Available online at www.sciencedirect.com

ScienceDirect

Meat Science 79 (2008) 423–436

MEAT SCIENCE

www.elsevier.com/locate/meatsci

A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina

A.M. Descalzo *, A.M. Sancho

Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA),
CC 77, B1708WAB Morón, Buenos Aires, Argentina
Facultad de Agronomía y Ciencias Agroalimentarias, Universidad de Morón, Cabildo 134, (B1708JPD) Morón, Buenos Aires, Argentina

Abstract

Meat derived from pasture feeding, is associated with a high level of antioxidants. Antioxidants are incorporated within cell membranes and protect tissues against oxidation from reactive oxygen species. This maintains the overall quality of meat and secondary products. This paper reviews the implications of incorporating natural antioxidants into fresh beef, focusing on the benefits of feeding cattle good quality pasture. Pasture samples typically have higher levels of α-tocopherol, β-carotene, ascorbic acid and glutathione than feedlot samples. These compounds retard lipid and protein oxidation in fresh and stored meat, and preserve the color and odor quality of beef. The significance of antioxidant enzymes is variable, because their behavior depends on individual redox status before slaughter. Understanding total antioxidant activity requires information on antioxidant and pro-oxidant status. With an abundance of pasture, Argentina has a natural advantage in producing meat with a high antioxidant value.

Keywords: α-Tocopherol; β-Carotene; Glutathione; Ascorbic acid; Antioxidant activity; Antioxidant enzymes; Lipid and protein oxidation; Argentine beef

1. Introduction

The use of nutritional strategies to improve meat quality combines both animal and food science. 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).

Antioxidants can be incorporated in muscle through dietary delivery. Among the nutritional strategies, supplementation of diets with vitamin E has shown to be effective in reducing lipid oxidation, improving meat color, and the consequent obtaining of meat products with extended shelf-life. Also grass-feeding supplies natural antioxidants that are efficiently incorporated into the muscle (Table 1). Indeed, antioxidants incorporated within cell membranes are more efficient that those added post mortem to preserve meat from oxidative damage (Kerry, Buckley, Morrisey, O'Sullivan, & Lynch, 1999).

Argentine meat has been traditionally produced on natural and planted pastures. However feed-lot strategies are also gaining place among producers (especially when low-cost corn is available). Meat produced on pasture or grain differs in their antioxidants, pro-oxidants and fatty acids composition. Pasture-fed cattle rendered meat with higher $n-3$ poly-unsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) content than their counterparts fed concentrate diets (Descalzo et al., 2005; García et al., 2005). In this context, unsaturated lipids are prone to lipid oxidation. Especially, the polar fraction of phospholipids contains the highest proportion of PUFA that exhibits a high susceptibility to oxidation (Ingene, Pearson, Dugan,

---

[*] Corresponding author. Address: Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 77, B1708WAB Morón, Buenos Aires, Argentina. Tel.: +54 11 4621 0446; fax: +54 11 4621 2012.

E-mail address: adescalzo@cnia.inta.gov.ar (A.M. Descalzo).

0309-1740/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2007.12.006

Table 1
Levels of α-tocopherol and TBARS in fresh beef

Authors	Animals	Basal diet	Vitamin E supplement	Muscle	Grass or pasture	Grass or pasture + E	Grain	Grain + E	SD (pooled)	Observations
				α-Tocopherol[A] in muscle (IU × 10^{-3}/g fresh meat) and TBARS[B] number when indicated						Vitamin E associated with diet:
Yang et al. (2002b)	Hereford cross: steers	Sorghum-based feedlot ration or pasture	2500 IU/head/day for 132 days	LD	6.7 (4.5) a	6.9 (4.6) a	2.7 (1.8) b	6.4 (4.3) a	0.3	Improved red color and lipid stability in fresh and aged meat
				SM	6.6 (4.4) a	6.4 (4.3) a	2.9 (2.0) b	7.9 (5.3) a	0.3	
				GM	8.6 (5.8) a	9.1 (6.1) a	3.6 (2.4) b	8.9 (6.0) a	0.3	
Descalzo et al. (2005)	Cross-breed: steers	Pasture or corn based diet	500 IU/head/day for 140 days	PM	4.6 (3.1) a	5.8 (3.9) a	2.2 (1.5) b	2.7 (1.8) b	1.1	Improved lipid stability, GSH, total antioxidant activity levels, SOD modulation
				PM TBARS	*0.09 a*	*0.1 a*	*0.28 b*	*0.26 b*	*0.08*	
Insani et al. (2007)	Cross-breed: steers	Pasture or corn based diet	Non	PM	3.1 (2.1) a		1.2 (0.8) b		0.03	Improved lipid and color stability retarded metmyoglobin formation and protein oxidation throughout refrigerated storage
				PM TBARS	*0.13 b*		*0.48 a*		*0.02*	
Mercier, Gatellier, and Renerre (2004)	Charolais: cows	Pasture or mixed diet	Non	LD	6.3 (4.2) a		5.4 (3.6) a		1.4	Improved lipid stability, GPX and SOD modulation, no effects on protein oxidation
Eikelenboom, Hoving-Bolink, Kluitman I., Houben, and Klont (2000)	Piemontese × Holstein Friesian cross: young bulls	Corn based diet	2025 UI/head/day for 136 days	LT			3.1 (2.1) b	6.6 (4.4) a	0.5	Improved lipid stability through ageing in LT and PM. No effect on color stability of fresh or aged LT muscle was found
				LT TBARS			*0.06*	*0.10*	*0.06*	
				PM			4.8 (3.2) b	12.4 (8.3) a	1.3	
				PM TBARS			*0.10*	*0.11*	*0.06*	
Realini et al. (2004a)	Hereford: steers	Pasture or corn based diet	1000 UI/head/day for 100 days	LD	5.8 (3.91)a		4.4 (2.9) b	5.6 (3.7) a		Improved lipid and color stability throughout refrigerated storage
				LD TBARS	*0.1 b*		*0.14 a*	*0.16 a*	*0.05*	
Formanek, Kerry, Buckley, Morrissey, and Farkas (1998)	Friesian cattle	Barley based diet	2000 mg α-tocopheryl acetate/kg diet	SM (freshly minced)			5.1 (3.4) a	3.1 (2.1) b	0.7	Improved lipid oxidation throughout refrigerated storage
				SM (80% O_2: 20% CO_2) TBARS			*1.78*	*2.26*	*0.13*	
Houben, van Dijk, Eikelenboom, and Hoving-Bolink (2000)	Piemontese × Holstein Friesian cross: young bulls	Corn-based diet	2025 mg/head/day for 136 days	BF (minced)			3.4 (2.3) b	7.9 (5.3) a	0.6	Improved lipid stability throughout refrigerated storage
				BF (minced) TBARS			*0.77 a*	*0.21 b*	*0.21*	

Lynch, Kerry, Buckley, Faustman, and Morrissey (1999)	Friesian cattle	Barley and grass silage (4 kg/day)	2000 mg/kg diet for 50 days	LD			3.5 (2.3) b	8.1 (5.4) a	0.2	Delayed Lipid oxidation and color deterioration in fresh and frozen meat
				GM			4.6 (3.1) b	6.6 (4.4) a	0.4	
				PM			6.7 (4.5) b	10.3 (6.9) a	0.1	
Walshe et al. (2006).	Cross-breed steers	Forage + concentrate C: conventional	Non	LD		Conventional		5.7 (3.8)	2.1	No differences were found in vitamin E levels. O samples were significantly higher in fat content than C samples and C samples had greater shelf life stability compared to O samples
						Organic		6.0 (4.1)		
		O: organic	Non	LD TBARS		Conventional		*0.10*	*0.05*	
						Organic		*0.11*		
O'Grady et al. (2001).	Charolais cross, Simmental cross, Limousin cross	Barley based concentrate	300 IU of DL-α-tocopheryl acetate/kg diet for 55 days	LD			1.2 (0.8) b	3.4 (2.3) a	NI	Improved lipid and oxymyoglobin stability throughout refrigerated storage (80% O_2:20% CO_2)
				LD TBARS			*0.24 a*	*0.11 b*	*0.05*	
O'Sullivan et al. (2002)	Charolais cross-bred heifers	Maize or grass silage	Non	LD	5.7 (3.8) a		3.1 (2.1) b		NI	Delayed lipid oxidation and metmyoglobin formation (greater color stability) throughout refrigerated storage
Harris, Huff-Lonergan, Lonergan, Jones, and Rankins (2001)	Angus × Gelbvieh heifers	Corn based diet	1000 IU of DL-α-tocopheryl acetate/head/day for 125 days	Strip Loin			3.2 (2.1) b	6.0 (4.0) a	0.03	Injection of $CaCl_2$ into beef with elevated levels of α-tocopherol resulted in accelerated tenderization. Vitamin E improved red color and lipid oxidation
				Strip Loin TBARS			*0.165*	*0.131*		
Dunne, Monahan, O'Mara, and Moloney (2005)	Continental cross-bred steers	Grass silage	40 kg of vitamin E per 1000 kg of mineral/vitamin mix VE/kg	ECR	3.6 (2.4) b	5.7 (3.8) a			0.3	VE supplementation of steers fed grass silage positively affected color stability, only of LD muscle
				LD	3.3 (2.2) b	4.2 (2.8) a			0.3	
				SM	4.0 (2.7) b	5.0 (3.3) a			0.3	

Implications for meat quality improvement.

