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Full Length Research Paper

Oxygen radical-scavenging capacities of peptides from swine blood

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In China, about five hundred million swine are slaughtered yearly, which represents about 45% of the world´s production. Swine blood is generally discarded except for the small amount that is used in soybean curd and other food products. This not only wastes resources, but also contaminates the environment. In this study, we found that peptides from swine blood had molecular masses of less than 2,100 Da and most were about 1,000 Da. Furthermore, the contents of Glu, Val, Met, Ile, Leu, Phe and Lys were higher than those in dried swine blood. Peptides from swine blood most strongly scavenged ·OH among different oxygen species. This is the first reported study on the oxygen radical-scavenging capacities of peptides from swine blood, and the results suggest that swine blood may be promising for use in food or feed.

Key words: Peptides of swine blood, components, scavenging capacities, oxygen radicals.

INTRODUCTION

The metabolic balance of oxygen radicals is a basic factor in maintaining life and health (Stadtman and Levine, 2003). In some pathological conditions, the abnormal production and scavenging of oxygen radicals leads to the accumulation of such radicals in the organism. A high level of oxygen radicals will damage the organism at the molecular, cellular, and organ levels, which can accelerate the senile process of the organism and may induce cancer, inflammation, and cardiovascular disease (Gutteridge 1994 and Tabatabaie et al. 2003). Oxidative stress also plays a crucial role in the development of complications in diabetes mellitus (DM) (Kapalla et al., 2005). Oxygen radicals include many kinds of oxygen compounds, such as superoxide anion free radicals ($O_2^{\cdot -}$), hydroxy free radicals ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), fat hydroperoxide, and singlet oxygen ($^1O_2$). The hydroxy free radical is the most active and also the most damaging to organisms (Gutterridge 1994; Tabatabaie et al. 2003; Peng et al., 2005; Zhang et al., 2005). Common oxygen radical scavengers include antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase (Fang et al., 2003), and endogenous and exogenous antioxidants such as vitamin C (VC), vitamin E (VE) and β-carotene. Recent reports have indicated that the hydrolyzed product of soybean protein and casein had certain antioxidant activity (Hou et al., 2008).

Swine blood is a valuable resource in China due to its high protein content. However, since the protein from dried blood powder is hard to digest and has poor palatability most of it is thrown out, which poses a serious threat to the environment (Fang et al., 2004). To solve these problems, peptides from swine blood (PSB) was obtained by the fermentation of swine blood (Fang et al. 2005) followed by micro-filtration, ultra-filtration, and spray-drying. These peptides have been shown to have some biological activities. In this study, the components of PSB were analyzed by a mass spectrometer, infrared spectrometer, and automatic amino acid analyzer. The ability of these peptides to scavenge ultra oxygen anion free radicals, hydroxy free radicals and hydrogen peroxide was examined by a chemical luminescence detection system.

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Abbreviations: PSB, Peptides of swine blood; **DM,** diabetes mellitus; **BHA,** butylated hydroxyanisole; **BHT,** butylated hydroxytoluene; **VC,** vitamin C; **VE,** vitamin E; **CL,** luminous intensity.

MATERIALS AND METHODS

Materials

Bacillus subtilis Strain A32 was obtained from a slaughterhouse (GenBank, Accession No. DQ631809), PSB was obtained by fermentation of swine blood with A32, micro-filtration, ultra-filtration, and spray-drying. Luminol (chromatography grade) and catechin were obtained from Sigma and Hunan Kinglong BIO-resources Products Industry Co., LTD, respectively.

