

## Antioxidant Enzymes in Relation to Oxidative Deterioration of Muscle Foods

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### 근육식품에서 지방산화와 관련된 항산화 효소

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#### Abstract

Antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are known to inhibit oxidative reactions by inactivating compounds responsible for the formation of free radicals. SOD transforms superoxide radical into hydrogen peroxide which is precursor to active free radicals. CAT reduces hydrogen peroxide to water. GSH-Px reduces hydroperoxides to corresponding alcohols.

Antioxidant enzyme activities of muscle are different by animal species, age, stress and exercise, muscle type and part, conditions of post mortem, storage and processing which are related to oxidative deterioration in muscle foods as well as oxidative defence in living systems. Antioxidant enzyme systems are enhanced rather than weakened in aging skeletal muscle. Red muscle contains higher antioxidant enzyme activity than white muscle. The antioxidant enzyme activities of poultry are higher in leg than in breast, and those of beef are higher in redder and more unstable muscles. It is clear that the effectiveness of the antioxidant enzyme in muscle foods seems to be influenced by meat processing operations. Both GSH-Px and CAT are inactivated by heat processing. NaCl also influence the efficiency of the antioxidant enzymes since its presence diminishes their catalytic activity.

Key words : antioxidant enzyme, catalase, glutathione peroxidase, superoxide dismutase, oxidative deterioration, muscle foods.

#### Introduction

Lipid oxidation is one of the major causes of deterioration in the quality of meat and meat products. Especially oxidative reactions can be accelerated by heat processing and sodium chloride resulting in quality deterioration due to the development of rancidity. Heat- and salt-induced oxidative reactions have been postulated to be accelerated by changes in iron distribution, alterations in the reactivity of iron and heme-containing proteins and disruption of cellular

membrane systems<sup>(1~8)</sup>.

There are many antioxidants including  $\alpha$ -tocopherol, carnosine, anserine, the  $\beta$ -alanine-containing dipeptides, and the antioxidant enzyme in skeletal muscle<sup>(9~11)</sup>. Antioxidant enzymes (AOE) such as catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase, and superoxide dismutase (SOD) play an important role in inhibiting lipid oxidation of meat. The biological function of these antioxidant enzymes is to control the concentration of hydrogen peroxide, lipid peroxides and superoxide anion all of which act as oxidation intermediates which are involved in the formation of free radicals<sup>(12~13)</sup>.

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Heat processing and sodium chloride could also influence oxidative stability by diminishing the activity of the multicomponent antioxidant system of skeletal muscle<sup>(14-16)</sup>. Any processing procedures or additives which influence the activity of the antioxidant enzymes would be important since the role of these enzymes is to control the oxidation intermediates, superoxide anion, lipid peroxides and hydrogen peroxide. These oxidation intermediates can interact with prooxidants (e.g. transition metals and heme-containing proteins) leading to the production of free radicals which are known to initiate and promote rancidity. It is not yet well known that there is a relationship between antioxidant enzymes activity and the development of oxidative deterioration of muscle food during storage and processing.

In this paper, the role of antioxidant enzymes in muscle foods will be reviewed. And change of antioxidant enzyme activity

related to oxidative rancidity in cooked and salted muscle foods will also be considered.

### Antioxidant enzyme in muscle

#### Glutathione peroxidase (GSH-Px)

Several peroxidases reduce hydrogen peroxide and hydroperoxides as shown in Table 1<sup>(17)</sup>. GSH-Px catalyzes the reduction of hydrogen or lipid peroxides (ROOH) with reduced glutathione (GSH)<sup>(18)</sup>.