Different letters within rows indicate significant differences ($P < 0.05$).

Abbreviations: *M. longissimus dorsi* (LD); *M. semimembranosus* (SM); *M. extensor carpi radialis* (ECR); *M. poas major* (PM); *M. gluteus medius* (GM); *M. semimembranous* (SM).

NI: not indicated.

[A] α-Tocopherol: in order to compare with literature, values in microgram per gram are indicated in parenthesis.

[B] TBARS: thiobarbituric acid reactive substances, number (mg MDA/kg meat) are indicated in italics.

& Price, 1980). Hence, lipid soluble antioxidants incorporated within the inner and outer sides of lipid membranes are capable to quench free radicals produced by post-mortem processing and storage of meat.

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 defenses are composed by non-enzymatic water and lipid soluble compounds like vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiols, and enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Together, enzymatic and non-enzymatic systems operate to counteract the action of pro-oxidants in muscle tissues (Decker, Livisay, & Zhou, 2000).

Therefore, in order to assess the quality of fresh meat, different factors that affect the oxidation of tissues and the antioxidant systems have to be considered.

2. Antioxidant vitamins and lipid stability

α-Tocopherol is the compound usually identified as vitamin E (Stinnett, 1983), although other tocopherols also present some vitamin E activity: D-α-tocopherol, D-β-tocopherol, D-γ-tocopherol, D-δ-tocopherol and D-α-tocotrienol showing, respectively, 1.49, 0.75, 0.30, 0.15 and 0.45 units of activity (Machlin, 1984).

Meat derived from pasture feeding, is associated with more antioxidants in the form of D-α-tocopherol, carotenoids and flavonoids (Wood & Enser, 1997), which stabilize the fatty acids. (Gatellier, Mercier, Juin, & Renerre, 2005; Gatellier, Mercier, & Renerre, 2004; Moloney, Mooney, Kerry, & Troy, 2001; Richardson, Nute, Wood, Scollan, & Warren, 2004).

Dietary vitamin E supplementation results in elevated concentrations of α-tocopherol within cell membranes (Ashgar et al., 1991; Monahan, Buckley, Morrissey, Lynch, & Gray, 1990). This supplementation resulted in an extension of 1.6–5 days of retail display life without compromising microbiological quality. It is believed that lipid oxidation products catalyse the oxidation of oxymyoglobin to metmyoglobin. The direct antioxidant action of α-tocopherol on membrane lipids is about 10^4 times faster than the propagation of lipid peroxidation, which is further spread to protein oxidation. Therefore, α-tocopherol may indirectly delay oxymyoglobin oxidation and meat decoloration (Morrisey, Buckley, & Galvin, 2000). At the same time the α-tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membrane phospholipids during storage which inhibits the passage of sarcoplasmic fluid through the muscle cell membrane (Gray, Gomaa, & Buckley, 1996).

For production systems in Uruguay and Argentina, beef finished on grass had higher vitamin E, β-carotene and ascorbic acid concentration and better lipid stability than those finished on concentrates (Descalzo et al., 2005; Insani et al., 2007; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004a).

In fresh meat the incorporation of vitamin E in the form of the α-tocopherol isomer was described by many authors, for different production systems and breeds with or without supplementation. Recent results, indicating tocopherol values and lipid oxidation in fresh beef, are compiled in Table 1. They indicate that vitamin E preserved meat from oxidation and its manifestations.

It can be concluded that the high herbage diet produced the most lipid and color stable meat and the high concentrate diet the least with other diets being intermediate. Cattle that grazed good-quality pasture had higher concentrations of α-tocopherol in skeletal muscle than cattle fed an unsupplemented high concentrate diet. These findings were widely described by Arnold, Arp, Scheller, Williams, and Schaefer (1993a).

However, most of these studies showed no differences in thiobarbituric acid reactive substances (TBARS) associated with vitamin E concentrations in fresh meat. The effect of vitamin E on lipid and color stability was detected after refrigerated storage or cooking of meat.

Data from three different experiments (Descalzo et al., 2005, 2007b; Insani et al., 2007) with cattle finished on pasture or grain and grazed buffaloes showed different initial TBARS levels, even in fresh beef. As shown in Fig. 1, the distribution of vitamin E (α-tocopherol isomer) and TBARS values showed a high variability among animals and experiments. Nevertheless, pasture-fed fresh meat had overall TBARS levels below 0.2 mg of malondialdehyde (MDA) equivalent per kilogram meat (MDA/kg), whereas α-tocopherol levels were over 2.98 × 10^{-3} IU/g

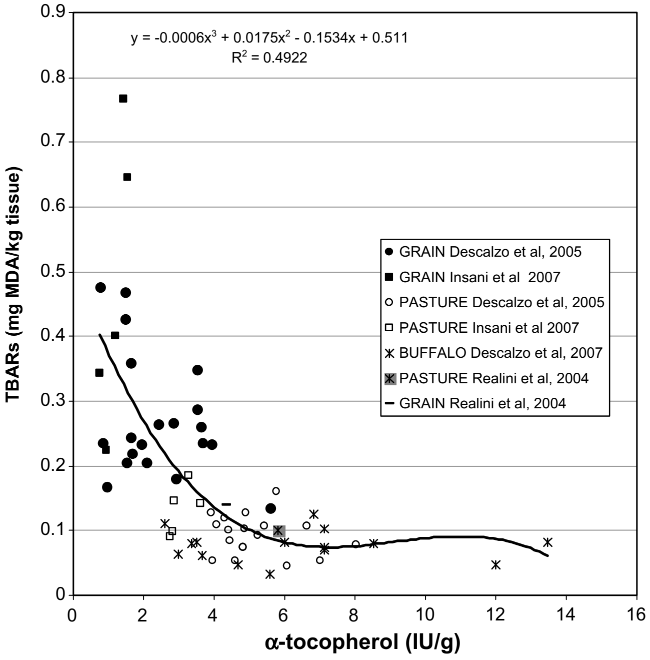

Fig. 1. Relationship between α-tocopherol and TBARS in fresh meat. Plot of TBARS vs. α-tocopherol from separated experiments.

(2 μg/g). For comparison with a similar feeding system in Uruguay, means reported by Realini et al. (2004a) were included in the graphic.

TBARS plotted against α-tocopherol values showed a cubic relationship in agreement with data published by Liu, Scheller, Arp, Schaefer, and Williams (1996). These authors showed higher TBARS numbers than the reported in this review, because they considered meat with 14 days of ageing in their plot. Nevertheless, tendency is similar to results in fresh beef thus indicating that α-tocopherol incorporated into the muscle could protect the tissue even at the onset of the lipid oxidation. Thereafter, during storage or processing, oxidative reactions progress in an exponential manner (Arnold, Scheller, Arp, Williams, & Schaefer, 1993b; Arnold et al., 1993a; Liu et al., 1996). These studies describe that the higher vitamin E incorporated into tissue, the lower TBARS progression during refrigerated storage. Yang, Lanari, Brewster, and Tume (2002a) found that supplementation did not increase the vitamin E content of the grazed animals but brought that of the feedlot animals up to the level of the grass-fed animals and reduced the rate of fat and color oxidation.

As shown in Table 1, different levels of α-tocopherol were incorporated into fresh meat through diverse feeding and supplementation strategies. The incorporation of α-tocopherol and the development of lipid oxidation may vary according to the age, muscle type, breed and feeding regime. In general cattle grazed in good quality pasture achieved α-tocopherol concentrations enough to preserve meat color and development of rancidity. However, lipid oxidation values (measured by TBARS) are low and it is difficult to relate them to the amount of α-tocopherol in fresh meat. Oxidative stability in meat is usually enhanced throughout refrigerated meat storage either for fresh or aged beef and is associated with the deterioration of red color and the formation of metmyoglobin. (Faustman, Chan, Schaefer, & Havens, 1998; McDowell et al., 1996; O'Sullivan et al., 2002).