Methods

The molecular weight distribution of PSB was measured by mass spectrograph (MS) 4307 (Bruker, Munich, German). Mode of operation: reflection; extraction mode: delayed; polarity: positive acquisition; manual; accelerating voltage: 200000 V; grid voltage: 68%; acquisition mass range: 850-5000Da; number of laser shots: 150/spectrum. The amino acid composition was measured by an automatic amino acid analyzer L - 8800 (Hitachi, Tokyo, Japan). Column: Na^+ exchange column (4.6 ID × 40 mm); detector: UV-visible light detector; reagent: ninhydrin/sodium acetate buffer; buffer system: citric acid buffer B1 (pH3.2), B2 (pH3.0), B3(pH 4.0), B4(pH4.9), column temperature: 55°C, reaction temperature: 135°C, flow rate: 0.4 ml/min. The ability to scavenge $O_2^{\cdot-}$ was determined according to the method of Xu et al. (2001). In this procedure, pyrogallol (10 µl mM) and luminol-carbonate buffer (940 µl and pH 10.2) were added to 50 µl samples and mixed completely, and the chemical luminescence power curve was measured for 20 s. The ability to scavenge $^{\cdot}OH$ was analyzed according to the method of Fan et al. (1998). Thus, 50 µl mM CuSO4, 50 µl mM 1,10-phenanthroline, and 700 µl boric acid-borax buffer (pH 9.0) were added to 50 µl samples with thorough mixing. Next, 100 µl mM antiscorbutic acid and 50 µl 0.1 mM H_2O_2 were added, and the chemical luminescence power curve was recorded for 150 s. The ability to scavenge H_2O_2 was determined according to the method of Xu et al. (2001). In this method, to 50 µl samples (distilled water as a control) were added 50 µl 0.15% H_2O_2 solution and 900 µl luminol-carbonate buffer (pH 10.2). After thorough mixing, the chemical luminescence power curve was measured for 60 s. The ability to scavenge oxygen free radicals was determined according to the method of Wang et al. (2003). A certain level of luminous intensity (CL) is associated with the oxygen free radical-scavenging capacity. Therefore, CL can be used to show the relative output of oxygen free radicals. Furthermore, materials that can scavenge oxygen free radicals can reduce CL, so a material's capacity to scavenge oxygen free radicals could be measured according to the decrease in CL:

Led inhibition rate = ($CL_{control}$ - $CL_{samples}$) / $CL_{control}$

A luminous inhibition curve was obtained with the sample concentration as the abscissa and the LED inhibition rate as the ordinate. The IC 50 was the concentration at which the rate was 50%. A small IC 50 was associated with strong oxygen radical-scavenging capacity, and vice versa (Wang et al. 2003). The assays were conducted in triplicate.

The amino acid composition of PSB was measured by an automatic amino acid analyzer L-8800 as described by Li et al (2008) and Kong et al (2008).

RESULTS

Molecular composition of PSB

Figures 1 and 2, respectively, show the molecular composition of swine blood powder and PSB. PSB was obtained from fermented swine blood by micro-filtration (0.05 µm ceramic membrane), ultra-filtration (1000 Da), and spray-drying. The–molecular mass of PSB was less than 2000 Da, and in most cases about 1000 Da.

Amino acid content of PSB

Figures 3 and 4 show that he contents of glutamine, glutaminic acid, valine, methionine, isoleucine, tyrosine, leucine, pheny-lalanine, lysine, histidine in PSB were about 10 - fold greater than those in swine blood powder. Half-cystine was increased nearly four times, and threonine was about doubled. PSB had a good balance of amino acids and abundant essential amino acids, and the imbalance of leucine and isoleucine was improved compared to that in swine blood powder. At the same time, the contents of taste-related amino acids such as glutamine (fresh) and glycine (sweet) were very high, and this improved the nutritional value and flavor of PSB.

Ability of PSB to eliminate free radicals

Figure 5A shows that all of the antioxidants tested decreased both the peak value and the area of luminous curves for H_2O_2. PSB, catechin and VC could scavenge H_2O_2, with IC 50 values of 1.27, 0.45, and 1.31 mg·mL-1, respectively (Table1). The ability of catechin to scavenge H_2O_2 was obviously higher than those of PSB and VC. The ability of PSB to scavenge H_2O_2 was slightly stronger than that of VC, but this difference was not significant.

Figure 5B shows that all of the antioxidants tested could decrease both the peak value and the area of luminous curves for $O_2^{\cdot-}$ PSB, catechin and VC could scavenge $O_2^{\cdot-}$, with IC 50 values of 3.42, 1.19, and 0.45 mg·ml-1, respectively (Table 1). The ability of VC to scavenge $O_2^{\cdot-}$ was obviously higher than those of catechin and PSB. The scavenging capacity of PSB was weaker than that of catechin.

PSB, catechin, and VC each reduced both the peak value and the area of luminous dynamics curves for ·OH. However, there were large differences in the ·OH-scavenging capacities of these materials. The peak value and area of luminous dynamics curves of PSB were much lower than those of caffeine and VC (Figure 5C). Their respective IC 50 values were 0.66, 14.23, and 16.74 mg·ml-1 (Table 1). The ·OH-scavenging capacity of PSB was nearly 22 times greater than that of catechin and 25 times greater than that of VC.

