



## ORIGINAL ARTICLE

# *In vitro* antioxidant actions of sulfur-containing amino acids

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### KEYWORDS

Sulfur containing amino acid;  
Antioxidant;  
Cysteine;  
Radical scavenging activity;  
Oxidative stress

**Abstract** Sulfur-containing amino acids such as methionine, cysteine, and taurine are present in animals and plants with biological functions. The aim of this study was to determine the antioxidant activities of representative sulfur-containing amino acids using various *in vitro* antioxidant assays including radical scavenger activities against DPPH<sup>•</sup>, ABTS<sup>•</sup>, and superoxide radicals, ferric reducing antioxidant power, hydrogen peroxide scavenging activity, and metal chelating activities. Of the three sulfur-containing amino acids, cysteine had the highest DPPH<sup>•</sup>, ABTS<sup>•</sup>, and O<sub>2</sub><sup>•−</sup> radical and H<sub>2</sub>O<sub>2</sub> scavenging activities, FRAP, and metal chelating activities except for Fe<sup>2+</sup> chelating. However, methionine and taurine failed to show DPPH<sup>•</sup> or ABTS<sup>•</sup> radical scavenging activity. Based on the results of the present study, sulfur-containing amino acids with excellent antioxidant abilities might be useful for the food processing industry as antioxidant additives to extend shelf-life of food or food products and offer beneficial pharmacological effect against cell damage caused by oxidative stress.

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## 1. Introduction

Lipid oxidation is one of the main reasons behind food decay. Many antioxidants have been researched for the purpose of inhibiting lipid oxidation. Lipid oxidation is largely induced by three different routes: enzymatic oxidation, free-radical-mediated oxidation, and non-radical oxidation (Niki et al., 2005). The process of lipid oxidation related to free-radical

chain mechanism is basically divided into three steps. In the initiation step, free radical is generated by the removal of unstable hydrogen atom bound with unsaturated fatty acids. In the step of propagation, hydroperoxides are produced by the initial reaction products with oxygen. In the step of termination, chemical reactions are terminated due to the formation of chemically stable form (Gray, 1978). Free radicals and reaction products can cause many biological problems such as cell injury and human diseases. Different antioxidants and antioxidant enzymes play important roles in scavenging free radicals (Fang et al., 2005).

Sulfur is involved in numerous biological and chemical reactions. Organic sulfur compounds are produced in plants and microorganisms. These organic sulfur compounds are then oxidized to sulfate which is then excreted through the urinary system in animals (Parcell, 2002). Sulfur can be combined with

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several chemical forms such as sulfur-containing amino acids (methionine, cysteine, homocysteine, and tauric acid) and glutathione (Brosnan and Brosnan, 2006). Sulfur containing amino acids (methionine and cysteine) are regarded as essential amino acids (Konashi et al., 2000). Song et al. (2013) have demonstrated that sulfur containing amino acids of serum in pigs are increased by feeding diet containing high sulfur content. Moreover, cysteine plays an important role in forming disulfide bond linkages between proteins such as insulin (Piste, 2013). Antioxidant effect of sulfur has been studied by some scientists to understand the mechanism of sulfur antioxidant (Atmaca, 2004; Battin and Brumaghim, 2009). Since sulfur amino acids can remove reactive oxygen species (ROS), sulfur amino acids can be used to reduce cell damage induced by oxidative stress (Moskovitz, 2005).

In previous studies, the antioxidant effects of sulfur containing amino acids have been theoretically explained. This study is aimed to investigate the effect of antioxidant capacity following as the different analytical measurements for finding antioxidant effects depends on sulfur-containing amino acids *in vitro*. Different sulfur containing amino acids may possess different antioxidant pathways depending on chemical forms (Parcell, 2002). Sulfur-containing amino acids with excellent antioxidant abilities might be useful for the food processing industry as antioxidant additives to extend shelf-life of food or food products. They might also offer beneficial pharmacological effect against cell damage caused by oxidative stress. Therefore, the objective of this study was to determine the antioxidant activities of sulfur-containing amino acids (Cys, Tau, and Met).

