Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle

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Received 3 April 2006; received in revised form 18 July 2006; accepted 18 July 2006

Abstract

The main goal of the present work was to determine the overall antioxidant status in fresh meat from animals fed different diets and to differentiate them through their odour profiles. Attributes were evaluated in beef from pasture or grain-fed animals with (PE and GE) or without supplementation (P and G) with vitamin E (500 UI/head/day).

Fresh meat produced on pasture (P and PE) had higher total ferric reducing antioxidant power (FRAP) levels than meat from grain fed-animals (G and GE) ($P < 0.05$). However, no differences were found on their ability to reduce ABTS$^+$/C5$^+$ (2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid)), indicating that total antioxidant activity was preferentially due to the reduction potential than to the quenching capacity of tissue homogenates. Two-fold glutathione (GSH and GSSG are the reduced and oxidised forms, respectively) levels were found in the P + PE group respect to G + GE meat ($P < 0.001$). In addition, meat from pasture-fed animals presented a higher glutathione redox potential compared to grain-fed animals ($156.1 ± 6.1$ and $158.1 ± 6.5$ vs. $148.1 ± 13$ and $149.8 ± 14.6$ for P, PE G and GE, respectively), showing that the antioxidant status in fresh meat was affected by diet.

Enzymatic activity of catalase and glutathione peroxidase were equivalent for all dietary groups. Only superoxide dismutase activity was slightly higher ($P < 0.05$) in the P + PE group than in G + GE samples.

Odour profile analysis was performed in relationship to antioxidant parameters. Significant linear correlation coefficients ($P < 0.05$) were found for a set of sensors and the FRAP values. E-nose methodology successfully discriminated the odour characteristics of samples corresponding to pasture- or grain-based diet. Hence, it was possible to describe an analytical relationship between the odour profile and the antioxidant power of fresh meat.

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Keywords: Argentine beef; Pasture- and grain- fed cattle; Vitamin E supplementation; E-nose; FRAP; Antioxidant enzymes; Antioxidant status; ABTS; GSH

1. Introduction

Meat quality covers inherent properties of meat, being the main attributes safety, nutritional value, flavour, odour, texture, water-holding capacity, colour, lipid content and composition, oxidative stability and uniformity. Multiple interacting factors like breed, genotype, feeding programs, slaughter and storage conditions influence meat quality. Among them, animal nutrition plays an important role due to its regulatory effect on biological processes in muscle that are reflected in the quality of meat (Andersen, Oksbjerg, Young, & Therkildsen, 2005).

Argentine beef has been traditionally produced on pasture. Grass feeding confers nutritional benefits such as...
lower cholesterol and saturated fatty acids, and higher
\( n - 3 \) polyunsaturated fatty acid and antioxidant vitamin
contents than meat produced in grain (Descalzo et al.,
2005; García, Pensel, Margarita, & Olga Rosso, 1999; Lar-
tick & Turner, 1989; Realini, Duckett, Brito, Dalla Rizza, &
De Matos, 2004; Yang, Lanari, Brewster, & Tume, 2002).
Meat is also a source of conjugated linoleic acid (CLA),
a component with anticarcinogenic and antiatherogenic
activities whose amounts are modulated by dietary regimes
in cattle (García et al., 2005).

Meat flavour is the result of a complex interaction of
precursors derived from the lean and fat components. Lip-
ids provide volatile compounds that may contribute to
meat aroma (Mottram, 1991). Indeed, short chain alde-
hydes such as hexanal and pentanal are commonly associ-
ated with lipid oxidation (Descalzo et al., 2005), which is
the main non-microbiological factor in the deterioration
of meat quality.

Previous results indicated that meat produced on pas-
ture showed less TBARS and volatile production than
meat from grain-fed animals, due to the protection con-
ferrered by natural antioxidants present in grass (Descalzo
et al., 2005) and protection conferred by natural antioxidants present in grass (Descalzo et al., 2005). Mercier, Gatellier, and Renerre (2004) demonstrated that finishing Charolais cows with pasture diet, protected muscle homogenates against lipid and protein oxidation in comparison with grain finished cows.

