

# **Relationship between Lipid Oxidation and Antioxidant Enzymes in Meat**

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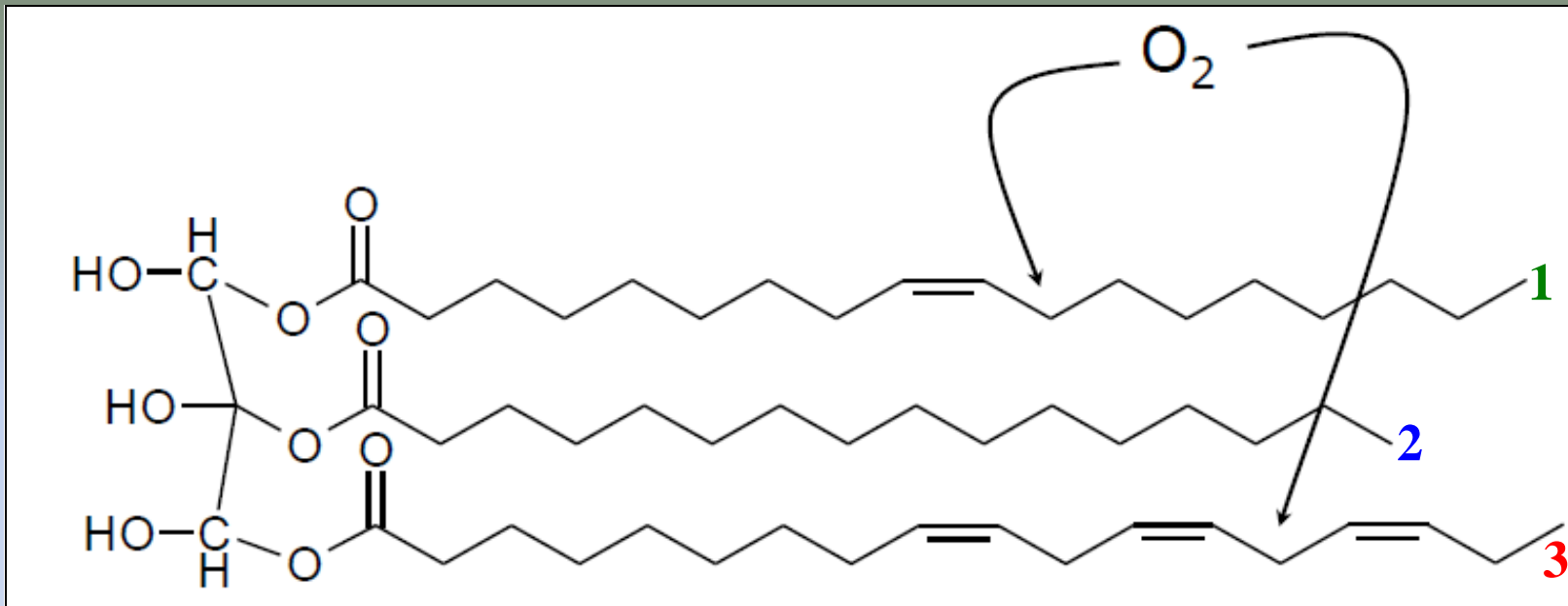
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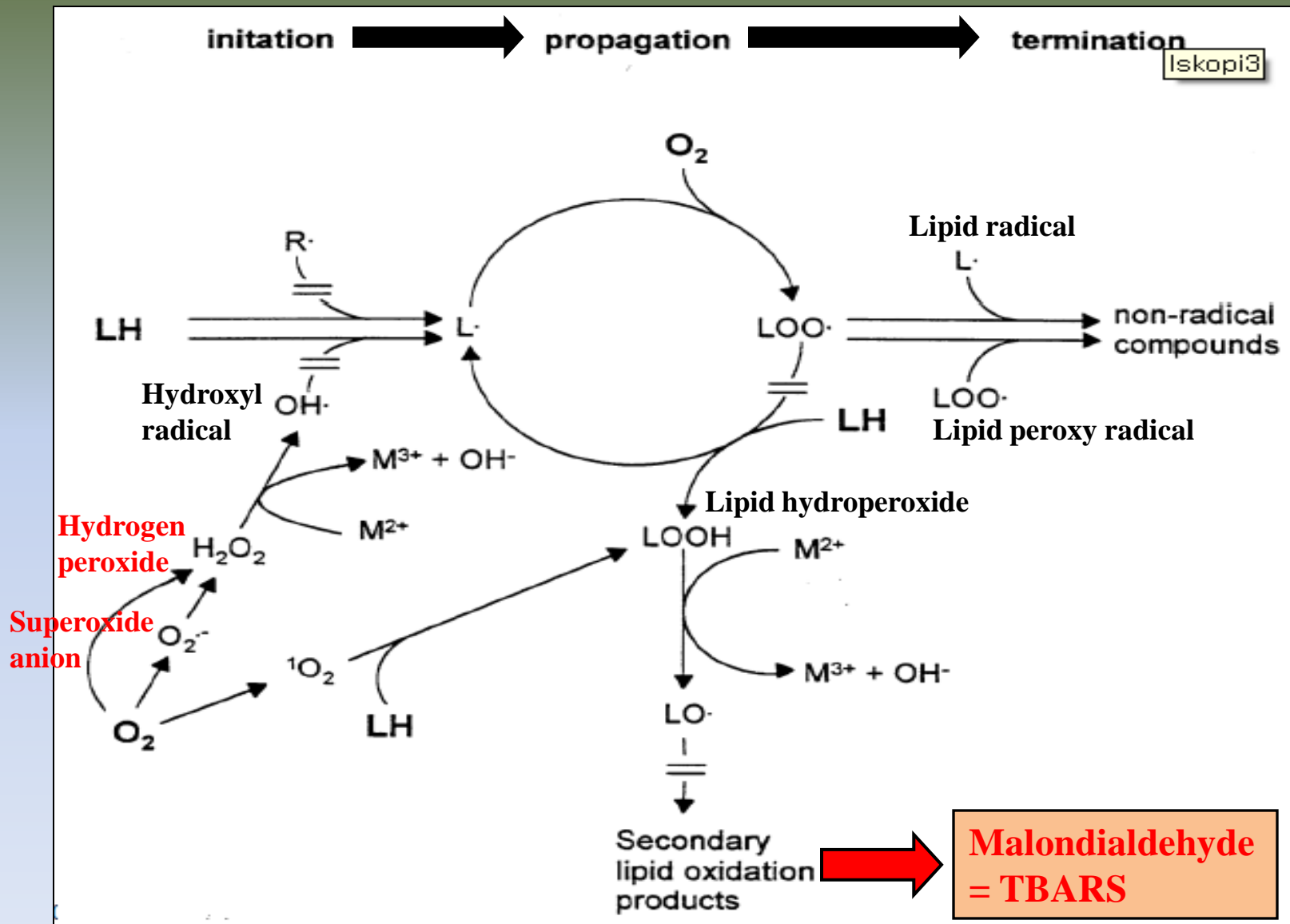
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# 1. Lipid Oxidation in Meat



- 1** : Oleic acid (C18:1n-9) : 1 double bond (Monounsaturated fatty acid)
- 2** : Palmitic acid (C18:0) : 0 double bond (Saturated fatty acid)
- 3** : Linolenic acid (C18:3n-3) : 3 double bond (Polyunsaturated fatty acid)