## 2. Material and methods

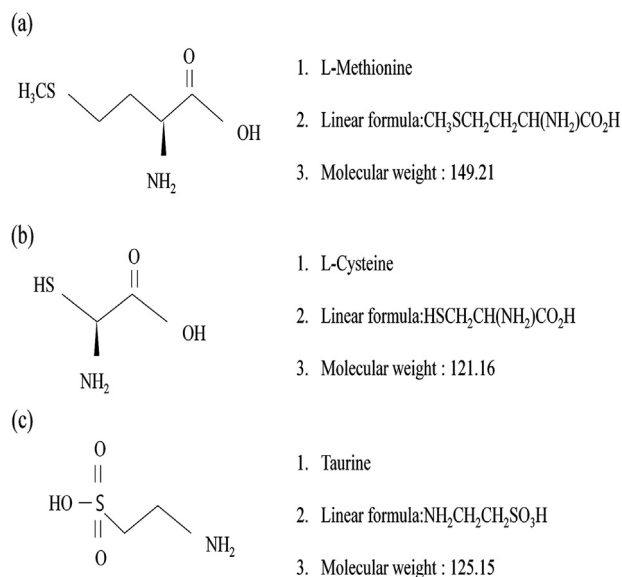
### 2.1. Chemicals and experimental design

Processed sulfur (97.9% inorganic sulfur) was purchased from Jungmin co., Ltd (Korea). L-Methionine, L-Cysteine and taurine were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals in analytical grade were purchased from Sigma-Aldrich (Fig. 1). Experimental design of this study is shown in Fig. 1.

### 2.2. Total antioxidant ability

#### 2.2.1. Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was conducted using published method of Oyaizu (1986) with slight modifications. Briefly, different concentrations (1000, 2000, and 4000 ppm) of each amino acid were prepared in distilled water as stock solutions. One mL of the stock solution was mixed with 2.5 mL of sodium phosphate buffer (0.2 M at pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was placed at 50 °C for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture. The upper layer of the mixture was taken. It was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>. The absorbance value of the solution was measured at wavelength of 700 nm using a spectrophotometer.



**Fig. 1** Structures of sulfur-containing amino acids: methionine, cysteine and taurine.

### 2.3. Metal chelating activity

#### 2.3.1. Ferrous ions ( $\text{Fe}^{2+}$ ) chelating activity

The ferrous ion-chelating ability of sulfur compound was measured using the method of Dinis et al. (1994). Briefly, 0.4 mL of the stock solution of each sulfur amino acid (20 µg/mL) was mixed with 0.2 mL of 2 mM FeCl<sub>2</sub>. Then 0.4 mL of ferrozine (5 mM) was added to the mixture to initiate the reaction. The reacted mixture was shaken for 10 min at room temperature. The absorbance of the mixture was measured at wavelength of 562 nm. The ferrous ion-chelating activity was calculated using the following equation:

$$\text{Chelating ability (\%)} = \{1 - (\text{A}_{\text{sample 562 nm}} / \text{A}_{\text{control 562 nm}})\} \times 100$$

where  $\text{A}_{\text{control 562 nm}}$  was the absorbance of the control reaction containing distilled water instead of sample and  $\text{A}_{\text{sample 562 nm}}$  was the absorbance of the sample in the presence of sulfur-containing amino acid.

#### 2.3.2. Copper ( $\text{Cu}^{2+}$ ) and zinc ( $\text{Zn}^{2+}$ ) chelating activity

Copper ( $\text{Cu}^{2+}$ ) and zinc ( $\text{Zn}^{2+}$ ) chelation activities was determined by the method of Asakura et al. (1990), the sulfur-containing amino acids were diluted into 20 µg/mL in distilled water. CuSO<sub>4</sub> and ZnCl<sub>2</sub> metal solutions were prepared at concentrations of 0.24 mM and 0.8 mM in the same buffer. One mL of the sample solution was mixed with 1 mL of metal solution followed by the addition of 0.1 mL of 1 mM tetramethylmurexide solution. Absorbance value was then recorded at A282/A530 for  $\text{Cu}^{2+}$  and A462/A530 for  $\text{Zn}^{2+}$  to obtain bound metal (%) using the following equation:

$$\text{Bound metal}^{2+} (\%) = (1 - (\text{Concentration of free metal} / \text{Concentration of total metal})) \times 100$$

In order to calculate the percentage of metal chelating activity, standard curves of copper and zinc were prepared. The range of  $\text{Cu}^{2+}$  was from 0.025 to 0.125 mM/L. The range for  $\text{Zn}^{2+}$  was from 0.2 to 1.0 mM/L.