Glutathione (GSH) provides a substrate for GSH-Px and can also sometimes "repair" radicals resulted from an attack by  $\text{OH}^\bullet$ <sup>(13)</sup>. GSH-Px contains selenium (Se) and is distributed in various mammalian tissues such as mitochondria and cytosol. The enzymes consist of four identical subunits, which contain 1 mole of selenium/mole of subunit as a selenium-containing

**Table 1. The role of peroxidase in living system(Niki, E., 1997)<sup>(17)</sup>**

Peroxidases	Role as protector from oxidative damage
Catalase(CAT)	Decomposition of hydrogen peroxide $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
Glutathione peroxidase(cellular) (GSH-Px)	Decomposition of hydrogen peroxide and free fatty acid hydroperoxides $\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG}$ $\text{LOOH} + 2\text{GSH} \rightarrow \text{LOH} + \text{H}_2\text{O} + \text{GSSG}$
Glutathione peroxidase(plasma) (GSH-Px)	Decomposition of hydrogen peroxide and phospholipid hydroperoxides $\text{PLOOH} + 2\text{GSH} \rightarrow \text{PLOH} + \text{H}_2\text{O} + \text{GSSG}$
Phospholipid hydroperoxide glutathione peroxidase	Decomposition of phospholipid hydroperoxides
Peroxidase	Decomposition of hydrogen peroxide and lipid hydroperoxides $\text{LOOH} + \text{AH}_2 \rightarrow \text{LOH} + \text{H}_2\text{O} + \text{A}$ $\text{H}_2\text{O}_2 + \text{AH}_2 \rightarrow 2\text{H}_2\text{O} + \text{A}$
Glutathione-S-transferase	Decomposition of lipid hydroperoxides

amino acid, selenocysteine, in the catalytic site.

GSH-Px is a more efficient scavenger of hydrogen peroxide at the levels found in tissue than CAT because of its low  $K_m$  for this component<sup>(19)</sup>. Two forms of GSH-Px have been existed in biological tissues of which one, phospholipid hydroperoxide glutathione peroxidase, is specific for phospholipid peroxides<sup>(20)</sup>. Although GSH-Px and CAT decompose hydrogen peroxide and fatty acid hydroperoxides, it is not clearly established if this antioxidant enzyme activity regulates the life span after slaughtering of mammalian tissues.

Activity of antioxidant enzyme in muscles is different by animal species and muscle type. The levels of GSH-Px, CAT and SOD activity of fish species are 30~230 nmol/min/g, 76~1523  $\mu$ mol/min/g, and 157~796 U/g (Table 2), and those of raw animal tissue are 0.23~2.4 U/g, 62.4~645 U/g, and 2118~4757 U/g, respectively (Table 3). Both GSH-Px and CAT of turkey thigh muscle are higher than those of breast muscle. GSH-Px and CAT of mackerel red muscle are also higher than those of white muscle<sup>(21)</sup>. Especially, red muscle of mackerel contains 34-fold higher GSH-Px activity than white muscle. GSH-Px ac-

**Table 2. Activity of glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) in skeletal muscle of different fish species (Aksnes and Njaa, 1981)<sup>(21)</sup>**

Species	GSH-Px (nmol/min/g muscle)	CAT ( $\mu$ mol/min/g muscle)	SOD (U/g muscle)
Blue whiting	100	801	157
Capelin	<30	424	164
Cod	40	76	412
Great silver smelt	80	184	386
Mackerel	230	1128	236
Norway pout	80	1057	209
Rainbow trout	160	592	796
Saithe	40	1523	277
Sprat	90	616	285

**Table 3. Activity of glutathione peroxidase (GSH-Px; U/g muscle), catalase (CAT; U/g muscle), and superoxide dismutase (SOD; U/g muscle) in uncooked and cooked skeletal muscle of different meat species**

Species	GSH-Px		CAT		SOD	
	Uncked	Cked*	Uncked	Cked	Uncked	Cked
Beef <sup>1</sup>	1.33	0.23	285	0	4757	5573
Pork <sup>1</sup>	0.23	0.99	645	0	2118	2266
Chicken <sup>2</sup>						
Breast	1.9**	--	--	--	--	--
Leg	2.4**	--	--	--	--	--
Turkey <sup>3</sup>						
Breast	0.40	--	62.4	--	--	--
Thigh	0.73	0.03	147.5	0	--	--

\*Internal temperature of 80°C was reached, samples were cooled in an ice batch.