As meat is a complex matrix, different models have been
developed for studying the balance and the interaction
between anti- and pro-oxidant substances. Antioxidant
defences are composed by non-enzymatic hydro and lipo-
soluble compounds like vitamin E, vitamin C, carotenoids,
ubiquinols, polyphenols, cellular thiols, and enzymes like
superoxide dismutase (SOD), catalase (CAT) and glutathi-
one peroxidase (GPX). Together enzymatic and non-enzym-
atic systems operate to counteract the action of pro-
oxidants in muscle tissues (Decker, Livisay, & Zhou,
2000, chap. 2).

We have previously described that pasture feeding con-
fers higher levels of vitamin E, \( \beta \)-carotene (in agreement
with other authors: Yang, Brewster, Lanari, & Tume
(2002), Gatellier, Mercier, & Renerre (2004)) and vitamin
C (Descalzo et al., 2005). However, it is desirable to estab-
lish a method that can measure the antioxidant activity of
meat.

Several methods have been developed to measure “total
antioxidant activity”. However, no single measurement of
antioxidant status is going to be sufficient, but a “battery”
of measurements, will be necessary to adequately assess
oxidative stress in biological systems (Prior & Cao, 1999).

Usually, flavour analysis is carried out by human assess-
ment. In view of the remarkable progress in instrumental
techniques, different methodologies have been proposed to
assess odour characteristics. Recently, there is a great
interest in using electronic nose in food odour analysis,
which allows volatile compounds to be assessed directly
in their original matrix. This device is a sensor-based
instrument designed to respond to the volatile compounds
present in the headspace of a sample, combined with a suit-
able pattern recognition routine. E-nose has been success-
fully used to classify samples with similar odour characteristics of agricultural products (Persaud, Khaffaf,
Hobbs, & Sneath, 1996), sheep meat (Braggins & Frost,
1997), ground beef (Spanier & Braggins, 1999), processed
beef (Grigioni, Margarita, Pensel, Sánchez, & Vaudagna,
2000), milk (Irurutela et al., 2005), etc.

The aim of this work was to study how feeding influ-
ences the overall antioxidant power in meat and the possible
relation with meat odour characteristics. With this
purpose, we describe the application of the Ferric Reducing
Antioxidant Power (FRAP) and ABTS\(^{+}\) assays together
with the redox potential of the GSSG/GSH couple and
the aroma profile of fresh meat samples.

2. Materials and methods

2.1. Animals and diets

This study is a part of a project to determine the influence
of diet on meat quality and oxidative stability of fresh
and aged meat from crossbreed steers as described previ-
ously in Descalzo et al. (2005).

Pasture: 20 crossbreed steers were grown on natural pas-
ture at a forage rate of 1000/2000 kg grass dry matter/Ha.
Ten pasture-fed animals were used as control and another
10 animals of this group received 500 U/day of vitamin E
(all – rac \( \alpha \)-tocopherol, Roche Argentina).

Grain Silage: 20 crossbreed steers were fed with 5 kg
corn/day/animal + 6 kg hay/day/animal. Ten of these
animals were supplemented with 500 units/day of vitamin
E whereas the rest remained as grain silage control. All ani-
mals were grown in the EEA Pergamino in Buenos Aires
Province, Argentina, and transported to the slaughter-
house after reaching 480 kg weight, where they were huma-
nely harvested.

Muscle \( psoas major \) (PM) was selected because of its
high export value (for argentine producers) and its elevated
susceptibility to oxidative deterioration during long periods
of storage, which causes quality deterioration, such as
surface darkening and the appearance of off-odours and off-
flavours notes. Muscle samples (right and left side) were
cut into four longitudinal pieces. Pieces from individual
animals were randomly distributed (to be used for matura-
tion assays), vacuum packaged and immediately refriger-
ated at 1–2 °C. Fresh samples (24 h post-slaughter), used
in the present work, were kept at –70 °C until use.

2.2. FRAP assay

The FRAP assay was originally described to measure
the total reducing power of biological fluids and tradition-
ally applied to plasma, beverages, fruit and vegetable
extracts (Ou, Huang, Hampsch-Woodill, Flanagan, & Dee-
er, 2002). Antioxidant compounds such as \( \alpha \)-tocopherol,
trolox, vitamin C, uric acid, billirubin, among others, are
able to reduce ferric to ferrous-TPTZ which develops a blue colour (Benzie & Strain, 1999). At low pH, a ferryl tris(pyridyltriazine) (Fe III-TPTZ) complex is reduced to ferrous (Fe II) form that develops an intense blue colour with an adsorption maximum at 593 nm.