#### 2.4. Hydrogen peroxide ( $H_2O_2$ ) scavenging activity

Hydrogen peroxide scavenging ability was measured according to the method of Ruch et al. (1989). First, 43 mM of  $H_2O_2$  stock solution was prepared in phosphate buffer (0.1 M at pH 7.4). Sulfur compound (1000 ppm) in 3.4 mL phosphate buffer was mixed with 0.6 mL of 43 mM of  $H_2O_2$  in phosphate buffer solution. The absorbance value of the mixture was recorded at wavelength of 230 nm. Phosphate buffer without hydrogen peroxide was used as blank solution. The concentration of hydrogen peroxide (mM) was determined by using a standard curve ( $r^2$ : 0.9865).  $H_2O_2$  scavenging activity (%) of sulfur compounds was calculated using the following equation:

$$H_2O_2 \text{ scavenging ability (\%)} = \{1 - (A_{\text{sample } 230 \text{ nm}}/A_{\text{control } 230 \text{ nm}})\} \times 100$$

where  $A_{\text{control } 230 \text{ nm}}$  was the absorbance of control reaction containing distilled water instead of sample and  $A_{\text{sample } 230 \text{ nm}}$  was the absorbance of sample in the presence of sulfur-containing amino acid.

#### 2.5. Radical scavenging activity

##### 2.5.1. DPPH free radical scavenging activity

DPPH free radical scavenging activity assay was carried out using the method of Blois (1958) with slight modifications. Briefly, 0.5 mL of 0.1 mM DPPH in ethanol was added to 1.5 mL of sulfur compound at different concentrations in ethanol. This mixture was reacted in a dark room for 30 min. The absorbance values of samples were recorded at wavelength of 517 nm. A standard curve was prepared using different concentrations of DPPH ( $r^2$ : 0.9985). The DPPH scavenging effect (%) was calculated using the following equation according to Gülcin et al. (2010).

$$\text{DPPH scavenging effect (\%)} = \{1 - (A_{\text{sample } 517 \text{ nm}}/A_{\text{control } 517 \text{ nm}})\} \times 100$$

where  $A_{\text{control } 517 \text{ nm}}$  was the absorbance value of the control reaction containing distilled water instead of sample and  $A_{\text{sample } 517 \text{ nm}}$  was the absorbance value of the sample in the presence of sulfur-containing amino acid.

##### 2.5.2. ABTS radical cation scavenging activity

The ABTS radical scavenging activity was analyzed using the method of Re et al. (1999). Briefly, 2 mM of ABTS in ethanol was reacted with 2.45 mM potassium persulfate ( $K_2S_2O_8$ ) to generate  $ABTS^+$  in a dark room for 4 h. The  $ABTS^+$  solution was diluted to obtain an absorbance of 0.75 at wavelength of 734 nm by using 0.1 M sodium phosphate buffer (pH 7.4). One mL of diluted  $ABTS^+$  solution was then reacted with 3 mL of sulfur compound in distilled water for 30 min.  $ABTS^+$  scavenging effect (%) was calculated using the following equation:

$$ABTS^+ \text{ scavenging effect (\%)} = (1 - A_{\text{sample } 734 \text{ nm}}/A_{\text{control } 734 \text{ nm}}) \times 100$$

where  $A_{\text{control } 734 \text{ nm}}$  was the absorbance of the control reaction containing distilled water instead of the sample and  $A_{\text{sample } 734 \text{ nm}}$  was the absorbance value of the sample in the presence of sulfur-containing amino acid.