\*\*Unit: (nmol/min/mg protein)

- not determined

<sup>1</sup>Mei, L. et al.(1994)<sup>(14)</sup>; <sup>2</sup>DeVore, et al.(1983)<sup>(22)</sup>; <sup>3</sup>Lee, et al.(1996)<sup>(15)</sup>

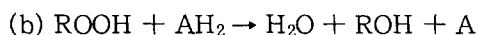
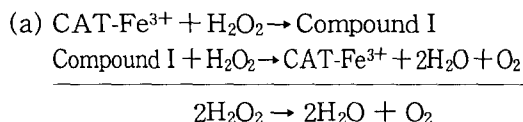
tivity of chicken red muscle is also 1.4-fold higher than that of white muscle<sup>(22)</sup>. GSH-Px of turkey breast and thigh is lower than that of beef, chicken breast and leg muscle, but higher than that of pork muscle<sup>(14)</sup>. Compared with fish and animal muscle in Table 2 and 3, in general, fish tissue contains higher GSH-Px activity and CAT activity, and lower SOD activity than animal muscle.

#### Catalase (CAT)

CAT is widely distributed in nature. It is tetrameric haemin-enzyme consisting of 4 identical tetrahedrally arranged subunits of 60,000 g/mol each. Therefore, it contains 4 ferriprotoporphyrin groups per molecule, its molecular mass being approx. 240,000.

CAT in the peroxisomes convert  $H_2O_2$  into water and  $O_2$  and help to dispose of  $H_2O_2$  generated by the action of oxidase enzymes located in mitochondria and cytosol. Namely, it catalyzes the following reactions<sup>(9,23)</sup>:

- decomposition of  $H_2O_2$  to give  $H_2O$  and  $O_2$  (see eqn. a),
- oxidation of H donors, e.g. methanol, ethanol, formic acid, phenols, with the consumption of 1 mol of peroxide (see eqn. b).



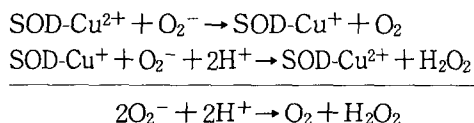
The predominating reaction depends on the concentration of H donor and the steady-state concentration or rate of production of  $H_2O_2$  in the system. In both cases the active catalase- $H_2O_2$ -complex I is formed first. The decomposition of  $H_2O_2$ , in which a second molecule of  $H_2O_2$  serves as H do-

nor for complex I, proceeds exceedingly rapidly, whereas peroxidative reactions proceed relatively slowly<sup>(24)</sup>.

The CAT activity of mammalian tissues varies greatly. For example, it is highest in liver and kidney and low in connective tissues. The CAT activity of both red muscle and white muscle in saithe and mackerel is similar<sup>(21)</sup>. Mei et al. (1994)<sup>(14)</sup> reported that CAT in turkey and thigh breast muscle was lower than in both beef and pork muscle. CAT was completely inactivated in ground turkey when cooked to 80°C as it was in pork and beef (Table 3).

#### Superoxide dismutase (SOD)

SOD is subsequently found to be a universal enzyme which exists in three different metalloforms where each form incorporates a transition metal ion at the active site. It catalyzes the reaction of superoxide anion radicals with hydrogen ions to yield molecular oxygen and hydrogen peroxide<sup>(25-26)</sup>.



Two types of SOD are found in eukaryotic organisms: one (Mn-SOD) containing Mn is located in mitochondria and is not inhibited by cyanide, the other (Cu,Zn-SOD) containing Cu and Zn is located in the cytosolic fraction and is inhibited by cyanide<sup>(27)</sup>. Human cells have a Mn-SOD in mitochondria.