For meat samples, this assay was modified to measure endogenous ions that could react with TPTZ developing blue colour (i.e. endogenous Fe II). Chopped meat samples were disrupted for 2 min at 3000 rpm with an Ultraturrax (IKA, Germany) homogeniser in potassium phosphate buffer pH 7.2. Homogenates were centrifuged at 10,000 g during 30 min and supernatant was collected. 83 µl aliquots were added to 2.5 ml FRAP buffer containing 10 mM TPTZ (Sigma–Aldrich, Argentina, SA) in 40 mM HCl and 20 mM FeCl₃ (Sigma–Aldrich, Argentina, SA) added to 300 mM acetate buffer. Samples were measured immediately after processing. Endogenous Fe II content (FRAPo) was determined with a TPTZ/HCl solution without the addition of FeCl₃ to the reaction mixture.

FRP activity of samples was calibrated with a ferrous sulphate (Fe₂SO₄·7H₂O, Sigma–Aldrich, Argentina, SA) curve within the range from 100 to 1000 µM and results were expressed as Fe II equivalent in µM.

2.3. ABTS⁺ radical cation decolorization assay

Meat antioxidant activity was determined following the adapted Trolox-equivalent antioxidant capacity (TEAC) assay as described by Re et al. (1999). ABTS [2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)] radical cation (ABTS⁺) was produced by reacting 14 mM ABTS with an equal volume of 4.9 mM potassium persulphate (final concentration: 7 mM ABTS in 2.45 mM potassium persulphate). The mixture was incubated in the dark at room temperature for 12–16 h before use. The ABTS⁺ solution was diluted with 5.5 mM PBS (pH 7.4) to an absorbancy of 0.70 (±0.02) at 734 nm (Sigma–Aldrich, Argentina, SA) and equilibrated at 30 °C. An aliquot of 10 µl of homogenate (prepared as described for FRAP assay) or Trolox standard (0–1.2 mM in PBS) (Fluka Chemie Gmbh, Buchs, Switzerland) was added to 1.0 ml of the diluted ABTS⁺ solution and the absorbancy was read at 30 °C exactly 1 min after the initial mix and up to 6 min after. The percentage inhibition of the blank absorbancy (0.70 ± 0.02) was calculated for each Trolox standard reference and meat sample, respectively.

2.4. Glutathione content in PM muscles

Homogenates were performed as indicated for the FRAP assay. Supernatants were tested for total and oxidized glutathione (GSSG) content as indicated by Tietze (1969), measuring the continuous formation of TNB from DTNB (5,5'-dithio-bis (2-nitrobenzoic acid), Sigma–Aldrich Chemie Gmbh, Steinheim, Germany), dependant on the original concentration of GSH (glutathione reduced form), in the presence of NADPH and glutathione reductase. Aliquots of 200 µl of supernatant were added to the reaction buffer containing 0.3 mM β-NADPH (Sigma–Aldrich, St. Louis, MO, USA), 6 mM DTNB in 0.05 M sodium phosphate buffer pH 7.5 and absorbancy of the blank was read at 412 nm. Reaction was initiated by addition of glutathione reductase (50 U/ml) (Sigma–Aldrich, St. Louis, MO, USA) and the increase of yellow TNB was followed at 412 nm for 5 min at 30 °C. Endogenous GSSG was measured by treating 500 µl of the homogenate with 10 µl of 97% 2-vinylpiridine (Fluka Chemie Gmbh, Germany) and 30 µl triethanolamine (Sigma–Aldrich, Argentina, SA) in order to avoid the oxidation of the GSH originally present and subsequently measured as above. GSH was determined as the difference between total glutathione and GSSG.

In order to calculate GSH and GSSG molar concentration, tissue volume was estimated after subtraction of intramuscular fat (2.8% for pasture samples and 4.4% for grain samples) and protein content (22% for all treatments).