##### 2.5.3. Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity was measured using the method of Li et al. (2006). Briefly, 0.2 mL of sulfur compound stock solution was mixed with 0.4 mL of 150  $\mu$ M of nitro blue tetrazolium (NBT) in phosphate-buffered solution (100 mM, pH 7.4), 0.4 mL of 460  $\mu$ M  $\beta$ -nicotinamide adenine dinucleotide ( $\beta$ -NADH), and 0.05 mL of 60  $\mu$ M phenazine methosulphate (PMS). After mixing well, the mixture was then placed at room temperature. After standing for 5 min, the absorbance value of the mixture was recorded at wavelength of 560 nm. Superoxide anion radical scavenging activity (%) was calculated using the following formula:

$$\begin{aligned} \text{Superoxide anion radical scavenging effect (\%)} \\ = \{1 - (A_{\text{sample } 560 \text{ nm}}/A_{\text{control } 560 \text{ nm}})\} \times 100 \end{aligned}$$

where  $A_{\text{control } 560 \text{ nm}}$  was the absorbance value of the control reaction containing distilled water instead of the sample and  $A_{\text{sample } 560 \text{ nm}}$  was the absorbance value of the sample in the presence of sulfur-containing amino acid.

#### 2.6. Statistical analysis

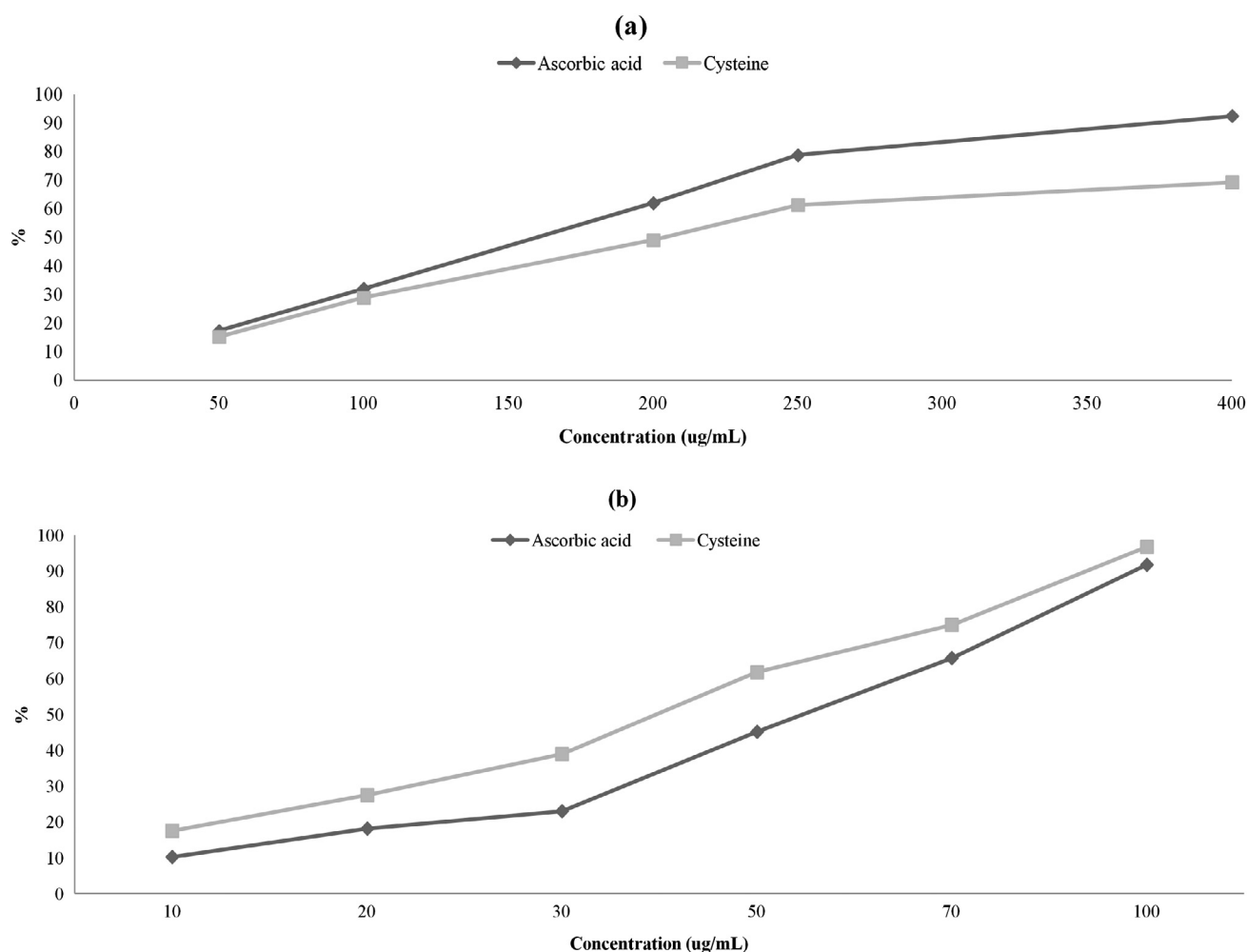
All experiments were conducted in triplicates and all data were analyzed with SPSS 19.0. Results are expressed as means  $\pm$  standard error of the means. One-way analysis of variance (ANOVA) was performed using ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. A  $p$  value of  $< .05$  was regarded as statistically significant.