The structure of Cu,Zn-SOD is largely composed of anti-parallel  $\beta$ -sheet. Eight stands of  $\beta$ -sheet fold into a cylinder (the  $\beta$ -barrel) which has two large non-helical loops protruding from opposite edges. These loops enclose the active sites. In all

SODs the metal ion prosthetic group is vital for catalytic activity. In Cu,Zn-SOD the Cu ion is responsible for catalysis and the role of zinc probably structural. Removal of Cu from Cu,Zn-SOD causes loss of enzymic activity which is restored only by its replacement.

SOD is powerful enough to increase the rate of dismutation of O<sub>2</sub> by several orders of magnitude at physiological pHs. The catalytic activity of Cu,Zn-SOD has been shown by direct assay to be constant over the pH range 5~9.5 and to decrease rapidly above pH 10<sup>(28)</sup>.

As shown in Table 2 and 3, activities of SOD in fish, pork and beef are 157~796 U/g, 2118 U/g, and 4757 U/g respectively. Mei et al. (1994)<sup>(14)</sup> reported that differences in the activity of SOD between ground pork and ground beef could be due to differences in muscle fiber content. And SOD was so heat resistant that its activity could not decrease until an internal tem-

perature of 90°C was reached.

### Role of antioxidant enzymes during aging and storage

The antioxidant enzyme activity of living skeletal muscle and the processes which occur during its conversion to meat are critical to an understanding of oxidative deterioration in muscle foods.

Renner et al. (1996)<sup>(29)</sup> reported that the GSH-Px activity of beef varied significantly by muscle type but not with time *post mortem*, although there was a small non-significant decrease stored between 1 and 8 days. The antioxidant enzyme (AOE) activities including GSH-Px, CAT and SOD in beef were higher in redder and more unstable muscles such as PM (psoas major) and D (diaphragma) muscles where oxidative stress was the highest. Nevertheless they found that, *post mortem*, the activity of the antioxidant enzymes did not appear to

**Table 4. Activity of glutathione peroxidase (GSH-Px) and catalase(CAT) and formation of TBARS in stored(4°C), cooked, ground turkey thigh muscle to which 1.3 U of glutathione peroxidase/g muscle and 170 U catalase/g muscle had been added, alone and in combination, after cooking (Lee et al., 1996)<sup>(15)</sup>.**

Enzyme added	Storage time				
	2 hr	16 hr	24 hr	48 hr	96 hr
GSH-Px activity (U/g muscle)					
Control*	0.07	0.05	0.04	0.02	0.01
CAT	0.09	0.07	0.07	0.05	0.02
GSH-Px	1.23	1.07	1.19	0.68	0.62
CAT + GSH-Px	1.27	1.08	1.23	0.72	0.66
CAT activity (U/g muscle)					
Control	ND**	ND	ND	ND	ND
CAT	130	101	95	65	81
GSH-Px	ND	ND	ND	ND	ND
CAT + GSH-Px	166	156	156	147	145
TBARS (mg/kg muscle)					
Control	18.2	45.3	58.6	64.6	85.7
CAT	17.4	46.9	57.4	63.8	79.3
GSH-Px	16.2	42.5	54.3	61.9	78.1
CAT + GSH-Px	16.9	41.8	50.4	59.4	75.7

\* Control samples had no added enzymes. \*\*ND = not detected

lead to increased protection against free radical damage. In conclusion, regulation of the AOE activity *post mortem* is not completely realized yet.

According to Nakano et al (1992)<sup>(30)</sup>, the GSH-Px activity of fish muscles increased significantly during storage at  $-50^{\circ}\text{C}$ . These findings suggest that the increase in the enzyme activity can protect fish muscles from the oxidative deterioration during storage and processing. However, Watanabe et al.(1996)<sup>(31)</sup> found that activity of the GSH-Px and level of GSH (reduced glutathione) decreased, lipid hydroperoxides were substantially formed during 5 days storage at  $4^{\circ}\text{C}$ . These results are elucidated that the increase in lipid hydroperoxides is due to losses in both enzyme and GSH, and differs the hypothesis of Nakano et al. (1992)<sup>(30)</sup>.