According to these results, it should be stated that the magnitude of benefits resulting from vitamin E supplementation in finishing diets may widely vary due to the basal diet (quality of pasture, natural grazing, grass silage, supplement nature) offered to cattle.

In addition to α-tocopherol, pasture supplied β-carotene (pro-vitamin A: 1 μg β-carotene equivalent to 0.56 IU) that is incorporated into different muscles. Yang, Brewster, Lanari, and Tume (2002b) found 0.16 μg/g (0.09 IU/g); 0.09 μg/g (0.05 IU/g) and 0.22 μg/g (0.12 IU/g) of β-carotene in *M. longissimus dorsi* (LD), *M. semimembranosus* (SM) and *M. gluteus medius* (GM), respectively, when cattle was fed on pasture. These levels where 10-fold higher than reported in the same muscles of grain-fed cattle. Results obtained in argentine beef agree with this statement. *M. psoas major* (PM) from pasture-fed-cattle presented significantly higher ($P < 0.05$) levels of β-carotene when compared to their grain-fed counterparts. Mean levels of β-carotene found in pasture and grain produced meat were 0.45 μg/g (0.25 IU/g) vs. 0.06 μg/g (0.034 IU/g) in Descalzo et al. (2005) and 0.74 μg/g (0.41 IU/g) vs. 0.17 μg/g (0.01 IU/g) in Insani et al. (2007). Also β-carotene and γ-tocopherol were efficiently incorporated in LD muscle of grazed buffalo (0.25 and 0.24 μg/g or 0.14 and 7.2×10^{-5} IU/g, respectively, reported in Descalzo et al., 2007b).

Walshe, Sheehan, Delahunty, Morrissey, and Kerry (2006) reported concentrations around 0.152 μg/g (0.085 IU/g) and 0.116 μg/g (0.065 IU/g) of β-carotene in LD muscles from organic and conventionally reared steers, respectively. In general, muscle levels of β-carotene showed a high variability among experiments. Average coefficients of variation ranged from 11% in Yang et al. (2002b); 24% in Descalzo et al. (2007b); 44% in Descalzo et al. (2005) and 64% in Walshe et al. (2006). This could indicate that incorporation of β-carotene into muscle depends not only on dietary delivery and muscle type, but also on the individual uptake capacity.

In addition to β-carotene, Walshe et al. (2006), reported around 0.11 μg/g retinol (active form of vitamin A equivalent to 0.37 IU/g) in LD muscle of beef produced under either organic or conventional systems. Sampels, Pickova, and Wiklund (2004) reported 0.03 μg/g (0.01 IU/g), 0.09 μg/g (2.7×10^{-5} IU/g) and 3.79 μg/g (5.6×10^{-3} IU/g) of retinol γ- and α-tocopherol, respectively, in SM of calves fed a pelleted feed mixture.

The significance of carotenoids, their retinoid derivatives and minor forms of vitamin E incorporated in muscle should be further stated. Their concentrations remain at least one order below α-tocopherol levels in vivo. However, their activities should be taken into account because they exert biological activities that prevent tissue damage. Particularly, γ-tocopherol has the ability to protect against nitrogen-based free radicals, whilst α-tocopherol cannot (Jiang, Christen, Shigenaga, & Ames, 2001).

Besides, β-carotene cooperates with tocopherols in the radical scavenging capacity within the inner part of lipid membranes (Tsuchihashi, Kigoshi, Iwatsuki, & Niki, 1995; Fig. 2). Therefore, carotenoids and tocopherols contribute to the protection of tissues against the deleterious effects of $^1O_2$. Moreover, β-carotene acts at low partial oxygen pressure as quencher of lipid peroxidation products (Kennedy & Lieber, 1992).

3. Radical oxygen species (ROS) detoxification

The most powerful natural chain breakers occurring in muscle are tocopherols. Many studies have shown that these chain breakers scavenge two peroxyradical molecules (Burton & Ingold, 1981; Niki, Saito, Kawakami, & Kamiya, 1984). Vitamin C and polyphenols also seem to be able to directly reduce peroxyradicals, but their hydrophilic nature and remoteness from lipophilic radicals seem to hinder all direct contact reactions. Vitamin C is involved mainly during regeneration (reduction) of tocopheroxyl (oxidized form of vitamin E) obtained through the antiradical

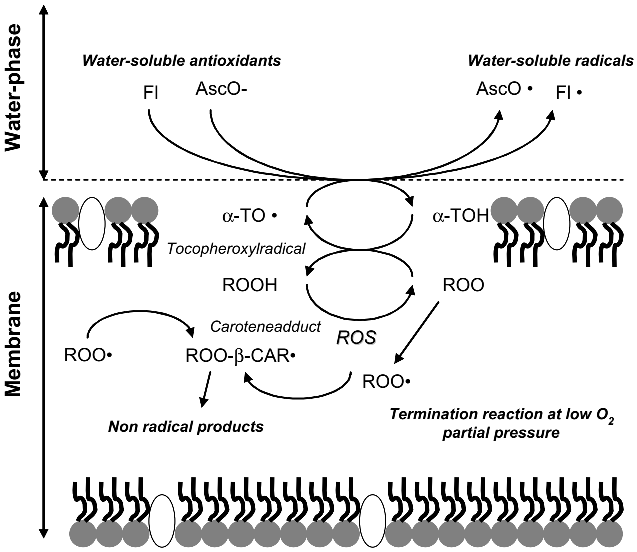

Fig. 2. Potential recycling mechanism between α-tocopherol (α-TOH), ascorbate (AscO$^-$) and flavonoid (Fl) during lipid oxidation (ROO$^{\cdot}$) through reactive oxygen species (ROS) and action of β-carotene (β-CAR) in membrane systems. Adapted from Laguerre et al. (2007) and Kennedy and Lieber (1992).

activity of tocopherol. Tappel (1968) was the first to suggest that these two molecules could act synergistically. This regeneration (reviewed in Packer & Kagan, 1993), is favored due to the relative low reducing potential of ascorbic acid ($E^0 \sim 0.28$) relative to α-tocopherol ($E^0 \sim 0.5$) (Becker, Nissen, & Skibsted, 2004). Ascorbic acid level in meat is not commonly reported. Nevertheless its presence in the cytoplasm side of cell membranes, close to tocopherol molecules, could help to maintain the antioxidant status within the tissue (Fig. 2).

Concerning polyphenols, many in vitro studies (Cos et al., 1998; Foti, Piattelli, Baratta, & Ruberto, 1996; Pietta, 2000; Rice-Evans, Miller, & Paganga, 1996) have shown that the antioxidant activity of phenolic compounds underlies in the 1,2-dihydroxy substitution on the B ring (catecholic structure). In vivo, it is probable that flavonoids with a catechol-like B cycle act in the same way as ascorbic acid (Cartron, Carbonneau, Fouret, Descomps, & Léger, 2001). This pathway seems to involve regeneration of the chromanoxyl radical of tocopherol into chromanol through polyphenol oxidation into phenoxyl radicals.

Descalzo et al. (2005) reported that pasture feeding could enhance ascorbic acid concentration from 15.92 (in grain-based diet) to 25.30 μg/g in PM muscle. Liu, Scheller, and Schaefer (1994) reported 16 μg/g in PM muscle of jugular infused animals, which is comparable to the values found in grain produced meat.

Realini, Duckett, and Windham (2004b), have shown that *post-mortem* addition of vitamin C to ground beef, was effective in retarding red color deterioration in grain or grass produced meat. However, lipid stability was improved only in the former samples. This observation is in agreement with an antioxidant network that retards ROS formation in muscle, conferred by feeding good quality pasture.

Regarding vitamin C activity, it is important to consider that ascorbyl radicals, formed after the reaction of ascorbic acid with higher reactive radicals, are strong metal-reducing agents. The reduced forms of these metals (especially iron) are able to decompose peroxides into radicals that can promote lipid and protein oxidation. Therefore, the net antioxidant capacity of vitamin C is a balance between its radical scavenging capacity and its influence on muscle prooxidants (Kanner, 1992).

4. Antioxidant enzymes in fresh beef

Antioxidant enzymes constitute an intracellular barrier against free radicals. Their activity in vivo is modulated by diverse factors like cell injury, stress, cancer and inflammatory reactions among others. In skeletal muscles the most important are catalase, superoxide dismutase and glutathione peroxidase.

After animal bleeding, all cells will be in anoxia and depleted of nutrients. In this conditions enzyme activity can be regarded only to the remnant at the onset of cell death. For that reason, research on antioxidant activity in beef shows diverse results.