### 3. Results and discussions

#### 3.1. Radical scavenging activity

##### 3.1.1. DPPH and ABTS radical scavenging assays

DPPH and ABTS free radical scavenging activities of cysteine were linearly increased with increasing concentrations. However, Met and Tau did not possess DPPH or ABTS free radical scavenging activity (Fig. 2). Kavlentis (1988) has reported that cysteine can inhibit the formation of DPPH complex whereas methionine does not affect the DPPH experiment. Ripoll et al. (2012) have shown the taurine does not have ABTS radical scavenging ability. The addition of taurine is unable to decrease ROS production or inhibit lipid peroxidation in glutathione-depleted cell culture (Heidari et al., 2014). In the present study, ascorbic acid has more powerful DPPH radical scavenging activity than cysteine at the same concentrations (50–400  $\mu$ g/mL). At 400  $\mu$ g/mL, cysteine and ascorbic acid showed 84.42% and 94.94% of DPPH radical scavenging activity, respectively. However, the ABTS radical scavenging activity of cysteine was significantly higher than that of ascorbic acid at concentrations from 10 to 100  $\mu$ g/mL. For instance, cysteine and ascorbic acid at 100  $\mu$ g/mL had 96.77% and 91.78% ABTS radical scavenging activities, respectively. In general, ascorbic acid is considered as a representative antioxidant that can remove water-soluble free radical. Ascorbic acid is converted into ascorbate radical when electron of ascorbate radical supplies lipid radical to terminate the oxidative chain reaction (Nimse and Pal, 2015). In general, DPPH assay is used for ethanol-soluble free radicals, whereas ABTS assay is



**Fig. 2** Radical scavenging activities of sulfur amino acids. (a) DPPH radical scavenging activity and (b) ABTS radical scavenging activity of different concentrations of sulfur-containing amino acids (cysteine, methionine and taurine) and ascorbic acid (reference antioxidant). Radical scavenging activities of methionine and taurine was not detected.

used to measure water-soluble free radicals. [Kaviarasan et al. \(2007\)](#) have found that ABTS assay is more sensitive than DPPH assay since DPPH radical is only involved in hydrogen ( $H^+$ ) transfer [DPPH $\cdot$  to DPPH-H] while ABTS radical is involved in the electron transfer pathway [ABTS $\cdot$  to ABTS]. According to the radical scavenging activity of cysteine, the presence of proteins containing disulfide bonds (Cys-Cys) might potentially increase the antioxidant ability of food.