# 2. Mechanism of Lipid Oxidation



# 3. Antioxidant Defense System in Meat

- **Decker, E. A. et al. (2000)**

- **Lipid-/water-soluble free radical scavengers**

- : tocopherol, ubiquinone, carotenoid, ascorbic acid

- **Thiols**

- : glutathione, lipoic acid

- **Nucleotides**

- : xanthine, hypoxanthine, uric acid

- **Polyamines**

- : putrescine, spermidine, spermine

- **Amino acids/peptides**

- : histidine, tyrosine, phenylalanine, carnosine

- **Transferrin/ferritin**

- **Antioxidant enzymes**

- : catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R), glutathione S-transferase (GSH-ST)

## 4. Antioxidant Enzymes

- **Antioxidant mechanism in the first and second steps of oxidative processes (Halliwell and Gutteridge, 1989)**
  - hydrogen peroxide, superoxide, fatty acid hydroperoxides
- **Stable in animal muscle during storage (Descalzo et al., 2000; Renner et al., 1996)**
- **Feedback mechanism in response to oxidative stress**
  - ageing (Rodriguez-Martinez and Ruiz-Torres, 1992)
    - increase of GSH-R activity
  - muscle disease (Murphy and Decker, 1986)
    - increase of CAT, SOD, GSH-Px, and GSH-ST activities
  - dietary PUFA supplementation (Venkatraman and Pinnavaia, 1998)
    - increase of CAT and SOD activities

## 4. Continued

**Table 1. Effect of tumor on the GSH content and GSH-related enzyme activities in human lung tissue**

Items	Normal	Tumor	P value <sup>1</sup>
Reduced GSH (micromole/g tissue)	3.21	3.10	NS
Oxidized GSH (micromole/g tissue)	0.0235	0.0195	NS
GSH-Px (Milliunits/mg protein)	7.83	22.69	*
GSH-R (Milliunits/mg protein)	92	184	*
GSH-ST (Milliunits/mg protein)	1.00	1.69	**

<sup>1</sup>NS : Not significant; \*p<0.05; \*\*p<0.01.

Cited from Saydam et al. (1997, Cancer Letters).

## 4. Continued

**Table 2. Effect of brain tumors on the antioxidant enzyme activities in human erythrocyte**

Items	Normal	Glioma	Meningioma	Acoustic neurinoma	Other types
CAT (Units/g Hb)	165300	124400	105300	93700	100600
SOD (Units/g Hb)	5186	4273***	4407***	3910***	4711
GSH-Px (Milliunits/g Hb)	7.93	6.59	7.35	5.59**	7.08
GSH-R (Milliunits/g Hb)	1.29	0.32*****	0.37*****	0.44*****	0.33*****
Ceruloplasmin	18.22	24.33****	21.27	20.76	22.20

\*p<0.05; \*\*p<0.01; \*\*\*p<0.005; \*\*\*\*p<0.001; \*\*\*\*\*p<0.0001.

Cited from Rao et al. (2000, Clinica Chimica Acta).



## 4. Continued

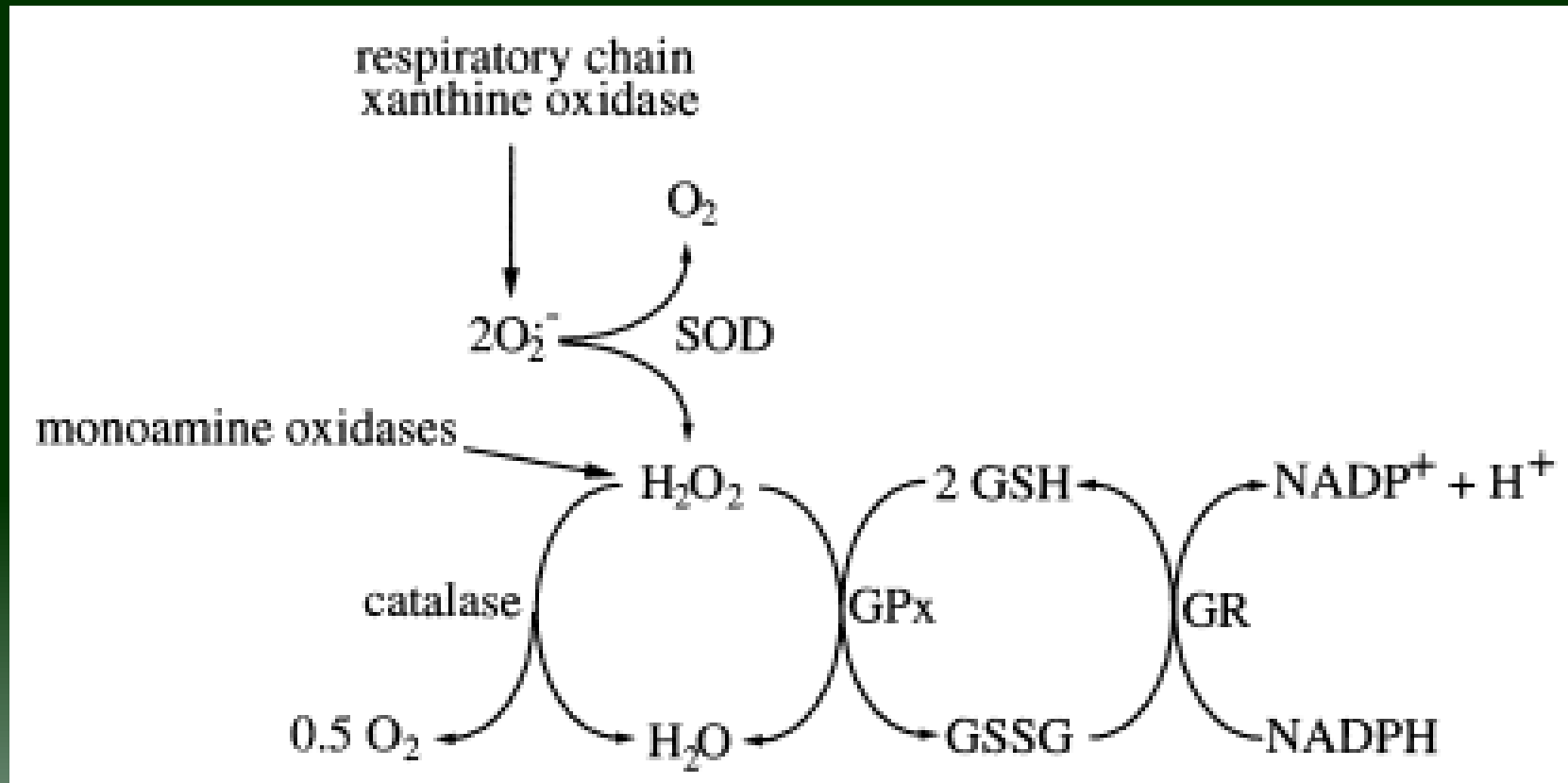
**Table 3. Effect of age on the catalase activity and TBARS level in rat tissues**

Items	Age (month)				
	1	6	12	18	24
<b>CAT (Units/g tissue)</b>					
Brain	-	2.2	2.4	2.2	1.7*
Liver	-	92.1	90.7	92.9	62.5**
Kidney	-	15.3	13.5	12.0	7.0**
<b>TBARS (mg MDA/kg tissue)</b>					
Brain	2.7	4.2*	4.3*	4.5*	3.8*

\*p<0.05; \*\*p<0.01.

Cited from Tian et al. (1998, Free Rad. Biol. Med.).

## 4. Continued



**Fig. 1. Antioxidant mechanism of CAT, SOD, GSH-Px, and GSH-R in human brain cells.** Cited from Berry et al. (1994, Prog. Neurobiol.), Dringen (2000, Prog. Neurobiol.), Fridovich (1995, Annu. Rev. biochem.), and Hille and Nishino (1995, FASEB J.).