Jia(1993)<sup>(32)</sup> reported that the levels of GSH-Px activity, GSH, and GSSG (oxidized glutathione) decreased in minced mackerel and minced blue fish during storage at  $-20^{\circ}\text{C}$ . The activity of GSH-Px was 25% greater in blue fish than in mackerel. The rate of total GSH loss was also greater in minced mackerel than in minced blue fish. According to Williamson (1989)<sup>(33)</sup>, the activity of glutathione S-transferase (0.4 mg /g muscle) in lamb muscle decreased during storage at  $1^{\circ}\text{C}$ . 20% of the activity was lost after 3 weeks. It was revealed that glutathione S-transferase inhibited copper-stimulated peroxidation of arachidonate in the presence of GSH. Therefore, this research suggests that glutathione S-transferase also play a role in inhibiting lipid oxidation in muscle foods.

During aging of mammals, oxidative stress induces tissue damage, and cell death, but it is not clear if this antioxidant enzyme activity regulates the life span. Antioxidant enzyme systems are enhanced in aging skeletal muscle of rat hindlimb,

with SOD and GSH-Px in both mitochondrial and cytosol increasing by 36 to 80% from 4 to 26 months age<sup>(34)</sup>. It seems that the cellular antioxidant enzyme systems are enhanced rather than weakened in senescent skeletal muscle.

Exercise also accelerates the oxidative stress on the body and cause various forms of cell damage. It was known that slow oxidative muscles exhibited a significantly greater CAT activity than fast oxidative muscles. GSH-Px activity in skeletal muscle increases immediately after exercise<sup>(34)</sup>. Ji et al.(1988)<sup>(35)</sup> reported that antioxidant enzymes in liver and skeletal muscle were capable of adapting to selenium deficiency and exercise in order to minimize oxidative injury caused by free radicals.

### Role of antioxidant enzymes in cooked muscle foods

The temperature-dependent acceleration of lipid oxidation in cooked muscle foods could be partially due to heat inactivation of antioxidant enzymes<sup>(14)</sup>. Lipid oxidation (as measured by thiobarbituric acid reactive substances; TBARS) increased rapidly during storage of pork and beef which had been cooked to temperatures  $\geq 70^{\circ}\text{C}$ . Internal temperatures  $\geq 70^{\circ}\text{C}$  also resulted in inactivation of GSH-Px and CAT while SOD activity did not decrease until the internal temperatures was  $90^{\circ}\text{C}$ .  $\alpha$ -Tocopherol concentrations were not altered at internal temperatures up to  $90^{\circ}\text{C}$ . The correlation between CAT and GSH-Px inactivation and TBARS formation ( $r > 0.80$ ) in both beef and pork suggested that these antioxidant enzymes may help protect against rancidity development in cooked muscle foods.

To better understand the relationship between antioxidant enzymes and the development of warmed-over flavor, experiments

were performed where catalase (CAT) and glutathione peroxidase (GSH-Px) were added to muscle which contained minimal activity of both enzymes (Table 4)<sup>(15)</sup>. This was accomplished by cooking ground turkey thigh muscle to an internal temperature of 80°C which decreased both CAT and GSH-Px to <5% of their original activity. CAT and GSH-Px were then added back to the cooked muscle and lipid oxidation rates were monitored by measuring TBARS formation during storage at 4°C for 4 days. When CAT (154U/g muscle) was added back to cooked turkey thigh muscle at concentrations similar to uncooked muscle, lipid oxidation was inhibited 9~12% for up to 48 hours after which time it became ineffective. Addition of CAT (366U/g muscle) at concentrations 2.5 fold higher than original muscle concentrations increased both the amount of TBARS inhibition (5~29%) and length of time during which the inhibition occurred (4 days). GSH-Px (1.2 U/g muscle) at concentration slightly higher than uncooked muscle was less effective, inhibiting lipid oxidation only 6% for the first 16 hours of storage. Concentrations of GSH-Px (3.6 U/g muscle) 5 times higher than concentration in uncoo-

ked muscle increased the time (48 hours) during which TBARS formation was inhibited but only slightly increased the total amount of inhibition (8~11%). The combination of CAT(130 U/g muscle) and GSH-Px (1.2 U/g muscle) was slightly more effective than either enzyme alone with TBARS being 8~12% lower than no enzyme controls for the entire 4 days of storage. GSH-Px (4.0 U/g muscle) was found to effectively inhibit oxidation in turkey homogenates made from raw but not cooked muscle. The ineffectiveness of GSH-Px in cooked muscle homogenates could be due to heat-induced structural changes in the muscle.