### 3.1.2. Superoxide anions radical scavenging activity

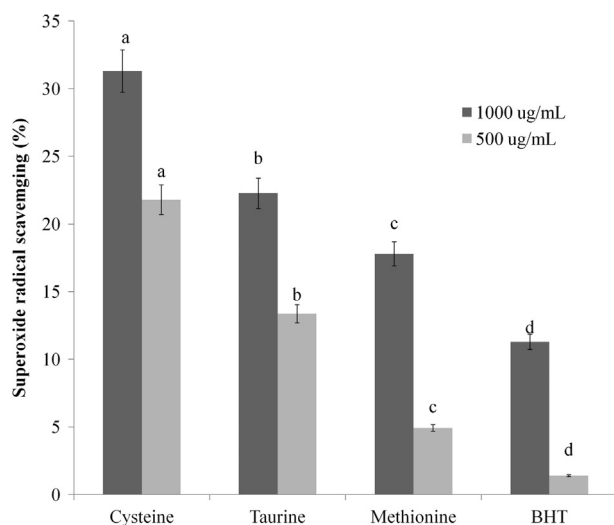
The principle of superoxide anion radical scavenging activity assay has been explained by [Gülcin et al. \(2010\)](#). The addition of PMS will induce the reduction of NBT with the generation of superoxide radicals ( $O_2^-$ ) in PMS-NADH system. Blue formazan at 560 nm is then formed through the NBT reduction. Therefore, inhibition of blue formazan production can be expressed as a decrease in absorbance at 560 nm due to  $O_2^-$  scavenging. At the same concentration (1000 ppm), cysteine, taurine, methionine, and BHT exhibited 31.32, 22.27, 17.8, and 11.29% superoxide anion radical scavenging activities, respectively ([Fig. 3](#)). Cysteine showed the highest ( $p < .001$ )

superoxide radical scavenging activity. The excellent superoxide radical scavenging activity of cysteine has been reported by [Hussain et al. \(1996\)](#). Likewise, methionine also possessed higher ( $p < .001$ ) superoxide radical scavenging activity than BHT. It has been reported that methionine can be easily oxidized to methionine sulfoxide by some oxidants such as oxygen, ozone, hydrogen peroxide, and superoxide ([Levine et al., 2000](#)). In a previous study, the superoxide radical scavenging activity (the reduction of NBT to form the blue formazan at 560 nm) of taurine has been observed at concentrations from 30 mM to 60 mM ([Oliveira et al., 2010](#)). [Kim and Cha \(2009\)](#) have found that taurine can reduce oxidative damage by altering superoxides into taurine chloramines.

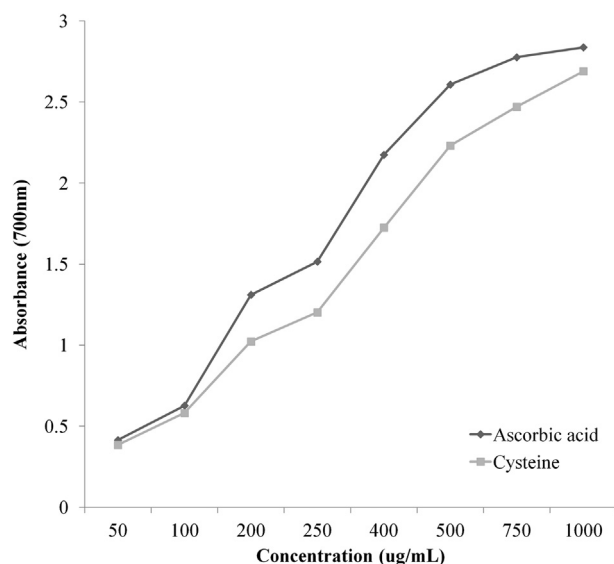
### 3.2. Reducing power

FRAP results of cysteine and ascorbic acid are shown in [Fig. 4](#). Methionine and taurine did not show any FRAP. FRAP measures the degree of residual  $Fe^{2+}$  generated from the reduction of  $Fe^{3+}$ . Although cysteine showed some FRAP, its reducing ability was lower ( $p < .05$ ) than ascorbic acid. At concentra-





**Fig. 3** Effect of sulfur-containing amino acids on superoxide anion radical scavenging (%). Different letters within the same concentration indicate significant differences ( $p < .05$ ).



**Fig. 4** Total ferric reducing power (FRAP) of different concentrations of sulfur-containing amino acids (cysteine, methionine and taurine) and ascorbic acid (reference antioxidant).