- **Hydrogen peroxide: Hydrogen peroxide oxidoreductase (E.C. 1.11.1.6)**
- **History**
  - 1811: first noticed (Louis Jacques Thénard)
  - 1900: named (Oscar Loew)
  - 1937: crystallized from bovine liver (James B. Summer)
  - 1938, 1969, 1981: M.W., amino acid sequence, 3D structure
- **Tetrameric haemin-enzyme : 4 tetramers + 4 ferriprotoporphyrins**
- **$2\text{H}_2\text{O}_2 \longrightarrow 2\text{H}_2\text{O} + \text{O}_2$**
- **Very high activity (million molecule of  $\text{H}_2\text{O}_2$  per sec)**
- **Definition of one unit**
  - micromole  $\text{H}_2\text{O}_2$ /min/g meat or nanomole  $\text{H}_2\text{O}_2$ /min/mg protein
- **Inhibitors**
  - azide, cyanide, cyanogenbromide, hydroxylamine, ascorbate, nitrite, fluoride, acetate, formate, methanol, ethanol

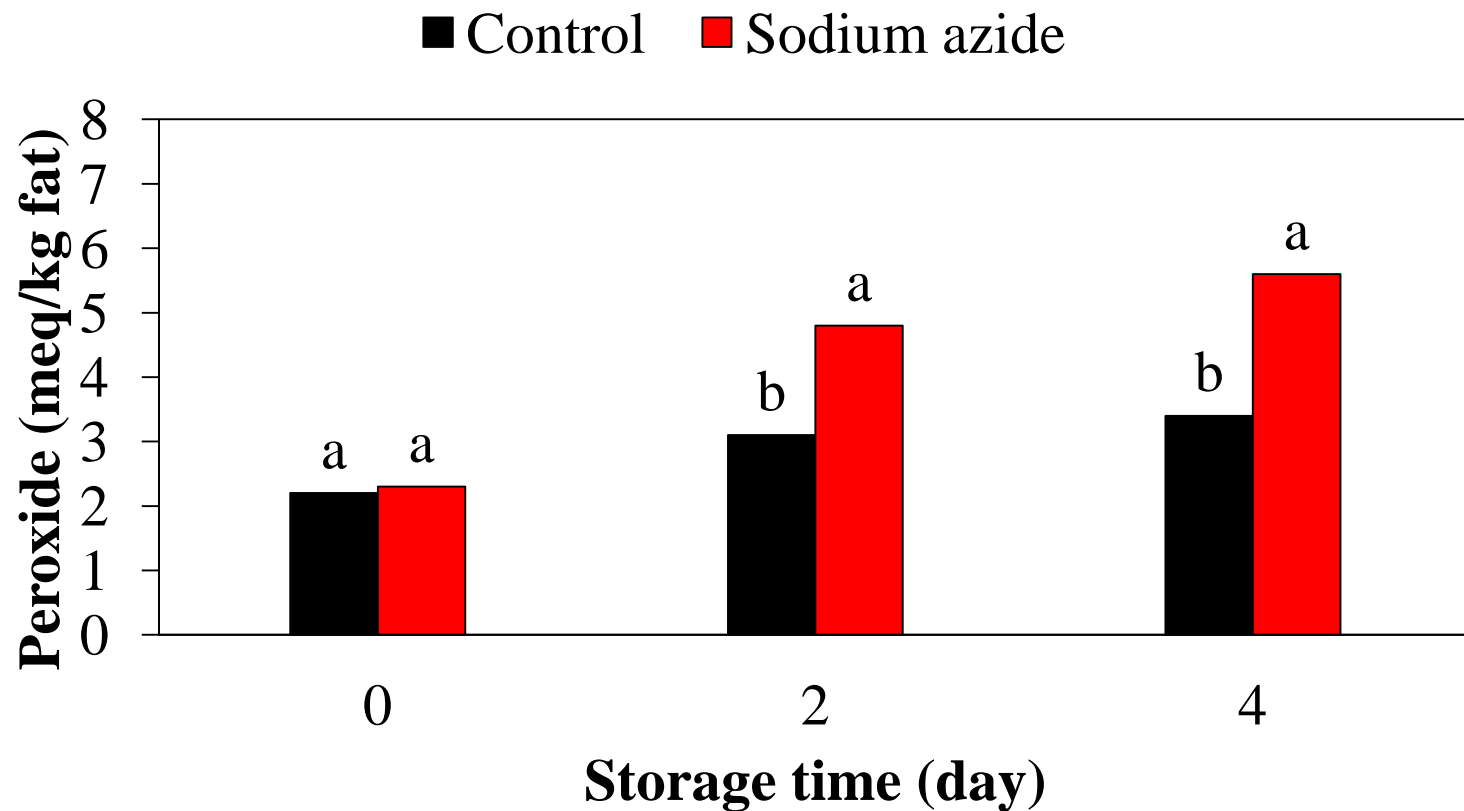
**Table 4. Effect of addition of catalase on the TBARS content in beef *semitendinosus* at 4°C for 6 days**

Item	Storage time (day)	Addition level of catalase (units)		
		0	1600	4000
TBARS (mg MA/kg meat)	1	3.39	2.91	3.41
	3	6.06	5.83	5.79
	6	7.96	6.96	7.10
	Means	5.80a	5.23b	5.43b

<sup>a-c</sup>Different letters indicate significant differences among catalase levels (p<0.05).

Cited from Pradhan et al. (2000, Meat Sci.).