4.1. Enzymatic reactions

Superoxide dismutase (SOD) and catalase (CAT) are coupled enzymes. SOD scavenges superoxide anion by the following reaction: $\mathrm{O}_2^{\cdot -} + 2\mathrm{H_2O} \rightarrow \mathrm{H_2O_2}$. Catalase removes hydrogen peroxide by: $\mathrm{H_2O_2} \rightarrow 2\mathrm{H_2O} + \mathrm{O_2}$. The reduced form of glutathione (GSH) may be oxidized by $\mathrm{H_2O_2}$ or organic peroxides to oxidized glutathione (GSSG) either spontaneously or via glutathione peroxidase (GPX) catalysis. Compensatory reduction of GSSG is catalysed by glutathione reductase (GR): $\mathrm{GSSG} + \mathrm{NADPH} + \mathrm{H}^+ \rightarrow 2\mathrm{GSH} + \mathrm{NADP}^+$.

4.2. Superoxide dismutase activity

SOD activity was measured in meat homogenates by the inhibition of epinephrine autooxidation, which proceeds through $\mathrm{O}_2^{\cdot -}$ formation (Misra & Fridovich, 1972). Meat from pasture-fed cattle had higher SOD activity than meat from grain-fed animals. It was found a positive correlation between SOD activity in PM and α-tocopherol content ($r = 0.434$; $P < 0.001$), which has been measured in the same muscles (Descalzo et al., 2007a). The same tendency, although not significant ($P > 0.05$), was found in a previous experiment for fresh meat (Descalzo, Insani, Eyherabide, Guidi, & Pensel, 2000). Renerre, Dumont, and Gatellier (1996) found that SOD activity was higher in oxidative and oxidative-glycolitic muscles (*diaphragm* and *psoas major*, respectively) and lesser in *M. tensor fasciae latae* (TFL) and *M. longissimus lumborum* (LL). They postulated that there is a natural protection which decreases the deleterious effects of oxy-radicals in these muscles. Gatellier et al. (2004), found that pasture diet significantly increased SOD activity in LD muscle of Charolais cows, heifers and steers (from four- to six-fold compared with mixed diet). Results in Descalzo et al. (2007a) with cross-breed steers indicated a moderate effect of pasture diet upon the modulation of SOD activity (Fig. 3A).

4.3. Catalase activity

Gatellier et al. (2004) showed a significant effect ($P < 0.01$) of sex (cows higher than steers or heifers) with a strong interaction between diet and catalase activity. CAT was significantly higher for heifers fed mixed vs. pasture diet. No differences were found attributable to diet in the case of cows and steers. Previous research in our laboratory (two different experiments) showed no differences in CAT activity in PM from steers fed pasture or grain diets (Descalzo et al., 2000, 2007a; Fig. 3B). In these experiments CAT activity was determined as described by Aebi (1984). Although CAT and SOD are coupled enzymes, they did not show the same pattern of activity in these experiments. Concerning to different muscles (young bulls, Français Frisone Pie Noir) Renerre et al. (1996) found higher activity in *diaphragm* (D) muscle ($P < 0.05$) when compared to LL, PM, and TFL and its activity did not change significantly after 8 days refrigerated storage. Pradhan, Rhee, and Hernández (2000) also reported stability of catalase throughout refrigerated and frozen storage of ground SM. They found that exogenously added catalase to beef samples failed to prevent lipid oxidation as did the endogenous enzyme. They postulated that the stability of catalase plays an important role in modulating lipid oxidation in fresh beef. Results presented in Descalzo et al. (2000) are in agreement with this finding, as CAT activity remained stable after 9 days storage in PM muscle.

4.4. Glutathione peroxidase activity

Basal diet seemed to influence GPX activity in muscle. As shown in Fig. 3C, separated experiments showed different results. In one case, grain diet induced higher GPX activity ($P < 0.05$) than pasture feeding. The second one did not show significant differences. Perhaps the high variation among each group did not allow statistical signification. GPX activity in meat samples is controversial. Gatellier et al. (2004) found a strong dependence of GPX with the diet. Meat from Charolais cattle fed mixed diet showed 2-fold GPX activity as compared with meat from pasture.

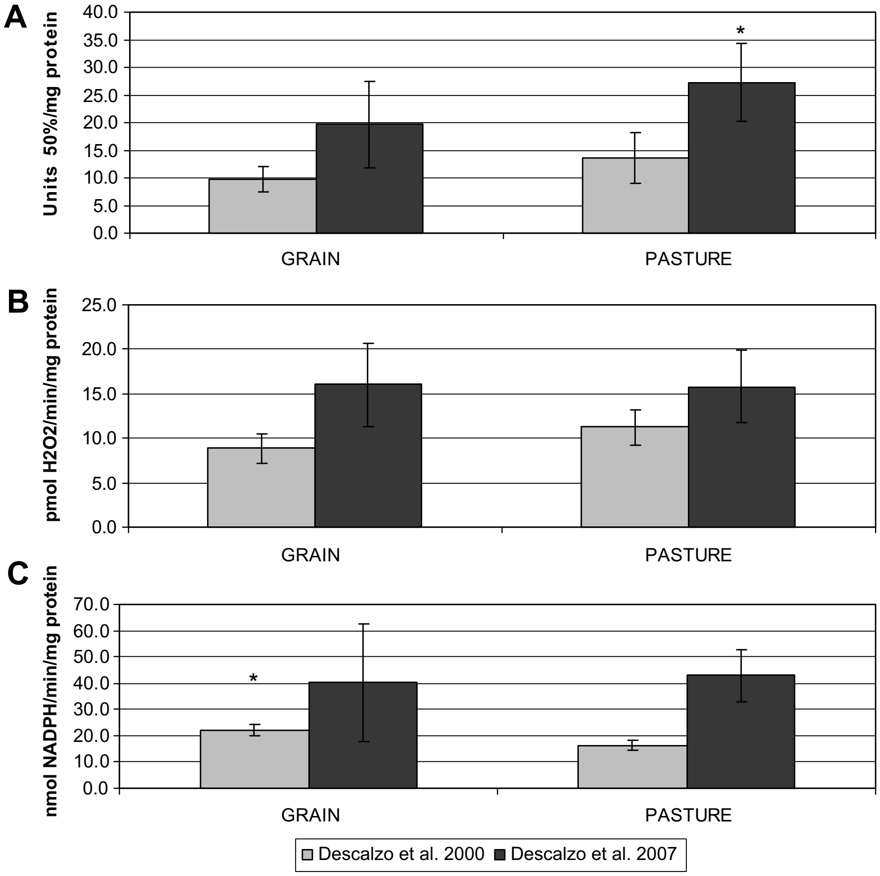

Fig. 3. Antioxidant enzymes in fresh beef: results compare pasture vs. grain beef from two separated experiments. A: SOD activity; B: CAT activity; C: GPX activity. Asterisk indicates significant differences ($P < 0.05$) within the same experiment.

As the main form of GPX is a seleno-dependent protein, it has been proposed that selenium (Se) content in the finishing diets is the major source of variation of GPX activity. De Vore and Greene (1982) reported a significant correlation between GPX and selenium in post-rigor muscle. On the other hand, O'Grady, Monahan, Fallon, and Allen (2001) found no differences neither in GPX activity nor in selenium content attributable to dietary supplementation. The authors concluded that the lack of an effect of dietary Se on muscle GPX activity was more likely a consequence of the relatively high native Se level in the basal diet.

In addition, GPX presented a negative correlation with oxidation levels, showing to be significantly sensible to oxidising conditions developed along 9 days refrigerated storage, for both, pasture and feedlot meat (Descalzo et al., 2000).

5. Glutathione redox system

Meat is an excellent source of total glutathione which is the major redox system within tissues. It is found in the reduced (GSH) and oxidized (GSSG) forms. 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 $OH^{\bullet}$, N_2O_3 and $ONOO^-$ (Wink & Mitchell, 1998). Therefore, measurement of glutathione content in tissues could be an indicator of the redox status of meat.

Reduced thiols are capable of donating hydrogens to free radicals. Among cellular thiols glutathione has probably the greatest potential as antioxidant due to its high concentration in skeletal muscle, and its ability to act as cofactor for the antioxidant enzyme GPX (Decker et al., 2000).

Meat homogenates from pasture-fed animals showed higher content of total glutathione than their grain counterparts ($P < 0.05$). Values were 194 ± 46 vs. 109 ± 48 mg/kg fresh beef for pasture and grain samples, respectively, despite of whether they were supplemented or not with vitamin E.

When the GSH/GSSG ratio was calculated for pasture or grain samples, no differences were found among the groups (8.74 and 8.54, respectively). Maintenance of this ratio could indicate that glutathione reductase activity is sufficient, and there should be no change in the GSH/GSSG ratio without any external induction of oxidative stress.

