine meat samples (Fig. 3); however, in this case, the CONTROL antioxidant activity was higher than those of the OREGANO and SAGE treatments only on day 12.

3.3. Cooked meat samples stored at 4 °C

Overall antioxidant activity of cooked meat in the treatments OREGANO and SAGE was significantly higher than that of the CONTROL, according to the TBA assay, the OREGANO being more effective as an antioxidant than the SAGE (Tables 1 and 2). Overall, oxidation significantly increased with storage time (Tables 1 and 2), the effect being significantly pronounced throughout (i.e. days 1–12) for the CONTROL treatment (Figs. 1B and 2B).

According to the DPPH assay, following cooking of meat, the overall antioxidant activity of the samples increased significantly $P \leqslant 0.05$ (Tables 1 and 2). The OREGANO treatment showed a significantly higher antioxidant activity than did the SAGE and CONTROL samples, throughout the experiment (Figs. 1D and 2D); however, the SAGE treatment showed an overall higher antioxidant activity than did the CONTROL in porcine samples (Table 1).

The results from the crocin assay (Table 3 and Fig. 3) showed that the antioxidant activity of the CONTROL and SAGE samples decreased after day 1 and then remained at low levels compared to the OREGANO that was higher throughout the experiment.

4. Discussion

The results of this study using three different assay methods demonstrate that the essential oil treatments, OREGANO and SAGE, gave generally higher, (in most cases significant), antioxidant activity, compared to the CONTROL. The treatments OREGANO and SAGE therefore resulted in lower oxidation of porcine and bovine meat upon storage at 4 °C, with the OREGANO treatment being the most potent. This is generally in agreement with other research studies that have investigated the effects of Oregano essential oil in meat protection from oxidation through feeding (Botsoglou, Fletouris et al., 2003; Botsoglou, Grigoroloulou et al., 2003). The antioxidant activity of various essential oil compounds has been measured in the past using a TBA assay in model meat systems, showing a potential for protecting meat from oxidation (Ruberto & Baratta, 1999). Extracts of Oregano and sage have been tested for antioxidant activity using a crocin test and a Rancimat test. Interestingly, while the sage and oregano extracts compositions are different from the corresponding essential oil compositions, the greater antioxidant activity seen for the sage samples was related to the phenolic compound content of the extracts (Pizzale et al., 2002). The extracts of 22 herbs and spices were tested at a concentration up to 2.5% w/w on pork meat. The results showed

Table 3
Antioxidant activity levels in porcine and bovine raw and cooked meat samples upon storage as determined by the crocin assay

Parameters	Treatment				Sampling time				
	CONTROL	OREGANO	SAGE	Sig.	Day 1	Day 4	Day 8	Day 12	Sig.
Porcine meat samples									
Raw	$0.98^{a} \pm 0.02$	$0.54^{b} \pm 0.02$	$0.31^{c} \pm 0.02$	***	$0.210^{a} \pm 0.002$	$0.297^{b} \pm 0.017$	$0.858^{c} \pm 0.043$	$1.07^{d} \pm 0.019$	***
Cooked	$0.09^{a} \pm 0.01$	$0.40^{b} \pm 0.01$	$0.09^{a} \pm 0.01$	***	$0.210^{a} \pm 0.002$	$0.233^{a} \pm 0.009$	$0.165^{b} \pm 0.004$	$0.168^{b} \pm 0.011$	***
Bovine meat samples									
Raw	$0.81^{a} \pm 0.02$	$0.71^{b} \pm 0.02$	$0.46^{c} \pm 0.02$	**	$0.293^{a} \pm 0.023$	$0.198^{a} \pm 0.032$	$0.796^{b} \pm 0.024$	$1.36^{c} \pm 0.043$	**
Cooked	$0.10^{a} \pm 0.02$	$0.45^{b} \pm 0.02$	$0.11^{a} \pm 0.02$	***	$0.293^{a} \pm 0.023$	$0.218^{b} \pm 0.015$	$0.208^{a,b} \pm 0.046$	$0.158^{a,b} \pm 0.028$	***

Data shown for each parameter represent means ± respective standard errors. Within each treatment or within each storage time, means with different superscripts (a–c) in each row differ significantly ($^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$ and $^{***}P \leqslant 0.001$).