## 4-1) Continued

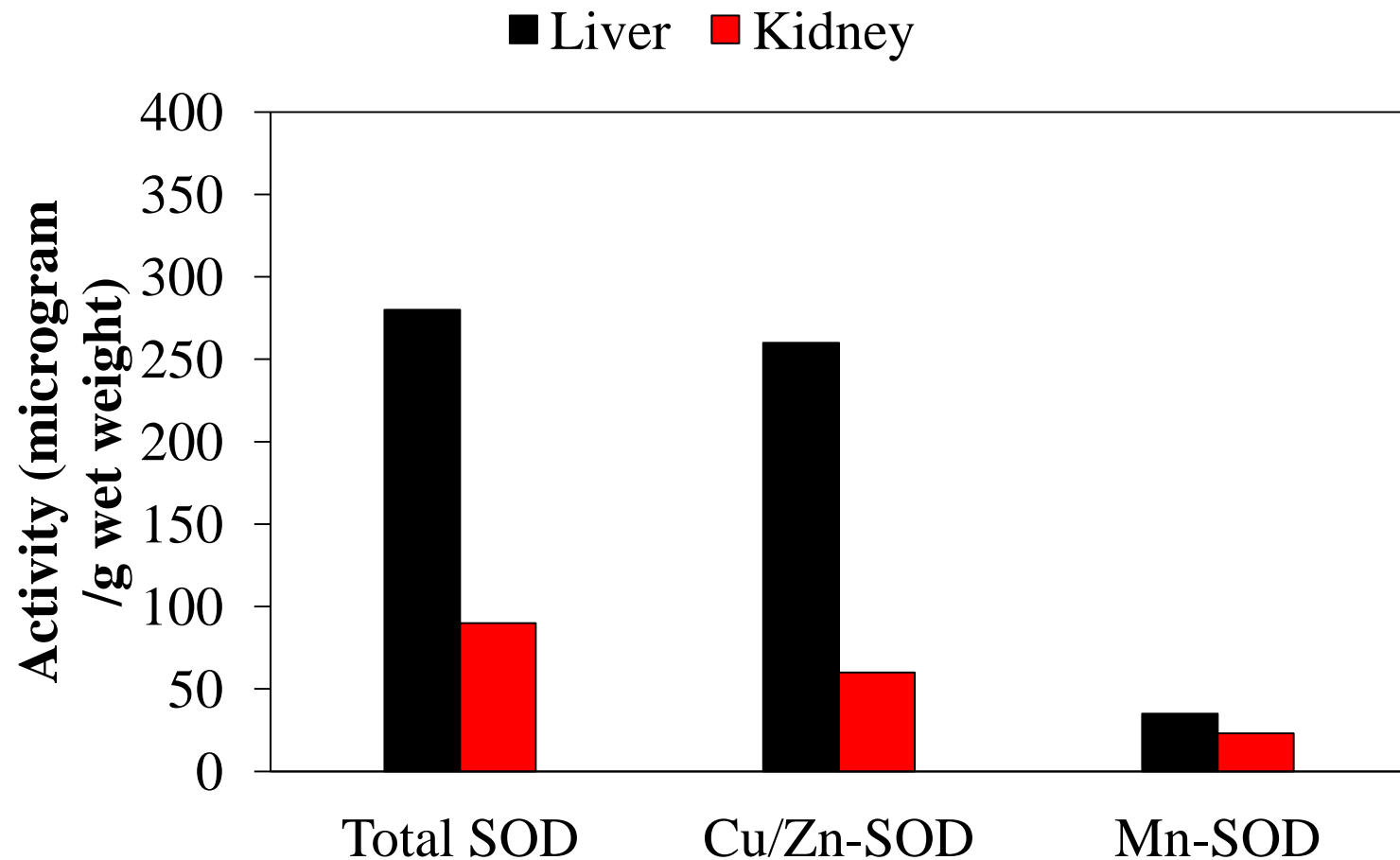


**Fig. 2. Effect of addition of sodium azide on the peroxide value in beef *semitendinosus* at 4°C for 4 days.** <sup>a-b</sup>Different letters indicate significant differences among treatments ( $p < 0.05$ ). Cited from Pradhan et al. (2000, Meat Sci.).

## 4-2) SOD

- **Superoxide dismutase: superoxide oxidoreductase (E.C. 1.15.1.1)**
- **First noticed by McCord and Fridovich (1969)**  
: erythrocyte protein → inhibition of reduction by cytochrome C
- **xanthine + O<sub>2</sub> → uric acid + O<sub>2</sub><sup>•-</sup> (xanthine oxidase)**  
**O<sub>2</sub><sup>•-</sup> + O<sub>2</sub><sup>•-</sup> + 2H<sup>+</sup> → H<sub>2</sub>O<sub>2</sub> + O<sub>2</sub> (SOD)**
- **Coupled-enzyme with CAT**
- **Definition of one unit : 50% inhibition/min/g meat or mg protein**
- **Total SOD activity = Cu/Zn-SOD + Mn-SOD**
  - Cu/Zn-SOD (mitochondria)
    - 90-95% of total SOD activity
    - cyanide (1 mM) sensitive (Behndig et al., 2001)
  - Mn-SOD (cytosol)
    - cyanide insensitive

## 4-2) Continued



**Fig. 3. Total SOD, Cu/Zn-SOD, and Mn-SOD activities in liver and kidney from 24-months-old rats.** Cited from Tian et al. (1998, Free Rad. Biol. Med.).

## 4-3) GSH-Px

- **Glutathione: Hydrogen peroxide oxidoreductase (E.C. 1.11.1.9)**
- **Selenoprotein : The mineral Se is a cofactor**
- **In 1957, first noticed by Gordon C. Mills**
- **Decomposition of  $\text{H}_2\text{O}_2$  and lipid hydroperoxides (Christopherson, 1969)**
  - $\text{H}_2\text{O}_2 + 2\text{GSH} \longrightarrow 2\text{H}_2\text{O} + \text{GSSG} \text{ (GSH-Px)}$
  - $\text{GSSG} + \text{NADPH} + \text{H}^+ \longrightarrow 2\text{GSH} + \text{NADP}^+ \text{ (GSH-R)}$
  - $\text{LOOH} + 2\text{GSH} \longrightarrow \text{LOH} + \text{H}_2\text{O} + \text{GSSG} \text{ (GSH-Px)}$
- **Definition of one unit**
  - micromole NADPH/min/g meat or nanomole NADPH/min/mg protein



- **NAD(P)H: oxidized glutathione oxidoreductase (E.C. 1.6.4.2)**
- **Flavoprotein (Buzrad and Kopko, 1963)**  
: one mole of flavin-adenine dinucleotide (FAD) per mole of GSH-R
- **In 1951, first described by Conn and Vennesland**
- **Found in both eukaryotic and prokaryotic cells**
- **Maintenance of the intracellular reduced glutathione concentration**
  - $\text{GSSG} + \text{NADPH} + \text{H}^+ \longrightarrow 2\text{GSH} + \text{NADP}^+$
- **Definition of one unit**
  - micromole NADPH/min/g meat or nanomole NADPH/min/mg protein