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:

$$\mathrm{GSSG} + 2\mathrm{H}^+ + 2\mathrm{e}^- \rightarrow 2\mathrm{GSH}$$

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

$$\mathrm{Ehc} = -240 - (59.1/2)\log([\mathrm{GSH}]^2/[\mathrm{GSSG}])\ \mathrm{mV}$$
$$\text{(where Eho is–240 mV)}$$

Therefore, a change in the concentration of GSH even without a change in the GSH/GSSG ratio could alter the cellular redox state (Schafer & Buettner, 2001). As reported in Descalzo et al. (2007a), meat produced on pasture showed higher reduction potential of glutathione ($P < 0.05$) than meat produced on grain (-170 ± 5.58 vs. -160 ± 12.11 mV, respectively) thus indicating that pasture diet conferred a reducing environment to the PM muscle.

6. Total antioxidant activity

The antioxidant potential in food is determined by the antioxidant composition and the antioxidative properties of constituents. By definition, the antioxidant activity (AOA) is the capability of a compound (composition) to inhibit oxidative degradation, e.g. lipid peroxidation. While the antioxidant capacity gives the information about the duration of antioxidative action, the reactivity characterizes the starting dynamics of antioxidation at a certain concentration of an antioxidant or antioxidant mixture. Individual antioxidants can react as chain-braking of oxidative reactions and the activity is related to the reactivity of the antioxidants to free radicals (Roginsky & Lissi, 2005). For that reason different approaches are applied to asses AOA in food and beverages.

In meat extracts, Gatellier et al. (2004) used the benzoate hydroxylation test which scavenges $OH^{\bullet}$ generated by Fenton reaction. $OH^{\bullet}$ is trapped by benzoate to give a fluorescent hydroxybenzoate. Antioxidants present in meat homogenates compete with benzoate within the scavenging reaction and reduce the fluorescence.

Meat homogenates from Charolais cattle fed mixed-diet showed higher $OH^{\bullet}$ scavenging activity when compared to pasture produced meat. These authors applied the Trolox equivalent antioxidant capacity (TEAC) test to the same samples. TEAC measures antioxidant activity against 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\bullet}+$) radical cation. An improved form of this test was described by Re et al. (1999). The TEAC–ABTS test failed to show differences attributable to diet in meat homogenates. Also Dunne, Monahan, O'Mara, and Moloney (2005) found that supplementation of grass silage with vitamin E had no effect on plasma total antioxidant status values of exercised and non exercised steers. This method was applied with a commercial kit (Randox kit for total antioxidant activity) but is also based on the reduction of the ABTS$^{\cdot}$+ radical. Results presented in Descalzo et al. (2007a) are in agreement with this finding. TEAC test showed similar scavenging capacity for meat homogenates produced on pasture or grain (Table 2).

ABTS$^{\cdot}$+ reacts with any hydroxylated aromatics independently of their real antioxidative potential as found for the titration of aromatic OH-groups including OH-groups which do not contribute to the antioxidation. Although pasture meat had higher levels of α-tocopherol (consider the aromatic nature of tocopherols) this test failed to exhibit dietary differences among homogenates.

Conversely, differences in AOA of pasture and grain meat homogenates were found with the ferric reducing antioxidant power (FRAP) test (Benzie & Strain, 1996; Pulido, Bravo, & Saura-Calixto, 2000) which is based on the ability of phenolics to reduce Fe(3+) to Fe(2+). When this occurs in the presence of 2,4,6-trypyridyl-*s*-triazine, the reduction is accompanied by the formation of a blue ferrous complex.

AOA in grain or pasture produced meat is summarized in Table 2. FRAP assay showed positive correlations with antioxidant vitamins and glutathione and inverse with TBARS. TEAC assay failed to correlate with the same variables. These results clearly differentiated the radical scavenging capacity from the ferric reducing potential of meat samples.

7. Protein oxidation

Nutritional and sensory properties of meat are deteriorated by oxidation. Proteins are damaged by the action of free radicals producing the loss of their functions. From the nutritional point of view, meat is an ideal source of proteins that can barely be substituted by other protein sources, especially in the infant age.

In addition to their nutritional properties, functional dipeptides were described in meat (Arihara, 2006). Both carnosine (*b*-alanyl-L-histidine) and anserine (*N*-*b*-alanyl-1-methyl-L-histidine) are antioxidative histidyl dipeptides and the most abundant antioxidants in meats.

Table 2
Antioxidant assays in meat homogenates and correlation with antioxidant vitamins and TBARS

Diet	FRAP: μM equiv. Fe(2+)	TEAC: mM Trolox
Grain	137.92 ± 40.8 b	2.89 ± 0.28 a
Pasture	222.88 ± 35.6 a	2.71 ± 0.27 a
Pearson correlation coefficient[A] *with*		
α-tocopherol	0.5758	−0.1657
β-carotene	0.4048	−0.2160
Ascorbic acid	0.5702	−0.1658
Total glutathione	0.6302	−0.1751
TBARS	−0.4618	0.0875

Different letters within the same column indicate significant differences ($P < 0.05$).

[A] $P < 0.05$, $n = 20$.

The concentrations of carnosine in meat range from 500 mg/kg of chicken thigh to 2700 mg/kg of pork shoulder. On the other hand, anserine is especially abundant in chicken muscle. Their antioxidant activities may result from their ability to chelate transition metals such as copper and iron (Brown, 1981).

Free radicals generated during lipid oxidation, in addition to the presence of transition metals, promote the accumulation of oxidized proteins and it is probable that meat active peptides could loss their functionality due to this process.

As shown by Insani et al. (2007), dietary differences among cattle had minor influence on protein oxidation in fresh meat. Oxidation has to be enhanced by ageing or storage of meat (PM) under commercial conditions. After 7 days of ageing, meat with higher lipid oxidation values showed also higher protein oxidation and metmyoglobin formation. Arnold et al. (1993a) demonstrated that lipid oxidation and metmyoglobin formation occurred simultaneously in beef with α-tocopherol levels below 3.0 μg/g (4.5×10^{-3} IU/g). However, they found that protein and lipid oxidation showed different development patterns.

In addition, Rowe, Maddock, Lonergan, and Huff-Lonergan (2004) showed that supplementation of diets with vitamin E prevented irradiated beef (LL) from protein oxidation. Levels of 4.19 μg/g (6.24×10^{-3} IU/g) of α-tocopherol decreased significantly ($P < 0.05$) the number and extent of oxidized sarcoplasmic and myofibrillar proteins. These authors discussed that protein oxidation can cause insoluble aggregates and denaturation processes that may compromise not only the functional properties of muscle proteins but may also be detrimental for meat tenderization.

8. Improving functional properties of meat

The development of foods which offer scope in helping to promote health and prevent disease is a major growth area and this is likely to continue as consumers demand more foods. In this respect, meat can be considered as a functional food itself. It can also be used as an ingredient to improve the functionality of meat based products. For this purpose, the use of good quality meat is mandatory. Inherent antioxidative capacity of muscle may help to protect functional components of meat.

As reviewed by Scollan et al. (2006) nutrition is the major route for increasing the content of beneficial fatty acids in beef. Feeding grass or concentrates containing linseed (rich in α-linolenic acid, 18:3*n*−3) in the diet increases the content of 18:3*n*−3and its longer chain derivative eicosapentaenoic acid (EPA, 20:5*n*−3) in beef muscle and adipose tissue, resulting in a lower *n*−6:*n*−3 ratio. Grass feeding also increases docasahexaenoic acid (DHA, 22:6*n*−3). The main conjugated linoleic acid (CLA) isomer in beef is CLA *cis*-9, *trans*-11 and it is mainly associated with the triacylglycerol lipid fraction and therefore is positively correlated with level of fatness. As stated by

Palmquist (2001), the level of CLA *cis*-9, *trans*-11 in beef is related to (1) the amount of this isomer produced in the rumen and (2) synthesis in the tissue, by delta-9 desaturase, from ruminally produced *trans* vaccenic acid (18:1 *trans*-11; TVA).

Diets containing a proportionally high level of linolenic acid in the fat, such as fresh grass, grass silage, and pasture feeding during the finishing periods, resulted in increased deposition of CLA *cis*-9, *trans*-11 in muscle (Enser et al., 1999; French, O'Riordan, Monahan, Caffrey, & Moloney, 2003; French et al., 2000; García et al., 2005; Scollan et al., 2001). Therefore, from a nutritional standpoint grass fed beef will provide approximately 123 mg CLA from a standard meat product at 10% fat, whereas the same product from grain-feed beef would provide 48.3 mg. These values should be higher if also the C18:1 *t*11 isomer (*trans* vaccenic acid) is considered. The human body can convert an average of 19–30% of VA into CLA. Therefore, in considering total CLA benefit derived from grass fed beef, one must consider the total CLA derived from both direct CLA and conversion of VA to CLA by the body (Turpeinen et al., 2002).