2.5. Antioxidant enzyme activity measurements

Ten grams of minced meat were mixed with 20 ml of a potassium phosphate buffer (0.05 M, pH 7.7). Samples were homogenised with an Ultraturrax (IKA, Germany) homogeniser at 3000 rpm for 2 min. Triton X-100 (Sigma–Aldrich, St. Louis, MO, USA) was added for a final concentration of 0.2% and left for 30 min on ice-bath with interval mixing. Two milliliters of this homogenate were separated for SOD determination. Twenty-five milliliters of the homogenate were centrifuged at 4 °C at 9500g and the supernatant kept at −70°C until processing. Protein concentration was determined according to Lowry, Rosebrough, Farr, and Randall (1951). Solvents used were analytical grade from Sintorgan-Argentina.

For the SOD homogenate, 0.5 ml of ethanol and 0.3 ml of chloroform (cooled to −20°C) were added to the 2 ml separated previously and kept in ice-bath for 30 min with agitation, 280 µl of di-distillate water were added and centrifuged for 30 min at 10,000g. Enzymatic activity was determined using a Perkin–Elmer LAMBDA BIO 20 UV/Vis spectrophotometer (Norwalk, CT, USA). Total SOD activity was measured according to the procedure of Misra and Fridovich (1972) using inhibition of 1 mM epinephrine (Sigma–Aldrich, St. Louis, MO, USA) in a basic medium (0.2 M glycine buffer pH 10.2). One unit was taken as homogenate volume per mg protein that inhibited epinephrine autoxidation by 50%. CAT activity was measured by the rate of disappearance of 10 mM H₂O₂ at 240 nm (Aebi, 1984) in 0.05 M potassium phosphate buffer pH 7.2 and expressed as units of enzyme per mg protein. Units were calculated as pmol of disproportionated H₂O₂ min⁻¹ mg⁻¹ protein using constant value for pure catalase of 3.4 × 10⁻⁷ mol⁻¹ s⁻¹. GPX activity was assayed with a GSH reduction coupled to a NADPH oxidation by glutathione reductase (Flohé & Günzler, 1984). β-NADPH (Sigma–Aldrich, St. Louis, MO, USA),
consumption was determined at 340 nm and units were expressed as nmol of oxidized NADPH mg\(^{-1}\) protein.

2.6. Electronic nose analysis

An Electronic Nose AromaScan A32S (OSMETECH PLC, Crewe, England) with a detector array of 32 conducting polymer sensors was used. The relative response of each sensor, measured as the change in resistance relative to the base resistance (\(\delta R/R\)), reflects the range of volatile compounds in the headspace of the sample. The system allows a fast and accurate identification of unknown samples by odour analysis using recognition software that includes an artificial neural network.

2.6.1. Sample preparation

A slice of each muscle was trimmed of visible fat and cut into strips (approximately 1.5 \(\times\) 1 \(\times\) 0.5 cm). Then, an aliquot of 10 g was placed into a 50 ml glass test tube with stoppered screw caps. During analysis the sample temperature was kept at 50 °C in a water bath with large thermal inertia.

2.6.2. Protocol of analysis

The instrumental method was optimised to give the following hardware control conditions and all samples were analysed following this protocol. Analyses were carried out in triplicate. In this assay, the dynamic stripping method was selected (Grigioni et al., 2000). Data acquisition was performed in one cycle: reference: 30 s, sample: 90 s, wash: 60 s, reference: 120 s and 2% n-butanol–water solution as cleaning agent. The length of each phase in the acquisition cycle was found to be sufficient to obtain a stable baseline, to collect volatile compounds and to allow recovery-up of the sensors between successive analyses. An average odour pattern for each sample was collected in a time interval between 180 s and 210 s within the plateau of the sensor response curve and calculated among three consecutive runs.

Standard compounds for sensor diagnostic were purchased from Sigma–Aldrich of Argentina SA.

2.7. Statistical analyses

Biochemical variables are reported as the mean ± standard deviation for 10 measurements on each animal group. Treatments were compared by analysis of variance using the GLM procedure (SAS 8.0, SAS Institute, Inc., Cary, NC) for a fixed effect model with two basal diets and two supplement levels: \(Y_{ijk} = \text{mean} + \text{diet}_i + \text{supplement}_j + (\text{diet} \times \text{supplement})_{ij} + \epsilon_{ijk}\) where \(Y_{ij}\) is the dependent variable; \(\mu\) is an overall mean response, \(x_i\) is the effect of the \(i\)th level of season; \(\xi_{ij}\) is the residual error with zero mean and variance \(\sigma^2\). Mean values were compared using the Tukey test at a significance level of 0.05.