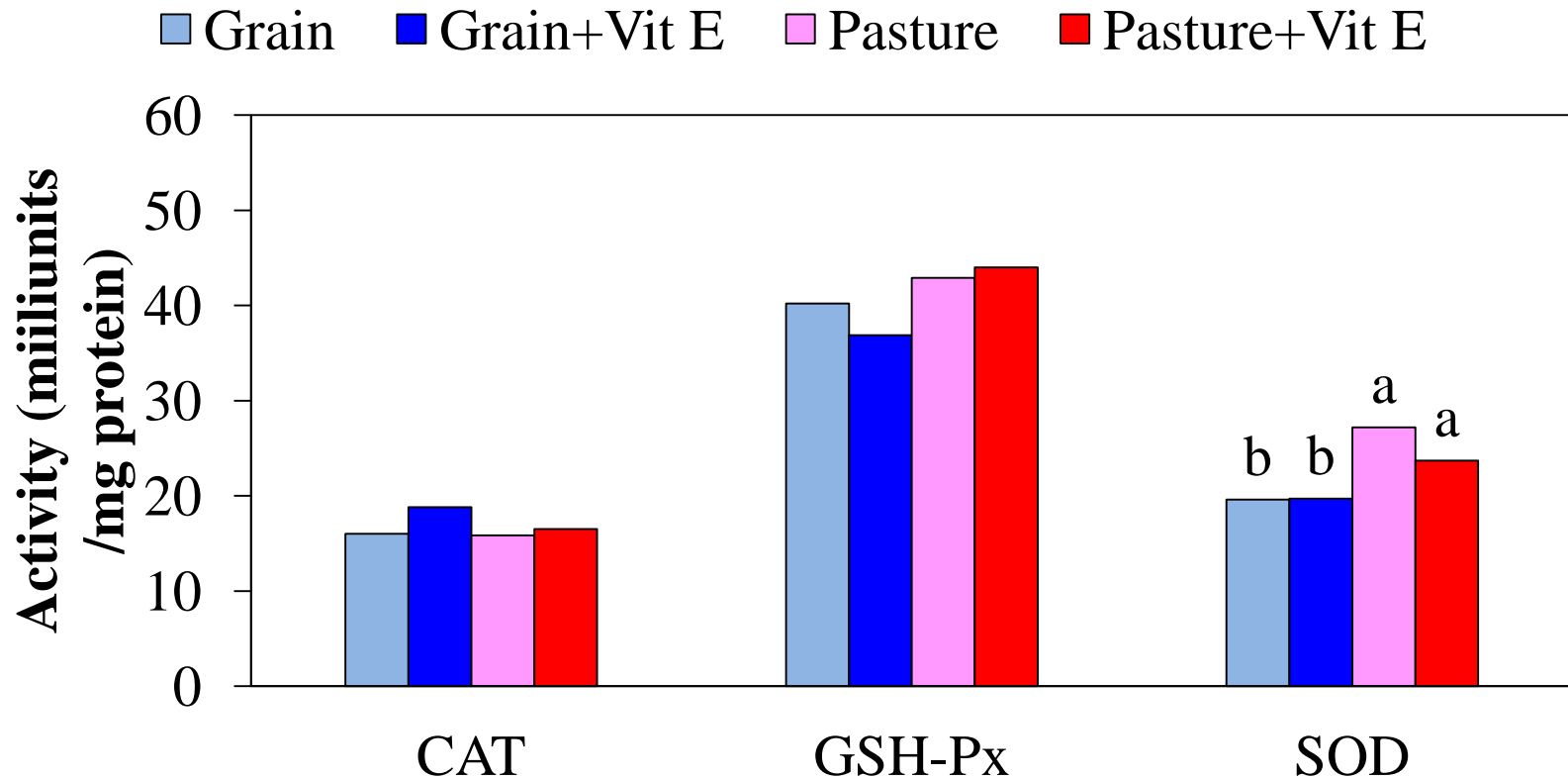
## 4-5) GSH-ST

- **GSH-ST(S-(hydroxyalkyl)glutathione lyase (E.C. 2.5.1.18)**
- **Present in both eukaryotes and prokaryotes**
- **In mammals, found in cytosolic or membrane-bound form (Mannervik, 1985)**
- **Detoxification of xenobiotics and lipid hydroperoxides (Leaver and George, 1998)**
  - Conjugation with sulfhydryl group of reduced glutathione
$$\text{GSH} + \text{CDNB} \longrightarrow \text{GS-DNB conjugate} + \text{HCl}$$
- **Antioxidant effect : Conjugation of hydroxyalkenals (Mosialou and Morgenstern, 1989)**
- **Definition of one unit**
  - micromole CDNB/min/g meat or nanomole CDNB/min/mg protein

# 5. Antioxidant Enzymes in Meat

- **Lee, S. K. (1998)**
  - Beef - CAT : 285 Units/g; GSH-Px : 1.33 Units/g; SOD : 4757 Units/g
  - Pork - CAT : 645 Units/g; GSH-Px : 0.23 Units/g; SOD : 2118 Units/g
  - Chicken - CAT : 1.9 Units/g
  - Turkey - CAT : 62.4 Units/g; GSH-Px : 0.40 Units/g
- **Gheisari, H. R. and Motamedi, H. (2010)**
  - Beef - CAT : 435.36 Units/g; GSH-Px : 1.63 Units/g
  - Chicken - CAT : 136.88 Units/g; GSH-Px : 0.89 Units/g
  - Camel - CAT : 470.43 Units/g; GSH-Px : 1.75 Units/g
- **Kang, S. M. (2011) : Hanwoo (Korean cattle) beef**
  - CAT : 249.78 Units/g meat or 3340.67 Milliunits/mg protein
  - GSH-Px : 1.00 Units/g or 13.33 Milliunits/mg protein
  - SOD : 176.25 Units/g or 2.36 Milliunits/mg protein
  - GSH-R : 0.16 Units/g or 2.19 Milliunits/mg protein
  - GSH-ST : 6.57 Units/g or 87.75 Milliunits/mg protein

## 5-1) Pasture



**Fig. 4. Effect of pasture-grazing and vitamin E supplementation on the antioxidant enzyme activities in beef *longissimus* homogenate.**

<sup>a-b</sup>Different letters indicate significant differences among dietary treatments ( $p < 0.05$ ). Cited from Descalzo et al. (2007, Meat Sci.).

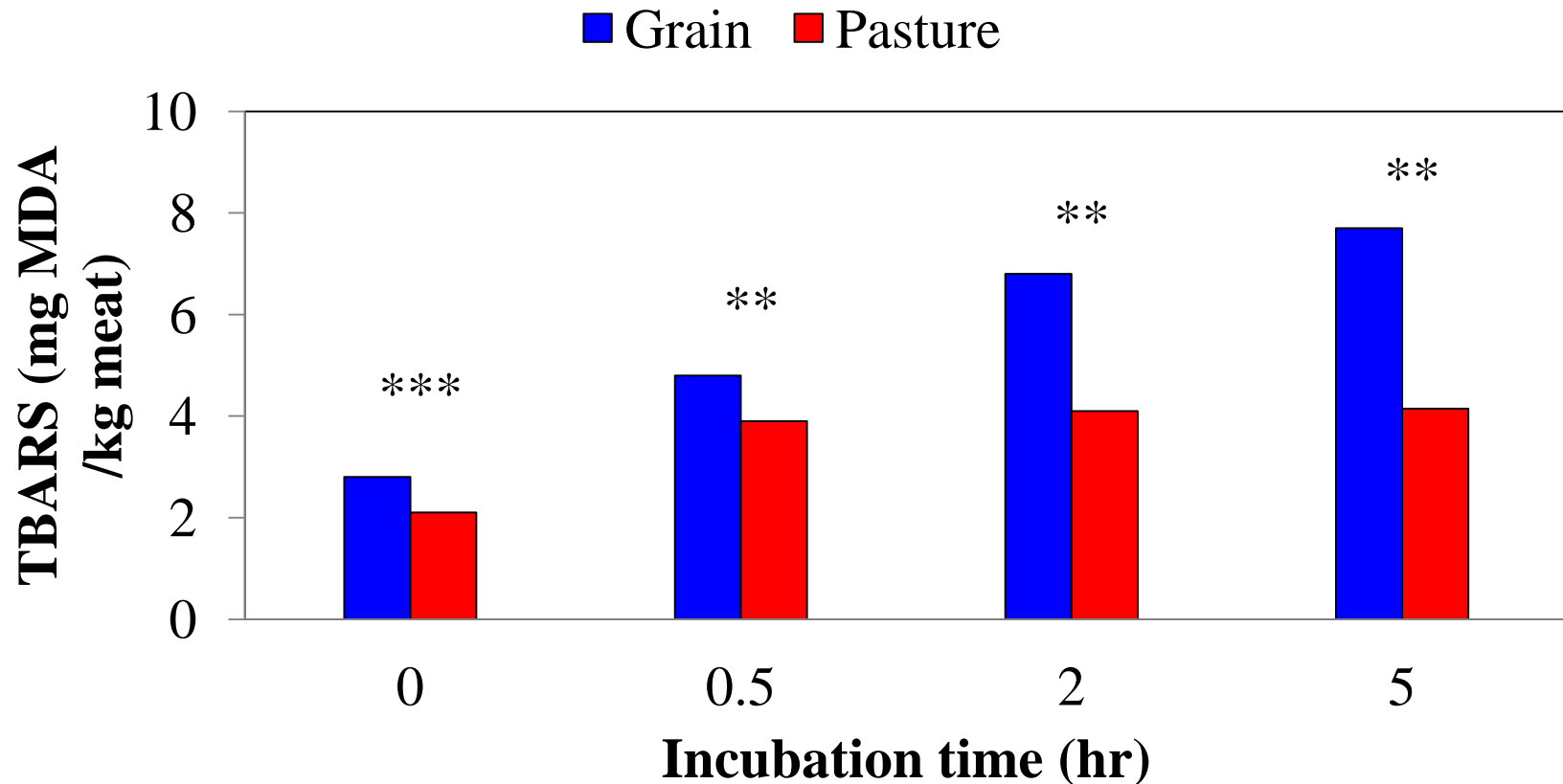
## 5-1) Continued

**Table 5. Effect of pasture-grazing on the antioxidant enzyme activities in beef under 1,900 lux at 4 °C for 9 days**

Items	Grain	Pasture
<b>CAT (Milliunits/mg protein)</b>		
1 day	8.9	11.3
9 day	12.0	13.4
<b>GSH-Px (Milliunits/mg protein)</b>		
1 day	22.3a	12.3b
9 day	16.3a -62.8%	10.8b -12.2%
<b>SOD (Milliunits/mg protein)</b>		
1 day	9.8	13.6
9 day	12.6	16.7

<sup>a-b</sup>Different letters indicate significant differences among dietary treatments ( $p < 0.05$ ).