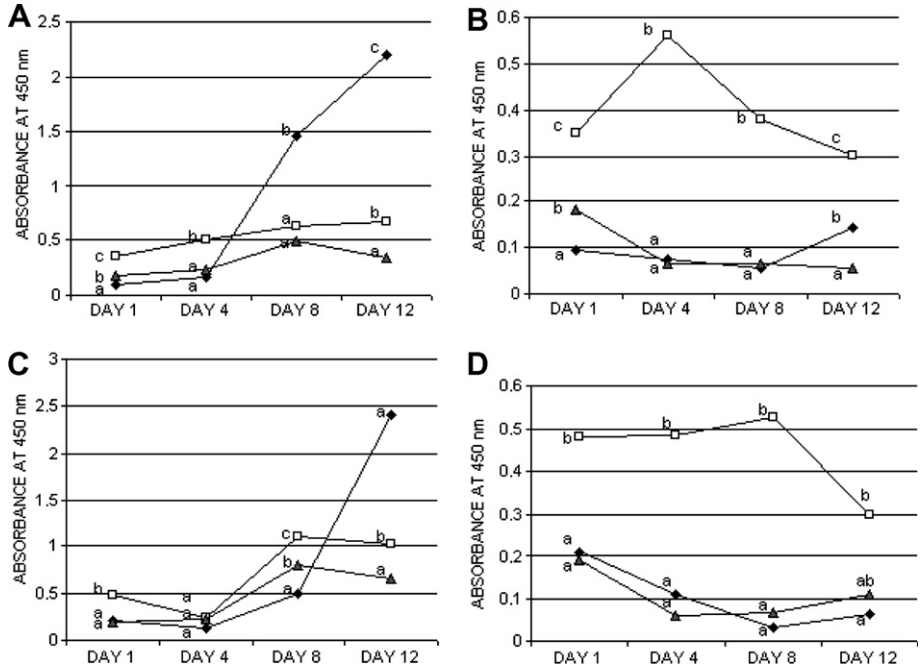

Fig. 3. Profiles of antioxidant activity level in porcine (A and B) and bovine (C and D) meat samples upon storage as determined by the crocin assay (A and C: raw samples, B and D: cooked samples) (CONTROL: —●—, OREGANO: —□—, SAGE: —▲—). Data represent means ± respective standard errors. Bars with different superscripts (a–c) within the same sampling day differ significantly ($P \leqslant 0.05$).

that, at such concentrations, all the tested extracts were capable of protecting the meat from oxidation, (compared to the control) using a TBA assay (Tanabe et al., 2002).

By comparing the TBA values between the porcine and bovine meat samples (Figs. 1A and 2A), it could be concluded, that the latter appears less vulnerable to oxidization than the former. This could be explained by the higher percentage of saturated fatty acids in beef than in porcine fat, known to be less prone to oxidation than unsaturated fats (Guillén-Sans & Guzmán-Chozas, 1998).

Furthermore it could be postulated that, apart from the added essential oils, other substances, such as proteins, present in the meat samples could have an effect on the antioxidant activity of the samples.

For the samples stored raw at 4 °C (Figs. 1A and 2A), irrespective of meat type, the TBA assay showed an increase in the oxidation of the samples upon storage that was greater for the CONTROL and less for the OREGANO and SAGE treatments. By contrast, according to the DPPH assay, the antioxidant activity of the samples increased (Figs. 1C and 2C). The latter finding is also supported by the crocin assay (Fig. 3). This could be explained by the fact that the oxidation of the meat leads to the creation of compounds that act as scavengers of free radicals (Guillén-Sans & Guzmán-Chozas, 1998) that could not be detected by the TBA assay that measures oxidation outcome (Guillén-Sans & Guzmán-Chozas, 1998).

Heat treatment of meat revealed significant differences between raw and corresponding cooked samples, upon storage at 4 °C. According to the TBA assay, much higher TBA values were noted for the cooked meat samples than for the corresponding raw ones (Tables 1 and 2). This could be explained by the production of pre-oxidized myoglobin that is susceptible to further oxidation. In addition, the presence of pre-oxidized myoglobin could also be interpreted as increased antioxidant activity from the DPPH assay (Tepe et al., 2005), irrespective of the cooked or raw state of meat (Tables 1 and 2). However, this does not appear to be the case with the crocin assay in that the increase seen in the antioxidant activity of the raw meat samples was not evident for the corresponding cooked ones (Table 3). This is probably due to reduced solubility of proteins following heat treatment, which renders them insoluble under the assay conditions and does not therefore allow them to be detected by the assay.

The TBA assay data showed that cooking increased the oxidation of meat upon storage. However, the addition of an antioxidant can offer sufficient protection. This can be seen from the higher TBA values in the CONTROL compared to the OREGANO and SAGE treatments that retained similar or even lower TBA values (compared to the corresponding ones from the raw meat samples). This clearly shows the importance of the addition of antioxidants to meat that is going to be stored in a cooked state.

Finally, it could be concluded that, among the three assays used, the TBA assay is the best for describing the oxidation taking place in the meat samples because the lipids seem to be the most affected. The DPPH assay (Mielnik, Aaby, & Skrede, 2003) and the crocin assay (Pizzale et al., 2002) prove to be useful in providing additional information as to the changes that occur during the cooking and storage of meat. In particular, the DPPH assay

seems to give a good measure of the total antioxidant activity of the meat as both water-soluble and non-soluble substances are detected. The crocin assay, however, seems to give a measure of the antioxidant activity only of the water-soluble substances. This was demonstrated by the different results produced by the two methods (DPPH and crocin) for the same samples. The crocin assay also seems to be the most sensitive for the detection of oxidative changes in the cooked meat samples.

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