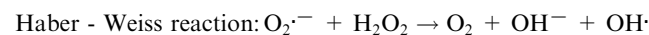
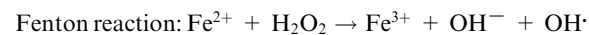
tion of 1000 ppm, the absorbance values of ascorbic acid and cysteine after reducing  $\text{Fe}^{3+}$  were 2.837 and 2.689, respectively. According to Giles et al. (2003), the thiol group (SH) of cysteine in proteins can modulate the redox reactions such as the formation of cysteine radical ( $\text{S}^\cdot$ ), cystine (S-S), cysteine sulfeninc acid (S-OH), and so on. Mancuso et al. (2010) have explained that the FRAP of human blood is increased when it is supplemented with cysteine-donor. Antioxidant systems are mostly associated with the inhibition of ROS production, metal chelating, or the removal of pro-oxidants by antioxidant enzyme activity, although the redox reaction is continuously occurring. Benzie and Strain (1996) have indicated that non-enzymatic antioxidants such as ascorbic acid and

$\alpha$ -tocopherol have FRAP. Therefore, the reducing power of cysteine might be caused by the presence of thiol group.

### 3.3. Hydrogen peroxide scavenging activity

$\text{H}_2\text{O}_2$  scavenging activities of sulfur-containing amino acids (cysteine, methionine, and taurine) are shown in Table 1. At the same concentration (1000 ppm), BHT showed significantly higher  $\text{H}_2\text{O}_2$  scavenging activity (79.81%) than sulfur-containing amino acids (Cys, 12.62%; Met, 20.66%; Tau, 52.01%). Although Met and Tau did not show radical scavenging activities against DPPH or ABTS, they showed higher ( $p < .001$ )  $\text{H}_2\text{O}_2$  scavenging activity than Cys.

Hydrogen peroxide is not directly toxic to human body. However, hydrogen peroxide continuously contributes as substrates to form ROS species through Fenton (Winterbourn, 1995) and Haber-Weiss reactions (Haber and Weiss, 1934):



Therefore, decomposition of  $\text{H}_2\text{O}_2$  is very essential in the antioxidant defense system to protect cell or food against oxidative stress. According to Halliwell and Rycker (1978), the thiol group in the cysteine residue can act as substrate for peroxidase to produce  $\text{H}_2\text{O}_2$ . During the scavenging of  $\text{H}_2\text{O}_2$  using cysteamine generated by the degradation of cysteine, the loss of thiol group has been detected by Aruoma et al. (1988). This result might prove that the hydrogen peroxide scavenging activity of cysteine is negatively associated with the presence of thiol group. In the case of methionine, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is decomposed due to its reaction with methionine. Therefore, methionine is converted into methionine sulfoxide which is then reconverted to the methionine throughout the oxidation of NADPH (Atmaca 2004; Brosnan and Brosnan, 2006).

### 3.4. Metal chelating activity

The three sulfur-containing amino acids failed to show  $\text{Fe}^{2+}$  chelating ability. However, EDTA standard at 40 ppm showed high (87.17%)  $\text{Fe}^{2+}$  chelating ability (Table 2). In terms of Fenton reaction, inhibition on DNA damage is not affected when  $\text{Fe}^{2+}$  and hydrogen peroxide are added into cell (Battin and Brumaghim, 2008). On the other hand, the three sulfur-containing amino acids expressed copper ( $\text{Cu}^{2+}$ ) and zinc ( $\text{Zn}^{2+}$ ) chelating abilities in this study. The metal chelating abilities of all samples were decreased ( $p < .001$ ) with increasing concentrations of metals. Relatively, cysteine showed higher chelating effects compared to other samples included with EDTA at same concentrations ( $p < .001$ ). Regarding the chelating activity for 0.25 mM  $\text{Cu}^{2+}$ , the chelating activity of cysteine was 81.87%. For other samples, their results were: EDTA, 56.26%; taurine, 27.32%; methionine, 46.91%; and ascorbic acid, 64.31%. Furia (1972) has reported that each sample can express different metal affinities (for example, cysteine:  $\text{Cu}^{2+}$ , 19.2;  $\text{Zn}^{2+}$ , 9.8; EDTA:  $\text{Cu}^{2+}$ , 18.8;  $\text{Zn}^{2+}$ , 16.5). Furia (1972) has reported that each sample can express different metal affinities (for example, cysteine:  $\text{Cu}^{2+}$ , 19.2;  $\text{Zn}^{2+}$ , 9.8; EDTA:  $\text{Cu}^{2+}$ , 18.8;  $\text{Zn}^{2+}$ , 16.5). Lower

**Table 1** Effect of sulfur-containing amino acids (1000 ppm) on H<sub>2</sub>O<sub>2</sub> scavenging activity (%).

	Cysteine	Methionine	Taurine	BHT	P-value
H <sub>2</sub> O <sub>2</sub> scavenging activity (%)	12.62 ± 0.68d	20.66 ± 1.36c	52.01 ± 0.57b	79.81 ± 0.10a	< .001
Different superscript letters in the same row indicate significant differences during aging time, <i>p</i> < .05.					