Cited from Insani et al. (2008, Meat Sci.).



**Fig. 5. Effect of pasture-grazing on the TBARS content in beef *longissimus* homogenate model system oxidized by ferrous ion and hydrogen peroxide. \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Cited from Mercier et al. (2004, Meat Sci.).**

## 5-2) Dietary fat

**Table 6. Effect of dietary fat source on the antioxidant enzyme activities in turkey breast meat**

Dietary treatments	CAT <sup>1</sup>	SOD <sup>1</sup>	GSH-Px <sup>1</sup>	GSH-R <sup>1</sup>
Tallow	248.57b	1.08	84.63c	2.12b
Rapeseed	321.71a	1.12	98.30b	2.62a
Soy	316.57a	1.11	110.09a	2.64a

<sup>a-c</sup>Different letters indicate significant differences among dietary treatments ( $p < 0.05$ ).

<sup>1</sup>Milliunits per mg protein.

Cited from Renerre et al. (1999, J. Agric. Food Chem.).

## 5-3) Dietary mineral

**Table 7. Effect of dietary Se supplementation on the antioxidant enzyme activities and TBARS content in veal meat**

Items	Dietary treatments		
	Control	Se	Se + Vit E
GSH-Px (Units/g meat)	$0.32 \pm 0.05b$	$0.50 \pm 0.06a$	$0.42 \pm 0.05a$
CAT (Units/g meat)	$31.4 \pm 5.2$	$34.0 \pm 5.8$	$36.4 \pm 3.9$
TBARS(mg MDA/kg)			
Day 0	$0.07 \pm 0.02$	$0.06 \pm 0.01$	$0.06 \pm 0.01$
Day 3	$0.83 \pm 0.20a$	$0.75 \pm 0.38ab$	$0.45 \pm 0.07b$
Day 6	$2.22 \pm 0.59a$	$1.82 \pm 0.74ab$	$1.29 \pm 0.21b$

<sup>a-b</sup>Different letters indicate significant differences among dietary treatments ( $p < 0.05$ ).

Cited from Skrivanova et al. (2007, Meat Sci.).



**Table 8. Effect of breed on the antioxidant enzyme activities in pork**

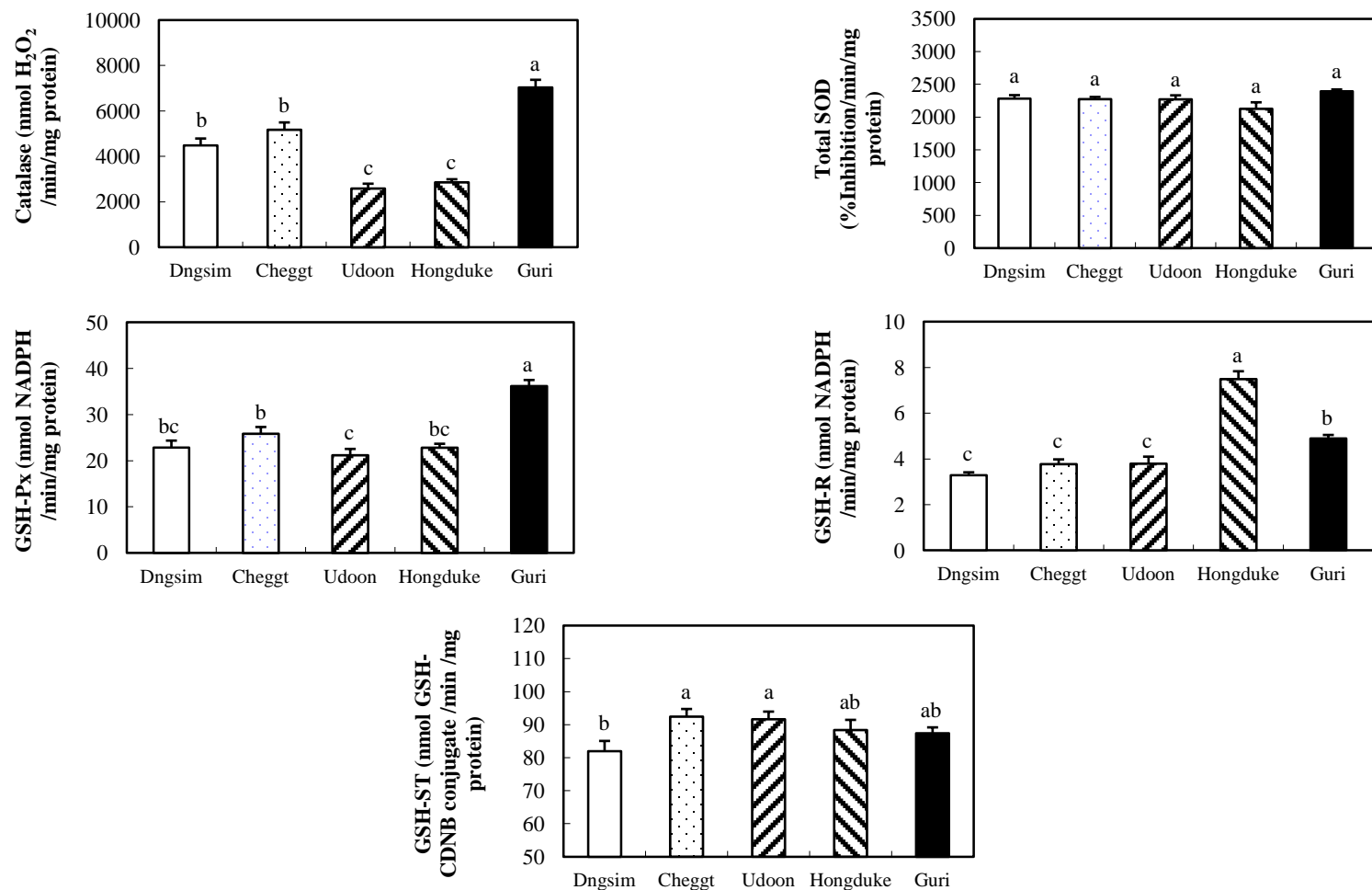
Items	Iberian	Iberian × Duroc	Landrace	Large White	Pietrain
<b>CAT<sup>1</sup></b>	<b>381a</b>	<b>324ab</b>	<b>294b</b>	<b>217c</b>	<b>187c</b>
<b>GSH-Px<sup>1</sup></b>	<b>739b</b>	<b>928a</b>	<b>637b</b>	<b>636b</b>	<b>657b</b>
<b>SOD<sup>1</sup></b>	<b>0.227</b>	<b>0.210</b>	<b>0.176</b>	<b>0.199</b>	<b>0.177</b>

<sup>a-c</sup>Different letters indicate significant differences among pig breeds (p<0.05).