### Role of antioxidant enzymes in salted ground pork

The influence of sodium chloride on antioxidant enzyme activity and lipid oxidation rates was studied in ground pork containing 0~2.0 % NaCl which was stored at -15°C for 10 weeks (Table 5)<sup>(16)</sup>. TBARS formation was found to increase in the ground pork as both storage time and sodium chloride concentrations increased. Formation of lipid peroxides did not in-

**Table 5. Activities (U/g muscle) of antioxidant enzymes and development of lipid oxidation (TBARS, mg/kg muscle; POV, meq peroxides/kg muscle) in frozen (-15°C) ground pork containing various concentrations of sodium chloride (Lee et al., 1997)<sup>(16)</sup>**

	Storage periods							
	1 day				10 weeks			
	NaCl(%)				NaCl(%)			
	0	0.5	1.0	2.0	0	0.5	1.0	2.0
	Enzyme activity							
CAT	421	457	417	446	385	407	395	386
GSH-Px	0.25	0.26	0.26	0.24	0.17	0.15	0.15	0.12
SOD	1694	1605	1450	1297	1236	1164	1254	1127
	Lipid oxidation							
TBARS	0.98	0.58	1.43	1.74	3.37	7.96	18.55	26.95
POV	0.02	0.02	0.03	0.03	0.03	0.07	0.41	0.70

crease during storage of pork containing 0 and 0.5% NaCl but increased 22.5 fold and 44.0 fold for pork containing 1 and 2% NaCl, respectively, after 10 weeks of storage. The activity of all of the antioxidant enzymes decreased during frozen storage with CAT, GSH-Px and SOD activity in 10 week old unsalted pork being 8, 32 and 27% lower, respectively, than 1 day old samples. While GSH-Px, SOD and CAT activity decreased during storage, their rate of inactivation was not accelerated by NaCl. Even though NaCl did not promote enzyme inactivation it is possible that its presence could impact enzyme activity through ion specific enzyme inactivation or by changing ionic strength. To test the effect of NaCl on enzyme activity, the antioxidant enzymes were extracted from muscle and their activity was measured as a function of NaCl concentration. Under these conditions, the activity of all the antioxidant enzymes decreased with increasing NaCl concentrations with CAT, SOD and GSH-Px being 18, 58 and 41% lower than 0% NaCl controls, respectively, when 2% NaCl was added to the enzyme assay. This loss of antioxidant enzyme activity as a function of NaCl concentration suggests that the ability of these enzymes to inhibit lipid oxidation could be diminished in salted muscle foods.

### Conclusion

Antioxidant enzymes including CAT, GSH-Px, glutathione S-transferase, and SOD are known to inhibit oxidative reactions by inactivating compounds responsible for the formation of free radicals. The levels of antioxidant enzyme activity in muscle are different by animal species, age, stress and exercise, muscle type and part, conditions of post mortem, storage and processing, etc. which are related to

oxidative deterioration in muscle foods. The antioxidant enzyme (CAT and GSH-Px) activities in muscle foods decreased by meat processing operations including heat processing. In addition to their inactivation, heating may produce structural changes in the muscle which may limit access of the enzymes to their substrates (e. g. lipid) as evidenced by inhibition of lipid oxidation by GSH-Px in raw but not in cooked muscle homogenates. NaCl also influence the efficiency of the antioxidant enzymes since its presence diminishes their catalytic activity.

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