**Table 2** Metal (Fe<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>) chelating activity (%) of sulfur-containing amino acids (methionine, cysteine and taurine) and reference chelators (EDTA and ascorbic acid).

	Fe <sup>2+</sup>	Cu <sup>2+</sup>		Zn <sup>2+</sup>	
		0.25 mM	0.8 mM	0.25 mM	0.8 mM
EDTA <sup>A</sup>	87.17 ± 0.26	56.26 ± 2.50c	55.12 ± 0.86c	78.29 ± 0.14b	78.30 ± 0.99a
Taurine	ND	27.32 ± 1.52e	1.87 ± 1.38e	63.41 ± 0.24e	45.69 ± 0.22c
Methionine	ND	46.91 ± 1.04d	22.11 ± 1.79d	67.89 ± 2.34d	45.53 ± 0.16c
Ascorbic acid	ND	64.31 ± 0.92b	53.58 ± 1.17b	72.20 ± 1.94c	51.87 ± 0.22b
Cysteine	ND	81.87 ± 1.44a	73.25 ± 2.85a	88.29 ± 0.65a	76.50 ± 0.89a
P-value	–	<i>P</i> < .001	<i>P</i> < .001	<i>P</i> < .001	<i>P</i> < .001

ND, Not detected.

Different superscript letters in the same column indicate significant differences during aging time, *p* < .05.

<sup>A</sup> Fe<sup>2+</sup> chelating reaction measurement at 40 ppm EDTA.

cysteine concentration is needed to show the same reduction for DNA damage caused by copper compared to methionine concentration (Battin and Brumaghim, 2008). Furthermore, the thiol group involved in cysteine structure has high heavy metal affinity (Piste, 2013). Therefore, the metal chelating ability of these sulfur-containing amino acids might be affected by several factors such as the number of binding-sites of chelator and metal affinity. Although zinc and copper are regarded as essential minerals in human body, toxic effects exist when the absorption of minerals is in excess (Cal et al., 2005). In particular, copper is regarded as a pro-antioxidant that produces free radical formation (Gaetke and Chow, 2003). Nandi et al. (2005) found that cysteine and methionine are considered as a metal chelator and reduction of oxidative stress in rats is proved by the cysteine and methionine supplementations. As shown in Fig. 1, cysteine only has a thiol group among sulfur containing amino acids. Thiol group is involved in oxidation-reduction reaction. It has antioxidant abilities such as metal chelating ability and free radical scavenging ability (Deneke, 2001). In addition, thiol groups present in protein and enzymes have strong metal affinity (Flora et al., 2008). The metal chelation activity of sulfur-containing amino acids could contribute to the excretion of toxic metal from the body (Flora and Pachauri, 2010).

#### 4. Conclusion

The results of this study revealed that sulfur-containing amino acids had valuable antioxidant activities based on *in vitro* assays, including DPPH, ABTS, superoxide radical scavenging activities, FRAP, hydrogen peroxide scavenging, and metal chelating activities. Cysteine showed DPPH and ABTS radical scavenging similar to standard antioxidant (ascorbic acid). However, methionine and taurine failed to show DPPH or ABTS radical scavenging activity. Superoxide radical and hydrogen peroxide scavenging effects of the three sulfur-containing amino acids were detected. Ferrous chelating activ-

ity was not observed for the three amino acids. However, they showed chelating activities against other metals (copper and zinc). Based on our results, addition or supplementation with sulfur-containing amino acids to food products or human body might be useful for inhibiting oxidative stress or diminishing cell damage. However, in terms of oxidative stability, further investigation is needed to determine the antioxidant effects of food added with sulfur-containing amino acids as additives both *in vitro* and *in vivo*.

#### Acknowledgement

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