The relationship between biochemical measurements and the response of the sensor array during E-nose analysis was evaluated using Pearson Correlation. E-nose data were analysed applying linear discriminant analysis (LDA) as a classification procedure, this method maximises the variance between and within categories. For variable selection Wilks’ lambda stepwise method was applied. The criterion used was the significance of \(F\) with a maximum of 0.05 to enter and a minimum of 0.10 to exit. First, the variables \(x_i\) were transformed into standardised \(z_i\) values where \(z_i = \frac{x_i - \mu_i}{\sigma_i}\).

SPSS® Advanced Statistics 12 software (SPSS Inc., Chicago, IL) was used.

3. Results and discussion

3.1. Antioxidant capacity in muscle

In a previous work (Descalzo et al., 2005), we reported the antioxidant vitamin status in fresh beef. Meat produced on pasture had higher content of antioxidant vitamins: \(\alpha\)-tocopherol, \(\beta\)-carotene and ascorbic acid than grain produced meat. Their values are summarised in Table 1.

In order to determine whether these results were related to the antioxidant capacity of fresh meat, total reducing activity was determined with the FRAP assay. Fig. 1 shows the response of antioxidant standards: ascorbic acid, \(\alpha\)-tocopherol, and GSH and their activities were compared to the response of Fe\(^{III}\) equivalents. As can be seen, \(\alpha\)-tocopherol and ascorbic acid reduced Fe\(^{III}\) in a similar way and the response of these vitamins corresponded to two equivalents of Fe\(^{III}\) standard within the concentration range of 100–1000 \(\mu\)M. On the contrary, GSH was unable to reduce the ferryl-TPTZ compound. Calibration of the FRAP method was in agreement with previous results (Benzie & Strain, 1999). As discussed by these authors, when FRAP was applied to EDTA plasma, the agents that reacted directly with TPTZ to form a blue chromogen were negligible. However, in order to apply this assay to meat homogenates, it was necessary to perform a blank reaction (FRAPo) with homogenate and TPTZ (without addition of Fe\(^{III}\)).

As shown in Fig. 2, meat homogenates contained agents that reacted directly with TPTZ. Endogenous Fe\(^{II}\) in meat homogenates could be responsible for the FRAP\(_o\) reaction. The difference between FRAP and FRAP\(_o\) curves showed the ferric reducing power of PM homogenates and was used to estimate the antioxidant potential of meat samples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(\alpha)-tocopherol ((\mu)g/g)</th>
<th>(\beta)-carotene ((\mu)g/g)</th>
<th>Ascorbic acid ((\mu)g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>1.50 ± 0.73a</td>
<td>0.06 ± 0.03a</td>
<td>15.92 ± 3.48a</td>
</tr>
<tr>
<td>Grain + E</td>
<td>1.76 ± 0.97a</td>
<td>0.05 ± 0.01a</td>
<td>17.39 ± 4.29a</td>
</tr>
<tr>
<td>Pasture</td>
<td>3.08 ± 0.45b</td>
<td>0.45 ± 0.21b</td>
<td>25.30 ± 10.23b</td>
</tr>
<tr>
<td>Pasture + E</td>
<td>3.91 ± 0.74b</td>
<td>0.63 ± 0.27b</td>
<td>21.98 ± 5.11b</td>
</tr>
</tbody>
</table>

Means (\(n = 10\) for each treatment) and standard deviation are indicated. Different letters within the same column differ (\(P < 0.05\)).

Table 1
Antioxidant vitamin levels in fresh beef
In addition, incubation of the samples with ascorbate oxidase and posterior FRAP measurement indicated the contribution of ascorbic acid (FRAPC) to the antioxidant capacity of the sample (Benzie & Strain, 1999).

Using this method, glutathione was unable to reduce Fe$^{III}$ (Fig. 1). However, it constitutes the major redox buffer in animal tissues. This result clearly shows that not all antioxidants match the ability to reduce a determined compound, and that a single measurement should not be used to indicate the overall antioxidant/oxidative status in the muscle.

The radical scavenging capacity of meat was determined by inhibition of the autooxidation of ABTS$^+$ cation. This method does not indicate the reducing potential of a sample; rather it indicates the radical scavenging capacity of different substances. The direct production of the blue/green ABTS$^+$ chromophore through the reaction between ABTS and potassium persulfate was used as previously described by Re et al. (1999).
Results shown in Fig. 3, compare both antioxidant approaches indicating that pasture samples presented higher reducing potential than grain samples, as assessed by FRAP. In addition, FRAPC assay (after subtraction of the reducing potential of ascorbic acid) showed the same tendency and the differences were also significant among pasture and grain diets.