However, increasing the content of $n-3$ PUFA in beef can influence color shelf-life and sensory attributes of the meat. As the content of $n-3$ PUFA increases then sensory attributes such as "greasy" and "fishy" score higher and color shelf-life may be reduced.

Under these situations, high levels of vitamin E are necessary to help stabilize the effects of incorporating high levels of long chain PUFA into meat. However, grass feeding not only increases $n-3$ PUFA and CLA but, due to its high content of vitamin E and β-carotene, oxidative stability is improved as discussed above.

Additionally to bioactive lipids, functional peptides can arise from meat processing. As discussed by Arihara (2006), the generation of bioactive peptides in meat products, such as fermented meat products, is a possible direction for introducing physiological functions. Bioactive peptides would be generated in fermented meat products since meat proteins are hydrolyzed by proteolytic enzymes during fermentation and storage.

Also the possible development of probiotic meat derivatives (Arihara et al., 1998; Hammes, Haller, & Gänzle, 2003; Hugas & Monfort, 1997) implies that the resulting product has to be stable.

Therefore, improving the antioxidant status in meat may help to maintain the integrity of its nutrients rather than constituting a natural source of food antioxidants itself.

9. Volatile compounds and odor produced in relationship with antioxidant properties of fresh beef

Aldehydes, produced during lipid oxidation, are the major contributors to off-flavors of beef. As discussed above, the presence or absence of vitamin E in animal tissues affects the stability of lipids during storage of meat. It is capable of quenching free radicals and thus protects phospholipids and cholesterol against oxidation.

Several studies report that animal diet can influence the fatty acid composition and natural antioxidants of meat products and therefore affect the volatile compounds production (Descalzo et al., 2005; Elmore, Mottram, Enser, & Wood, 1999; Elmore et al., 2004; Larick & Turner, 1990; Priolo, Cornu, Prache, & Krogmann, 2004; Young, Lane, Priolo, & Fraser, 2003). Therefore, altering the fatty acid composition of beef muscle can affect its flavor characteristics. For example, the relative levels of linoleic acid (C18:2$n-6$) and α-linolenic acid (C18:3$n-3$) in grain and forage are largely responsible for the differences in volatile composition, and hence the flavor, of beef finished on these diets (Larick & Turner, 1990; Larick et al., 1987).

Sensory analysis showed that flavor scores correlated significantly with C14:l. C18:0, C18:l and C18:3 of the neutral lipids, with C18:3 of the polar lipid and with water soluble sugar content (Melton, Amiri, Davis, & Backus, 1982). In general, high-energy grain diets produced a more acceptable or a more intense flavor in red meats than low-energy forage or grass diets. Other studies have shown that beef produced on pasture had similarly intense or equally acceptable flavor as beef produced on grain. The underlying reasons for these results are unknown. Perhaps this could be due to differences in sensory panels or to the high quality of some pastures (Melton, 1990). These results are described in cooked beef. However, differences in volatile aldehydes attributed to dietary treatments are found even in fresh meat. Grain-produced beef (PM) had higher contents of 3-methyl-butanal, hexanal, pentanal, octanal and heptanal than grass-produced beef and the presence of these volatile compounds correlated positively ($P < 0.05$) with TBARS (Biolatto, Descalzo, & Sancho, 2007).

Supplementation of diets with 500 IU/head/day of vitamin E did not improve significantly the stability of beef, thus indicating that antioxidants from good-quality pastures were efficiently incorporated into muscle and therefore could mask the action of vitamin E supplement (Descalzo et al., 2000, 2005).

E-Nose measurements on the same samples showed that a group of sensors could associate beef odor with vitamin E (Grigioni, Descalzo, Insani, Pensel, & Margaría, 2000) and antioxidant activity (Descalzo et al., 2007a). The discriminant functions found for the sensors array differentiated beef from grain and grain supplemented with vitamin E animals. Therefore, the E-nose profile of fresh beef samples can be related not only to classical sensory descriptors, but also to antioxidant potential in fresh meat. 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 pasture supplemented and non supplemented samples can be attributed to the quality of antioxidants provided by the pasture (Descalzo et al., 2007a).

10. Descriptive statistical analyses

The use of descriptive statistics to differentiate dietary groups is a powerful tool to evaluate the classification of experimental data, which are taken together without a previous hypothesis or any external information concerning the studied sample. Therefore, this kind of analysis allowed determining the relationship among dependent experimental variables and their influence over the meat samples. Discriminant analysis is used (1) for detecting the variables that allow the researcher to discriminate between different (naturally occurring) groups, and (2) for classifying cases into different groups with a better than chance accuracy. Therefore, this approach was applied to the complete set of measurements in fresh beef.

The Stepwise method selected α-tocopherol, TBARS, pentanal, heptanal and β-carotene as the variables which contributed most to the classification. Four linear Fischer's discriminant functions were defined according to dietary treatments with a success rate of correct classification of each sample of 100% and 97.4% for the original cases and after cross-validation. As shown in Fig. 4, fresh beef from grain or pasture with or without vitamin E treatments were clearly differentiated among each other.

In agreement with Laguerre, Lecomte, and Villeneuve (2007) there is not a unique method to describe the antioxidant capacity of biological samples. The complexity and diversity of mechanisms that contribute to the onset of oxidation and the mechanisms that counteract oxidative reactions involve multiple pathways. Therefore, it is difficult to determine the exact weight to be allocated to each variable to ensure the consistency and interpretation of this set of diverse data.

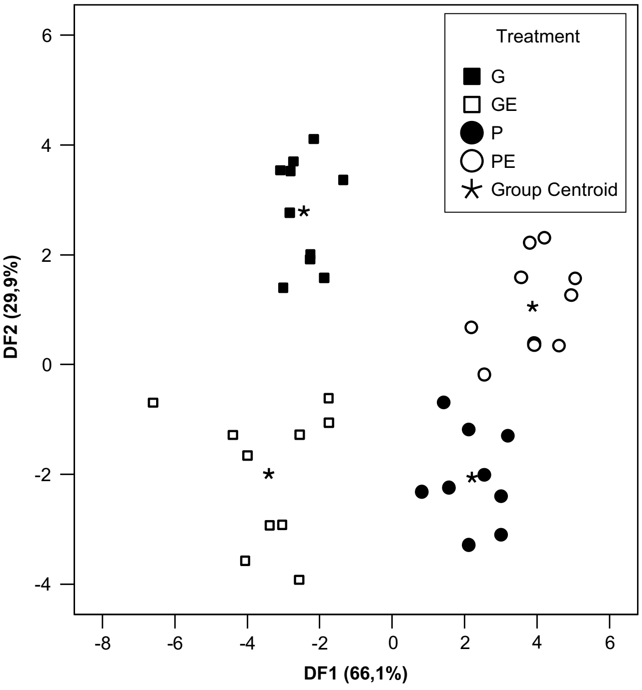

Fig. 4. Discriminant Analysis. Treatments: (■) grain-fed meat; (□) grain + 500 IU vitamin E/head/day; (●) pasture-fed meat; (○) pasture + 500 IU vitamin E/head/day.

Multivariate approach applied to a set of biochemical variables related to a defined set of samples pictures the global understanding of complex biochemical mechanisms occurring in meat.

11. Concluding remarks

Argentine meat has been traditionally produced on pasture. However, to comply with some market requirements, grain finishing is becoming more common among producers. This affects the quality of the meat. This review considers several factors concerning the antioxidant status and the development of oxidation in fresh meat obtained under different production systems.

Fatty acid composition was clearly affected by diet. Fresh meat produced on grain had higher fat and cholesterol content than meat from pasture-fed-animals. On the other hand, beef from pasture-fed cattle had higher percentage of linolenic acid, less linoleic acid and, overall, higher percentage of polyunsaturated fatty acids than beef from grain-fed animals. Nevertheless, oxidation markers derived from lipid oxidation, as TBARS number and volatile levels of hexanal, pentanal, heptanal, octanal and 3-methylbutanal, were higher in grain than in pasture samples. A plausible explanation is that pasture diet improved an overall antioxidant and redox status to fresh meat when compared to a grain-finishing diet, regardless of their supplementation with vitamin E. Therefore, pasture diet contributes natural antioxidants in sufficient amounts and is an efficient way to prevent lipid oxidation in fresh beef.

Antioxidant enzymes showed slight differences between samples, indicating 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 odor profile of meat samples with different antioxidant status. Moreover, it contributed to differentiate vitamin E supplemented from non supplemented grain-produced meat.