<sup>1</sup>Milliunits per mg protein.

Cited from Hernández et al. (2004, Meat Sci.).

# 5-5) Muscle Type



**Fig. 6. Effect of muscle type on the antioxidant enzyme activities in Hanwoo (Korean cattle) beef.** <sup>a-c</sup>Different letters indicate significant differences among beef muscles. Cited from Kang et al. (2012).

**Table 9. Effect of muscle type on the antioxidant enzyme activities, lipofuscin content (lipid oxidation), and metmyoglobin (MetMb) concentration in beef**

Items	<i>Longissimus lumborum</i>	<i>Psoas major</i>	<i>Diaphragma</i>
CAT <sup>1</sup>	1702.8b	2051.8b	3480.3a
GSH-Px <sup>1</sup>	21.6b	24.4b	51.8a
<b>SOD<sup>2</sup></b>	<b>2.3b</b>	<b>2.6a</b>	<b>3.0a</b>
<b>Lipofuscin</b>	<b>0.7b</b>	<b>0.9a</b>	<b>1.0a</b>
<b>MetMb (%)</b>	<b>22.8c</b>	<b>29.2b</b>	<b>34.3a</b>

a<sup>c</sup>Different letters indicate significant differences among beef muscles (p<0.05).

<sup>1</sup>Milliunits per mg protein.

<sup>2</sup>Units/g meat.

Cited from Renerre et al. (1996, Meat Sci.).

**Table 10. Comparison of drip loss, SOD and GSH-PX activities, and TBARS content between normal pork and PSE pork**

Items	Normal	PSE
<b>Drip loss (%)</b>		
48 hr <i>postmortem</i>	$1.20 \pm 0.42$	$5.13 \pm 0.13^{**}$
96 hr <i>postmortem</i>	$2.42 \pm 0.94$	$6.42 \pm 1.02^{**}$
<b>SOD (Milliunits/mg protein)</b>	$34.37 \pm 1.06$	$31.71 \pm 1.01$
<b>GSH-Px (Milliunits/mg protein)</b>	$1.96 \pm 0.14$	$1.02 \pm 0.08^{*}$
<b>TBARS (mg MDA/kg meat)</b>	$0.18 \pm 0.03$	$0.38 \pm 0.05^{*}$

\* $p < 0.05$ ; \*\* $p < 0.01$ .

Cited from Chen et al. (2010, Meat Sci.).

## 5-7) Antioxidants

**Table 11. Effect of addition of *Rhus verniciflua* Stokes (RVS) extract on the antioxidant enzyme activities, total reducing ability (TRA), and ABTS radical scavenging activity (TEAC) in Hanwoo (Korean cattle) beef patties**

Items	Control	0.02% gallic acid	0.02% RVS	0.04% RVS
<b>CAT<sup>1</sup></b>	249.78a	195.96b	<b>227.70b</b>	<b>213.90b</b>
<b>GSH-Px<sup>1</sup></b>	1.00a	0.93b	1.00a	0.98a
<b>SOD<sup>1</sup></b>	176.25a	163.75b	<b>170.00ab</b>	<b>165.00b</b>
<b>GSH-R<sup>1</sup></b>	0.16	0.17	0.18	0.19
<b>GSH-ST<sup>1</sup></b>	6.57	6.87	6.69	6.48
<b>Ceruloplasmin<sup>2</sup></b>	75.20	97.76	94.75	89.49
<b>TRA</b>	0.26d	0.77a	<b>0.47c</b>	<b>0.57b</b>
<b>TEAC<sup>3</sup></b>	18.97c	24.90a	<b>19.67b</b>	<b>19.97b</b>

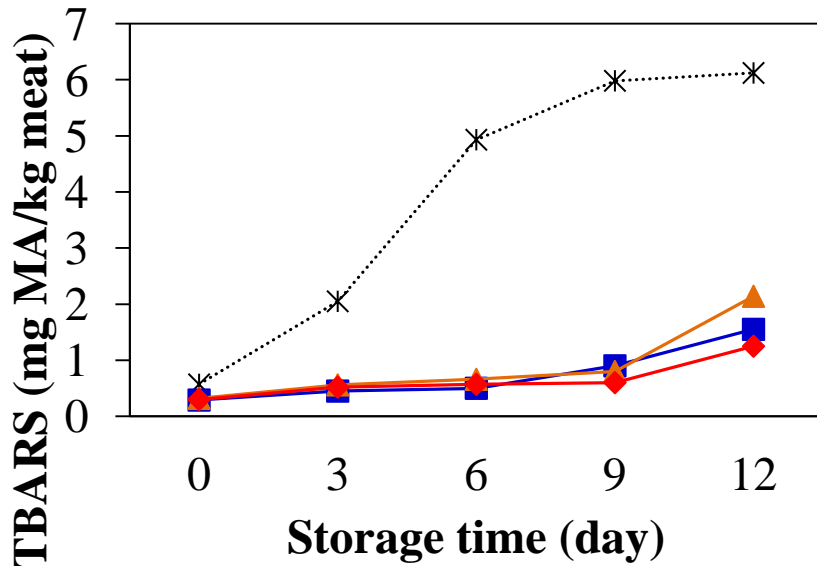
<sup>a-d</sup>Different letters indicate significant differences ( $p < 0.05$ ).

<sup>1</sup>Milliunits per mg protein. <sup>2</sup>Microgram per g meat.

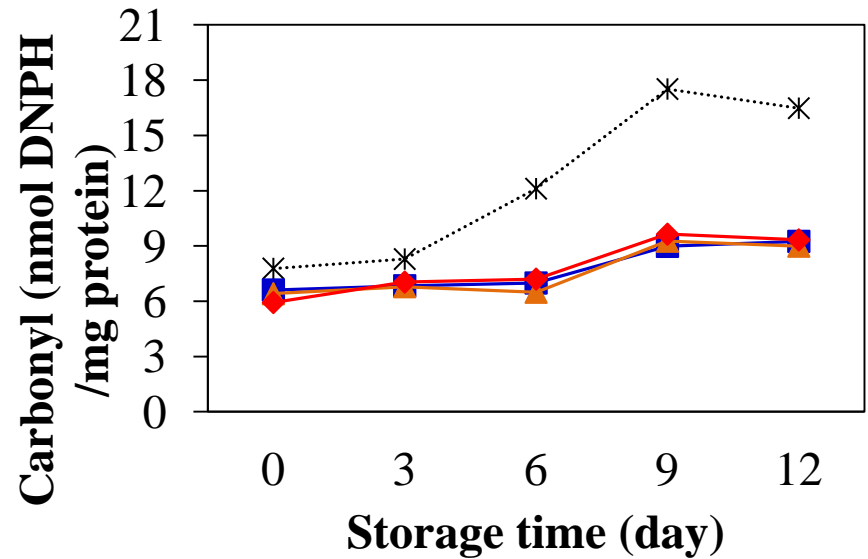
<sup>3</sup>Trolox equivalent antioxidant capacity (nanomole trolox per g meat).

Cited from Kang (2011).