Contrasting with the FRAP assay, no differences were found between pasture and grain groups in the ABTS assay. This observation is in agreement with Gatellier et al. (2004). These authors described no significant effect of diet on the TEAC test, which is also based on the reduction of the ABTS⁺ cation. These results clearly differentiated the radical scavenging capacity from the ferric reducing potential of meat samples.

When FRAP values were correlated with antioxidants and TBARS (data from Descalzo et al. (2005) for the same set of samples), positive correlations (P < 0.05) were found for α-tocopherol (r = 0.573), vitamin C (r = 0.571) and glutathione content (r = 0.628) and inverse correlation with TBARS (r = −0.462).

Supplementation of diets with vitamin E had no effect on either FRAP or ABTS reduction activity, indicating that the differences found in FRAP and FRAPC for the different diet groups could be attributed to basal diets (pasture or grain).

3.2. Glutathione content

The GSSG/2GSH couple contributes principally to the overall redox environment of the cell. Therefore, glutathione is considered the major thiol-disulfide redox buffer in animal tissues. Oxidative stress results in the formation of GSSG at the expense of 2GSH molecules (Schafer & Buettner, 2001).

Disposal of hydrogen and lipid peroxides is catalysed by isoforms of GPX. As a consequence, GSH is oxidized to GSSG, which is then reduced back to GSH by glutathione reductase at the expense of NADPH, thereby forming a redox cycle (Awasthi, Dao, & Saneto, 1980). In addition to enzymatic disposal of peroxides, GSH can also react non-enzymatically with OH, N₂O₃ and ONOO⁻ (Wink & Mitchell, 1998). Therefore, measurement of glutathione content in tissues could be an indicator of the redox status of meat.

Meat homogenates from pasture-fed animals showed higher content of total glutathione than their grain counterparts. Significantly higher amounts of glutathione, either reduced or oxidized, were found in pasture samples despite of whether they were supplemented or not with vitamin E (Table 2).

When the GSH/GSSG ratio (Table 2 third column) was calculated for pasture or grain samples, no differences were found among the groups. Maintenance of this ratio could indicate that the glutathione reductase activity is sufficient, and there should be no change in the GSH/GSSG ratio without any external induction of oxidative stress.

However, quantities of both species of glutathione do not indicate the reduction potential within the tissue. As the redox state is a second-order function of GSH concentration, a change in the concentration of GSH even without a change in the GSH/GSSG ratio could alter the cellular redox state.

One molecule of GSSG is formed at the expense of two molecules of GSH and the reduction potential may be calculated for the half-cell reaction:

\[ \text{GSSG} + 2H^+ + 2e \rightarrow 2\text{GSH} \]

The Nernst equation for the reduction potential of the redox couple GSSG/2GSH will be:

\[ E_{hc} = -240 - (59.1/2) \log([\text{GSH}]^2/[\text{GSSG}]) \text{ mV} \]

(where Eho is −240 mV)

Therefore, the reduction potential of meat from pasture-fed animals (Table 2 fourth column) was significantly higher (P < 0.05) than the reduction potential of meat from grain-fed animals, thus indicating that pasture diet conferred a reducing environment to the PM muscle.

3.3. Antioxidant enzyme activity

Results shown in Table 3, indicate that meat from pasture fed animals had higher SOD activity than meat from grain-fed animals.

Also, Gatellier et al. (2004) showed higher SOD activity in meat from Charolais cattle that consumed pasture diets.

| Table 2 |
| Glutathione molar concentration (GSH and GSSG) and redox potential in muscle samples |
| Treatment | GSH (M)ᵇ | GSSG (M)ᵃ | GSH/GSSGᵃ | Redox potentialᵇ⁺ |
| Grain | 2.78 ± 1.22 × 10⁻⁴ y | 3.38 ± 1.21 × 10⁻⁵ y | 8.5 ± 3.7 y | −160 ± 12.11 y |
| Grain + E | 2.81 ± 1.35 × 10⁻⁴ y | 3.36 ± 2.17 × 10⁻⁵ y | 9.9 ± 4.6 y | −161 ± 12.52 y |
| Pasture | 5.14 ± 1.23 × 10⁻⁴ z | 6.29 ± 1.90 × 10⁻⁵ z | 8.6 ± 2.4 y | −170 ± 5.88 z |
| Pasture + E | 4.85 ± 1.30 × 10⁻⁴ z | 4.77 ± 1.22 × 10⁻⁵ z | 10.6 ± 2.8 y | −172 ± 5.89 z |

ᵇ Redox potential was calculated by application the Nernst equation as explained above.