The combination of traditional statistics to define differences among treatments, together with the use of descriptive methods, resulted valuable tools to evaluate the whole picture when it is believed that many attributes (biochemical *post-mortem* processes within the muscle) explain differences between samples. Thus, naturally incorporated α-tocopherol, β-carotene and glutathione seemed to be the main factors influencing the oxidative/antioxidant status of fresh meat and could be used for meat differentiation.

These considerations apply to fresh meat in general. Hence, Argentina, as the country with higher meat consumption *per capita*; more than 3×10^3 tonnes produced; and in the fifth place among beef exporters (Rearte, 2007), may take advantage of its extensive production system which renders a high quality meat.

References

Aebi, H. (1984). Catalase in vitro. In L. Packer (Ed.). *Oxygen radicals in biological systems. Methods in enzymology* (Vol. 105, pp. 121–126). New York: Academic Press.

Andersen, H. J., Oksbjerg, N., Young, J. F., & Therkildsen, M. (2005). Feeding and meat quality – A future approach. *Meat Science, 70*, 543–554.

Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science, 74*, 219–229.

Arihara, K., Ota, H., Itoh, M., Kondo, Y., Sameshima, T., Yamanaka, H., et al. (1998). Lactobacillus acidophilus group lactic acid bacteria applied to meat fermentation. *Journal of Food Science, 63*, 544–547.

Arnold, R. N., Arp, S. C., Scheller, K. K., Williams, S. N., & Schaefer, D. M. (1993a). Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science, 71*, 105–108.

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., & Schaefer, D. M. (1993b). Dietary α-tocopheryl acetate enhances beef quality in Holstein and beef breed steers. *Journal of Food Science, 58*(1), 28–33.

Ashgar, A., Gray, J. I., Booren, A. M., Gomaa, E. A., Abouzied, M. M., Miller, E. R., et al. (1991). Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. *Journal of the Science of Food and Agriculture, 57*(1), 31–41.

Awasthi, Y. C., Dao, D. D., & Saneto, R. P. (1980). Interrelationship between anionic and cationic forms of glutathione *S*-transferases of human liver. *Biochemical Journal, 191*(1), 1–10.

Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality or health effects. *European Food Research and Technology, 219*, 561–571.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Biolatto, A, Descalzo, A. M., & Sancho, A. M. (2007). Evolución de compuestos volátiles como indicadores de oxidación durante el almacenamiento refrigerado de carne producida en la región de Pampa Húmeda Argentina. In *Proceedings of the XI congreso Argentino de ciencia y tecnología de los alimentos* (p. 26).

Brown, C. E. (1981). Interactions among carnosine, anserine, ophidine and copper in biochemical adaptation. *Journal of Theoretical Biology, 88*, 245–256.

Burton, G. W., & Ingold, K. U. (1981). Autoxidation of biological molecules. 1: The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *Journal of the American Chemical Society, 103*, 6472–6477.

Cartron, E., Carbonneau, M. A., Fouret, G., Descomps, B., & Léger, C. L. (2001). Specific antioxidant activity of caffeoyl derivatives and other natural phenolic compounds: LDL protection against oxidation and decrease in the proinflammatory lysophosphatidylcholine production. *Journal of Natural Products, 64*, 480–486.

Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Piel, B., et al. (1998). Structure–activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products, 61*, 71–76.

De Vore, V. R., & Greene, B. E. (1982). Glutathione peroxidase in post-rigor bovine semitendinosus muscle. *Journal of Food Science, 47*, 1406–1409.

Decker, E. A., Livisay, S. A., & Zhou, S. (2000). Mechanisms of endogenous skeletal muscle antioxidants: Chemical and physical aspects. In Eric Decker, Cameron Faustman, & Clemente J. López-Bote (Eds.), *Antioxidants in muscle foods* (pp. 25–60). John Wiley & Sons Inc.

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J., et al. (2007a). Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Science, 75*, 299–307.

Descalzo, A. M., Insani, E. M., Biolatto, A., Sancho, A. M., García, P. T., Pensel, N. A., et al. (2005). Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science, 70*, 35–44.

Descalzo, A. M., Insani, E. M., Eyherabide, A., Guidi, S. M., & Pensel, N. A. (2000). Antioxidant enzymes activity in *Psoas major* beef muscle from different production systems. In *Proceedings of the 46th international congress of meat science and technology* (Vol. 2, pp. 560–561).

Descalzo, A. M., Rossetti, L., Sancho, A. M., García, P. T., Biolatto, A., Carduza, F., et al. (2007b). Antioxidant consumption and development of oxidation during ageing of buffalo meat produced in Argentina. *Meat Science*. doi:10.1016/jmeatsci.2007.10.020.

Dunne, P. G., Monahan, F. J., O'Mara, F. P., & Moloney, A. P. (2005). Colour stability, under simulated retail display conditions, of *M. longissimus dorsi* and *M. semimembranosus* from steers given long-term daily exercise and supplemented with vitamin E. *Meat Science, 70*, 480–489.

Eikelenboom, G., Hoving-Bolink, A. H., Kluitman, I., Houben, J. H., & Klont, R. E. (2000). Effect of dietary vitamin E supplementation on beef colour stability. *Meat Science, 54*, 17–22.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1999). Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry, 47*, 1619–1625.

Elmore, J. S., Warren, H. E., Mottram, D. S., Scollan, N. D., Enser, M., Richardson, R. I., et al. (2004). A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein–Friesian steers fed diets based on silage or concentrates. *Meat Science, 68*(1), 27–33.

Enser, M., Scollan, N. D., Choi, N. J., Kurt, E., Hallet, K., & Wood, J. D. (1999). Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Animal Science, 69*, 143–146.

Faustman, C., Chan, W. K. M., Schaefer, D. M., & Havens, A. (1998). Beef color update: The role for vitamin E. *Journal of Animal Science, 76*, 1019–1026.

Foti, M., Piattelli, M., Baratta, M. T., & Ruberto, G. (1996). Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system structure–activity relationship. *Journal of Agricultural Food Chemistry, 44*, 497–501.

French, P., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., & Moloney, A. P. (2003). Fatty acid composition of intramuscular triacylglycerols of steers fed autumn grass and concentrates. *Livestock Production Science, 81*, 307–317.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monohan, F. J., Caffrey, P. J., et al. (2000). Fatty acid composition, including conjugated linoleic acid of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science, 78*, 2849–2855.

Formanek, Z., Kerry, J. P., Buckley, D. J., Morrissey, P. A., & Farkas, J. (1998). Effects of dietary vitamin E supplementation and packaging on the quality of minced beef. *Meat Science, 50*, 203–210.

García, P. T., Pensel, N. A., Latimori, N. J., Kloster, A. M., Amigone, M. A., & Casal, J. J. (2005). Intramuscular lipids in steers under different grass and grain regimen. *Fleischwirtschaft International, 1*, 27–31.

Gatellier, P., Mercier, Y., & Renerre, M. (2004). Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. *Meat Science, 67*, 385–394.

Gatellier, P., Mercier, Y., Juin, H., & Renerre, M. (2005). Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. *Meat Science, 69*, 175–186.

Gray, J. I., Gomaa, E. A., & Buckley, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science, 43*, s111–s123.

Grigioni, G. M., Descalzo, A., Insani, M., Pensel, N. A., & Margaría, C. A. (2000). Effect of vitamin E supplemented feed regimen on beef odour assessed by a conducted polymer sensors based electronic nose.

In *Proceedings 46th international congress of meat science and technology* (pp. 608–609).

Hammes, W. P., Haller, D., & Gänzle, M. G. (2003). Fermented meat. In E. R. Farnworth (Ed.), *Handbook of fermented functional foods* (pp. 251–275). Boca Raton, FL: CRC Press.

Harris, S. E., Huff-Lonergan, E., Lonergan, S. M., Jones, W. R., & Rankins, D. (2001). Antioxidant status affects color stability and tenderness of calcium chloride-injected beef. *Journal of Animal Science, 79*, 666–677.

Houben, J. H., van Dijk, A., Eikelenboom, G., & Hoving-Bolink, A. H. (2000). Effect of dietary vitamin E supplementation, fat level and packaging on colour stability and lipid oxidation in minced beef. *Meat Science, 55*, 331–336.

Hugas, M., & Monfort, J. M. (1997). Bacterial starter cultures for meat fermentation. *Food Chemistry, 59*, 547–554.

Ingene, J. O., Pearson, A. M., Dugan, A. M., & Price, J. F. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry, 5*, 263–276.

Insani, E. M., Eyherabide, A., Grigioni, G., Sancho, A. M., Pensel, N. A., & Descalzo, A. M. (2007). Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. *Meat Science*. doi:10.1016/j.meatsci.2007.10.01.