## 5-7) Continued



---\*--- Control  
—■— 0.02% gallic acid  
—▲— 0.02% RVS extract  
—◆— 0.04% RVS extract



---\*--- Control  
—■— 0.02% gallic acid  
—▲— 0.02% RVS extract  
—◆— 0.04% RVS extract

**Fig. 7. Effect of addition of *Rhus verniciflua* Stokes (RVS) extract on the TBARS and carbonyl content in Hanwoo (Korean cattle) beef patties stored with high oxygen-modified atmosphere package at 8 °C for 12 days. Cited from Kang et al. (2011).**

# 5-8) Oxygen

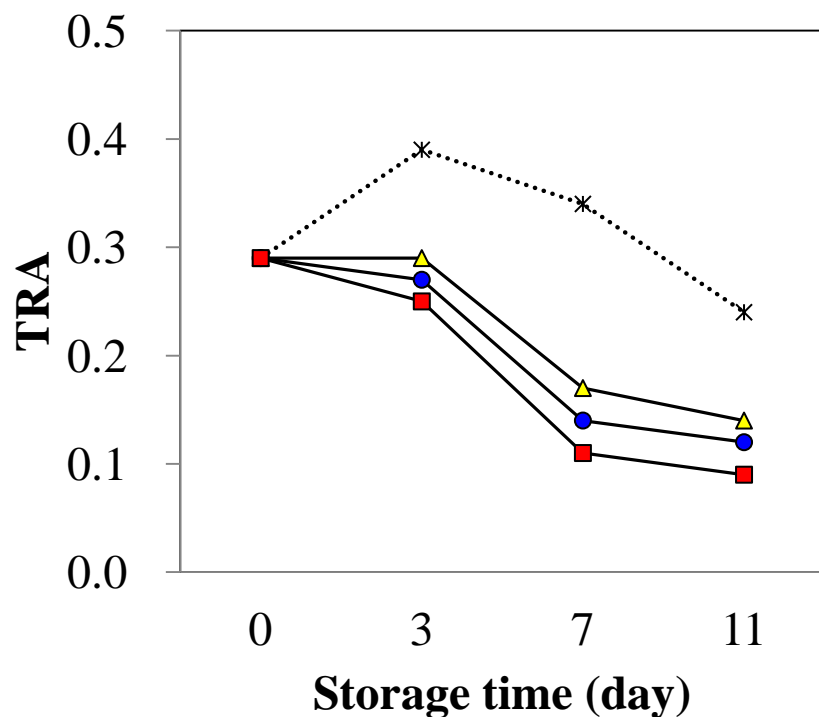
**Table 12. Effect of oxygen concentration in modified atmosphere package on the antioxidant enzyme activities in Hanwoo (Korean cattle) beef at 11 °C for 11 days**

Items	Storage time (day)	O <sub>2</sub> /CO <sub>2</sub> /N <sub>2</sub> (%) in MAP			
		0/20/80	25/20/55	50/20/30	75/20/5
CAT (Units/g meat)	0	333.68±12.73 A	334.51±15.61 A	336.17±12.03 A	332.86±16.02 A
	3	303.05±10.88 B	305.53±11.22 B	304.70±14.63 B	306.36± 7.48 B
	7	270.48± 5.66 C	276.00± 9.32 C	274.62±14.62 C	271.86± 9.68 C
	11	293.94± 5.24 B	296.01± 6.28 BC	296.70± 5.01 BC	297.39± 7.13 B
GSH-Px (Units/g meat)	0	0.87±0.02 A	0.88±0.02 A	0.88±0.03 A	0.88±0.01 A
	3	0.84±0.01aB	0.83±0.02aB	0.81±0.01abB	0.77±0.03bB
	7	0.85±0.01aB	0.84±0.02aB	0.79±0.02bB	0.72±0.01cC
	11	0.81±0.02aC	0.80±0.02aC	0.71±0.02bC	0.58±0.03cD
SOD (Units/g meat)	0	148.36± 3.90	147.64± 4.29 A	149.09± 3.28 A	150.55± 2.39 A
	3	143.38± 4.91a	132.31± 4.32bB	131.69± 5.04bB	131.08± 6.07bB
	7	139.52± 2.89a	93.44± 2.89bD	88.96± 3.97cD	86.40± 3.43cC
	11	144.62±10.81a	99.08± 4.91bC	94.15± 3.87bC	92.92± 2.78bC

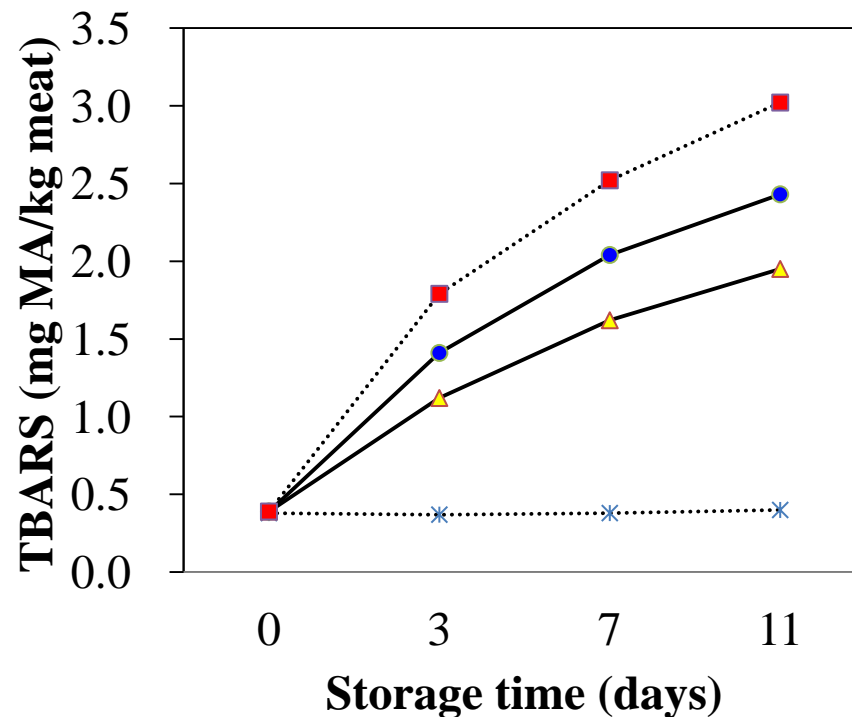
<sup>a-c</sup>Means±S.D. in the same row with different letters differ significantly (p<0.05).

<sup>A-D</sup>Means±S.D. in the same column with different letters differ significantly (p<0.05).

## 5-8) Continued



···\*··· 0% O<sub>2</sub>    —▲— 25% O<sub>2</sub>  
 —●— 50% O<sub>2</sub>    —■— 75% O<sub>2</sub>



···\*··· 0% O<sub>2</sub>    —▲— 25% O<sub>2</sub>  
 —●— 50% O<sub>2</sub>    ···■··· 75% O<sub>2</sub>

**Fig. 8. Effect of oxygen concentration in modified atmosphere package on the total reducing ability (TRA) and TBARS content in Hanwoo (Korean cattle) beef at 11 °C for 11 days. Cited from Kang (2011).**



# 6. Conclusions

- **Antioxidant enzymes are:**
  - important for the inhibition of lipid oxidation in muscle.
    - catalase < glutathione peroxidase, superoxide dismutase
  - influenced by various factors.
    - pasture, animal breed, animal diet, muscle type, glycolysis, antioxidants, oxygen etc.
  - the indicators of shelf-life.
- **There is little information on some antioxidant enzymes.**
  - Glutathione reductase
  - Glutathione S-transferase
  - Ferroxidase (particularly, ceruloplasmin)
- **The biological mechanism of antioxidant enzymes is still not clear.**

# THANK YOU FOR YOUR ATTENTION

우) 441-706 경기도 수원시 권선구 축산길 77(오목천동 564) 국립축산과학원 축산물이용과  
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