ᵇ⁺ Means (n = 10 for each treatment) and standard deviation are indicated. Different letters within the same column differ (P < 0.05).
when compared to the analogous mixed-diet group. These authors also found a significant correlation \( r = 0.591; P < 0.001 \) between SOD activity and vitamin E content in muscle. In the present work, we also found a positive correlation between SOD activity and \( \alpha \)-tocopherol content \( r = 0.434; P < 0.001 \), which has been measured in the same muscles (reported previously by Descalzo et al. (2005)).

Regarding the positive correlation between \( \alpha \)-tocopherol and SOD activity, the authors Li, Saldeen, Romeo, and Mehta (1999), reported that both \( \alpha \) and \( \gamma \)-tocopherol increased SOD expression either in plasma or in arterial tissues of Sprague–Dowley rats.

Therefore, further investigation in dietary content of vitamin E and SOD activity should be done in order to determine the role of dietary vitamin E content in the modulation of bovine SOD expression.

In contrast, CAT activity was similar in meat from animals fed pasture or grain diets (Table 3). Although CAT and SOD are coupled enzymes, they did not show the same pattern of activity. Similar results were described by Mercier et al. (2004).

3.4. Beef odour profile

The relationship between antioxidant status related variables (FRAP, ABTS\(^+\), CAT, SOD, GPX, GSSG and GSH) and the patterns generated from the sensor array were analysed using Pearson Correlation coefficients. The \( \alpha \)-tocopherol and TBARS values for the same samples (reported in Descalzo et al. (2005)) were also included. Table 4 shows the correlation results: s1, s6 and s31 vs. \( \alpha \)-tocopherol values; s6 vs. ABTS measurements; s12 and s13 vs. SOD activity; sensors s2-, s7-, s8-, s9-, s10-, s15-, s16-, s20-, s21-, s29 and s32 vs. FRAP values.

E-nose data were analysed applying linear discriminant analysis (LDA) to investigate the grouping of the fresh beef odour profiles as a function of feeding. Two discriminant functions were obtained that explained 57.9% and 24.9% of the total variance, respectively, with a success rate of correct classification of each sample into their respective group of 94.5% for the original cases and 46.5% after cross-validation. In this analysis, a superposition of

<p>| Table 3 | Antioxidant enzyme activity in meat homogenates |
|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>CAT(^a)</th>
<th>SOD(^a)</th>
<th>GPX(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>16.03 ± 4.68 y</td>
<td>19.6 ± 7.7 y</td>
<td>40.2 ± 22.2 y</td>
</tr>
<tr>
<td>Grain + E</td>
<td>18.81 ± 7.94 y</td>
<td>19.7 ± 5.1 y</td>
<td>36.9 ± 13.3 y</td>
</tr>
<tr>
<td>Pasture</td>
<td>15.84 ± 4.01 y</td>
<td>27.2 ± 7.1 z</td>
<td>42.9 ± 9.8 y</td>
</tr>
<tr>
<td>Pasture + E</td>
<td>16.51 ± 7.81 y</td>
<td>23.7 ± 6.3 z</td>
<td>44.0 ± 11.6 y</td>
</tr>
</tbody>
</table>

\( \text{Significance level of } ANOVA (P<0.05) \)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Supplement</th>
<th>Diet x suppl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5501</td>
<td>0.4123</td>
<td>0.6103</td>
</tr>
<tr>
<td>0.0010</td>
<td>0.4212</td>
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<tr>
<td>0.1903</td>
<td>0.8221</td>
<td>0.4177</td>
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\( a \) Means (n = 10 for each treatment) and standard deviation are indicated. Different letters within the same column differ \( (P<0.05) \).