Jiang, Q., Christen, S., Shigenaga, M. K., & Ames, B. N. (2001). Gamma-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *American Journal of Clinical Nutrition, 74*(6), 714–722.

Kanner, J. (1992). Mechanisms of nonezymic lipid peroxidation in muscle foods. In *Lipid oxidation in foods*, A. J. St. Angelo (Ed.), *American chemical society symposium series* (Vol. 500, pp. 55–73). Washington, DC: American Chemical Society Books.

Kennedy, T. A., & Lieber, D. C. (1992). Peroxyl radical scavenging by β-carotene in lipid bilayers. Effect of oxygen partial pressure.. *The Journal of Biological Chemistry, 267*(7), 4658–4663.

Kerry, J. P., Buckley, D. J., Morrisey, P. A., O'Sullivan, K., & Lynch, P. B. (1999). Endogenous and exogenous α-tocopherol supplementation: effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine *M. longissimus dorsi* roasts held in aerobic and vacuum packs.

Laguerre, M., Lecomte, J., & Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges.. *Progress in Lipid Research, 46*, 244–282.

Larick, D. K., & Turner, B. E. (1990). Headspace volatiles and sensory characteristics of ground beef from forage and grain fed heifers. *Journal of Food Science, 54*, 649–654.

Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., Garner, G. B., et al. (1987). Flavor constituents of beef as influenced by forage- and grain-feeding. *Journal of Food Science, 52*(2), 245–251.

Liu, Q., Scheller, K. K., & Schaefer, D. M. (1994). Jugular infusion of vitamin C and color stability of beef. *Journal of Animal Science, 72*(Suppl. I), 372.

Liu, Q., Scheller, K. K., Arp, S. C., Schaefer, D. M., & Williams, S. N. (1996). Titration of fresh meat colour stability and malondialdehyde development with steers fed vitamin E-supplemented diets. *Journal of Animal Science, 74*, 117–126.

Lynch, M. P., Kerry, J. P., Buckley, D. J., Faustman, C., & Morrissey, P. A. (1999). Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Science, 52*, 95–99.

Machlin, L. J. (1984). Vitamin E. In L. J. Machlin (Ed.), *Nutritional, biochemical and clinical aspects* (pp. 99–145). New York: Marcel Dekker.

McDowell, L. R., Williams, S. N., Hidiroglou, N., Njeru, C. A., Hill, G. M., Ochoa, L., et al. (1996). Vitamin E supplementation for the ruminant. *Animal Feed Science and Technology, 60*, 266–273.

Melton, S. L. (1990). Effects of feeds on flavor of red meat: A review. *Journal of Animal Science, 68*(12), 4421–4435.

Melton, S. L., Amiri, M., Davis, G. W., & Backus, W. R. (1982). Flavor and chemical characteristics of ground beef from grass-, forage-grain- and grain-finished steers. *Journal of Animal Science, 55*, 77–87.

Mercier, Y., Gatellier, P., & Renerre, M. (2004). Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Science, 66*, 467–473.

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry, 247*(10), 3170–3175.

Moloney, A. P., Mooney, M. T., Kerry, J. P., & Troy, D. J. (2001). Producing tender and flavoursome beef with enhanced nutritional characteristics. In: Animal nutrition and metabolism group symposium on 'Quality inputs for quality foods'. *Proceedings of the Nutrition Society, 60*, 221–229.

Monahan, F. J., Buckley, D. J., Morrissey, P. A., Lynch, P. B., & Gray, J. I. (1990). Effect of dietary α-tocopherol supplementation on α-tocopherol levels in porcine tissues and on susceptibility to lipid peroxidation. *Food Science and Nutrition, 42*(4), 203–212.

Morrisey, P. A., Buckley, D. J., & Galvin, K. (2000). Vitamin E and the oxidative stability of pork and poultry. In Eric Decker, Cameron Faustman, & Clemente J. López-Bote (Eds.), *Antioxidants in muscle foods* (pp. 263–287). John Wiley & Sons Inc.

Niki, E., Saito, T., Kawakami, A., & Kamiya, Y. (1984). Inhibition of methyl linoleate in solution by vitamin E and vitamin C. *Journal of Biological Chemistry, 259*, 4177–4182.

O'Grady, M. N., Monahan, F. J., Fallon, R. J., & Allen, P. (2001). Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. *Journal of Animal Science, 79*, 2827–2834.

O'Sullivan, A., O'Sullivan, K., Galvin, K., Moloney, A. P., Troy, D. J., & Kerry, J. P. (2002). Grass silage versus maize silage effects on retail packaged beef quality. *Journal of Animal Science, 80*, 1556–1563.

Packer, L., & Kagan, V. E. (1993). In L. Packer & J. Fuchs (Eds.), *Vitamin E in health and disease*. New York: Marcel Dekker Inc., p. 179.

Palmquist, D. L. (2001). Ruminal and endogenous synthesis of CLA in cows. *The Australian Journal of Dairy Technology, 56*, 134–137.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035–1042.

Pradhan, A. A., Rhee, K. S., & Hernández, P. (2000). Stability of catalase and its potential role in lipid peroxidation. *Meat Science, 74*, 385–390.

Priolo, A., Cornu, A., Prache, S., & Krogmann, M. (2004). Fat volatiles tracers of grass feeding in sheep. *Meat Science, 66*(2), 475–481.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by modified ferric reducing/antioxidant power assay. *Journal of Agriculture and Food Chemistry, 48*, 3396–3402.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radicals in Biology and Medicine, 26*(9/10), 1231–1237.

Realini, C. E., Duckett, S. K., Brito, Q. W., Dalla Rizza, M., & De Mattos, D. (2004a). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*, 567–577.

Realini, C. E., Duckett, S. K., & Windham, W. R. (2004b). Effect of vitamin C addition to ground beef from grass-fed or grain-fed sources on color and lipid stability, and prediction of fatty acid composition by near-infrared reflectance analysis. *Meat Science, 68*, 35–43.

Rearte, D. (2007). *Meat production program*. Coordinator: Daniel Rearte. <http://www.inta.gov.ar/info/doc/rearte.pdf>.

Renerre, M., Dumont, F., & Gatellier, P. (1996). Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Science, 43*, 111–121.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radicals in Biology and Medicine, 20*, 933–956.

Richardson, R. I., Nute, G. R., Wood, J. D., Scollan, N. D., & Warren, H. E. (2004). Effects of breed, diet and age on shelf life, muscle vitamin E and eating quality of beef. *Proceedings of the British Society of Animal Science*, 84.

Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry, 92*, 235–254.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Influence of early postmortem protein oxidation on beef quality. *Journal of Animal Science, 82*(3), 785–793.

Sampels, S., Pickova, J., & Wiklund, E. (2004). Fatty acids, antioxidants and oxidation stability of processed reindeer meat. *Meat Science, 67*, 523–532.

Schafer, F. Q., & Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radicals in Biology and Medicine, 30*(11), 1191–1212.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating of fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition, 85*, 115–124.

Scollan, N., Hocquette, J.-F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, I. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science, 74*, 17–33.

Stinnett, J. D. (1983). *Nutrition and the immune response*. Boca Raton, FL: CRC Press.

Tappel, A. L. (1968). Will antioxidant nutrients slow aging process? *Geriatrics, 23*, 97–105.

Tsuchihashi, H., Kigoshi, M., Iwatsuki, M., & Niki, E. (1995). Action of β-carotene as antioxidant against lipid peroxidation. *Archives of Biochemistry & Biophysics, 323*(1), 137–147.

Turpeinen, A. M., Mutanen, M., Antti, A., Salminen, I., Basu, S., Palmquist, D. L., et al. (2002). Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *American Journal of Clinical Nutrition, 75*, 504–510.

Walshe, B. E., Sheehan, E. M., Delahunty, C. M., Morrissey, P. A., & Kerry, J. P. (2006). Composition, sensory and shelf life stability analyses of *Longissimus dorsi* muscle from steers reared under organic and conventional production systems. *Meat Science, 73*, 319–325.

Wink, D. A., & Mitchell, J. B. (1998). Chemical biology of nitric oxide: Insights into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide. *Free Radicals in Biology and Medicine, 25*(4–5), 434–456.

Wood, J. D., & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition, 78*(1), S49–S60.

Yang, A., Lanari, M. C., Brewster, M., & Tume, R. K. (2002a). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science, 60*, 41–50.

Yang, A., Brewster, M. J., Lanari, M. C., & Tume, R. K. (2002b). Effect of vitamin E supplementation on α-tocopherol and β-carotene concentrations in tissues from pasture- and grain-fed cattle. *Meat Science, 60*, 35–40.

Young, O. A., Lane, G. A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne, or maize. *Journal of the Science of Food and Agriculture, 83*, 93–104.