Table 4 | Pearson correlation coefficients for antioxidant related variables and E-nose sensor response |
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\( * \) TBA mg eq. MDA/Kg tissue and \( \alpha \)TOC \((\alpha\text{-tocopherol}) \) \( \mu \)g/g tissue as determined in Descalzo et al. (2005).

Significance level: \( \ast P < 0.01 \), \( \ast \ast P < 0.05 \).
samples was observed corresponding to G with GE and P with PE groups. For that reason, LDA with Wilks’ lambda stepwise method for variable selection was applied, considering only the classification into grain (G + GE) or pasture (P + PE) diets. In this case, a success rate of correct classification of each sample was 95.2% for both the original cases and after cross-validation.

Changes in the classification scheme were obtained when E-nose data were analysed by LDA considering only those sensors that showed significant correlation coefficients ($P < 0.05$) with FRAP. In this case, two discriminant functions were obtained that explained 57.1% and 42.9% of the total variance, respectively, with a success rate of correct classification of each sample of 82.3% and 72.6% for the original cases and after cross-validation. In this case three groups were differentiated that corresponded to G and GE diets, and a third group that showed a superposition of P and PE groups. Fisher’s linear discriminant functions are presented in Table 5. These results clearly indicate that E-nose profile of fresh beef samples can be related not only to classical sensory descriptors, but also to antioxidant potential in fresh meat.

Among the set of measurements utilised in this assay, E-nose methodology allowed the discrimination of beef from supplemented and non-supplemented grain-fed animals, but this differentiation was not observed for pasture diet. The lack of discrimination between P and PE samples can be attributed to the quality of antioxidants provided by the pasture. Dietary delivery of natural antioxidants from pasture significantly improved the antioxidant status in muscle when compared to the supplement contribution. Among antioxidant vitamins, results shown in Table 1 indicate an almost ten-fold increase in α-carotene, 30% increase in ascorbic acid content and 51–54% increase in α-tocopherol in meat from pasture compared to grain (G + GE) samples.

This feature is not a surprise. All rac-α-tocopherol contains eight stereoisomers in equal proportions. Only one of these (12.5% of the total mixture) is the RRR-α-tocopherol, identical to natural-source vitamin E, which is selectively incorporated into mammalian tissues. The other seven stereoisomers have different molecular configurations and lower biological activities. The hepatic cytosolic α-tocopherol transfer protein (α-TTP), and another ubiquitous tocopherol associated protein (α-TAP) regulate the distribution of vitamin E into the tissues (Ricciarelli, Zingg, & Azzi, 2001). α-TTP is selective for the natural RRR-α-tocopherol form (100% affinity). This protein shows much less affinity for other tocopherols and tocotrienols: including 38% for β-tocopherol, 9% for γ-tocopherol, 2% for δ-tocopherol, and 12% for α-tocotrienol (Hosomi et al., 1998). This selectivity is probably associated to the regulatory role of vitamin E in mammalian tissues review (reviewed in Azzi, Ricciarelli, & Zingg, 2002).

Therefore, it is probable that only a low percentage of the vitamin E present in the supplement is efficiently used. Instead, pasture diet offers the natural form of vitamin E, which is the ligand of the tocopherol transporting proteins. For that reason, it is difficult to enhance vitamin E levels in meat with 500 UI/head/day of a synthetic supplement. Further studies should be conducted in order to determine the importance of vitamin transporting mechanisms into the muscle.

4. Conclusions

Pasture diet conferred an improved overall antioxidant and redox status to fresh meat when compared to a grain-finishing diet. As antioxidant enzymes showed slight differences between the assayed populations, our results indicate that non-enzymatic antioxidants contributed preferentially to differentiate pasture or grain produced meat. The use of an electronic nose proved to be a useful instrumental method to discriminate the aroma profile of meat samples with different antioxidant status. Moreover, it contributed to differentiate vitamin E supplemented from unsupplemented grain produced meat. Dietary modulation of natural antioxidant mechanisms in muscle and their significance in meat quality enhancement should be further stated.

Acknowledgements

The authors gratefully acknowledge the enthusiastic interest and skilled assistance of Ms. Ilda Avila and Juan Carlos Fernández for his technical support. We also thank Roche Argentina for providing the vitamin E preparations.

This study was financed by the National Agency of Science and Technology (ANPCyT) PICT 09-03517, BID 1201/OC-AR.

References