DISCUSSION

PSB had a molecular mass of less than 2000 Da, and most were about 1000 Da. When the molecular mass of peptides is less than 850 Da, the results obtained with the

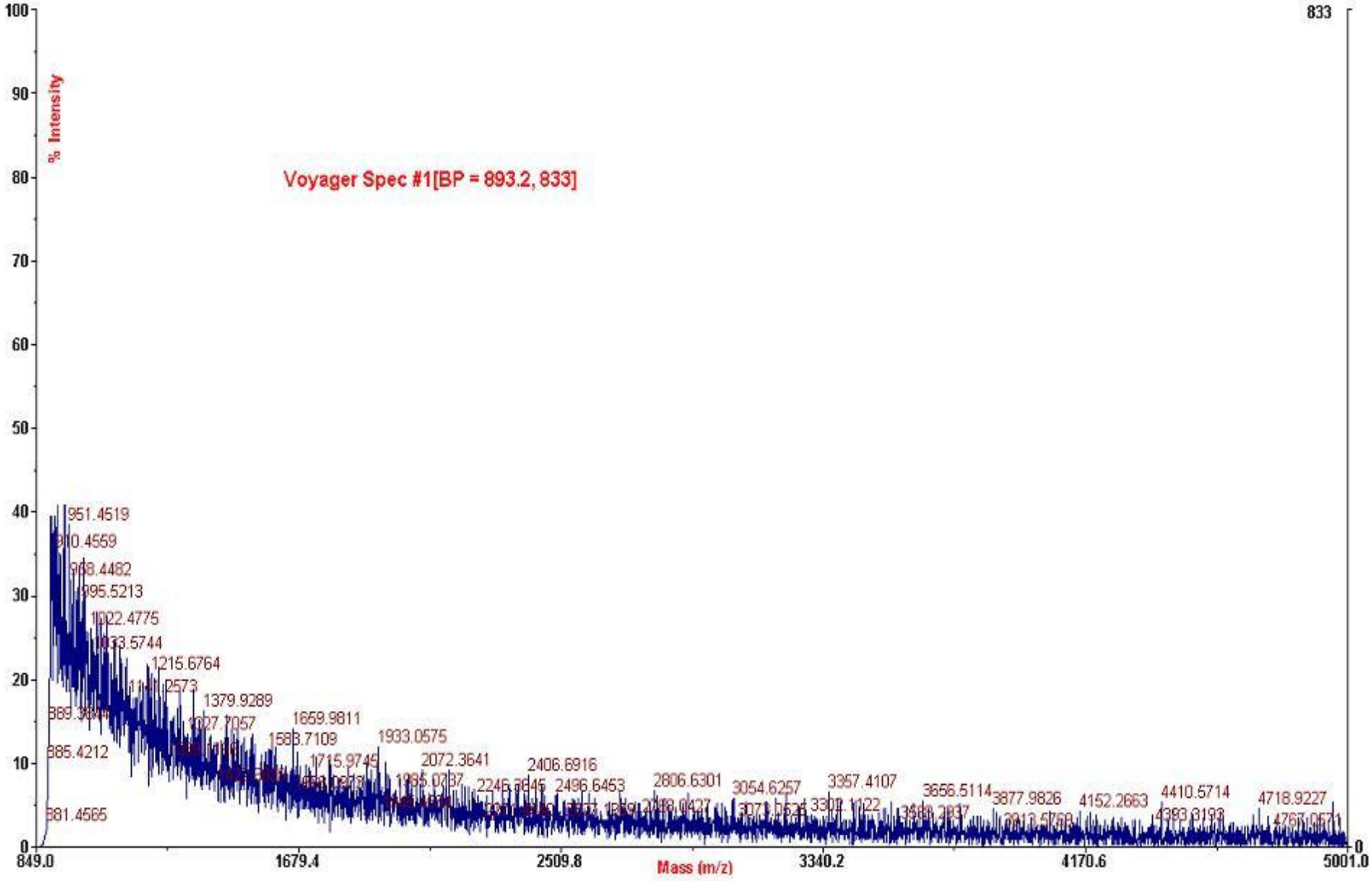

Figure 1. The molecular weight distribution of swine blood which was spray-dried was measured by a mass spectrograph.

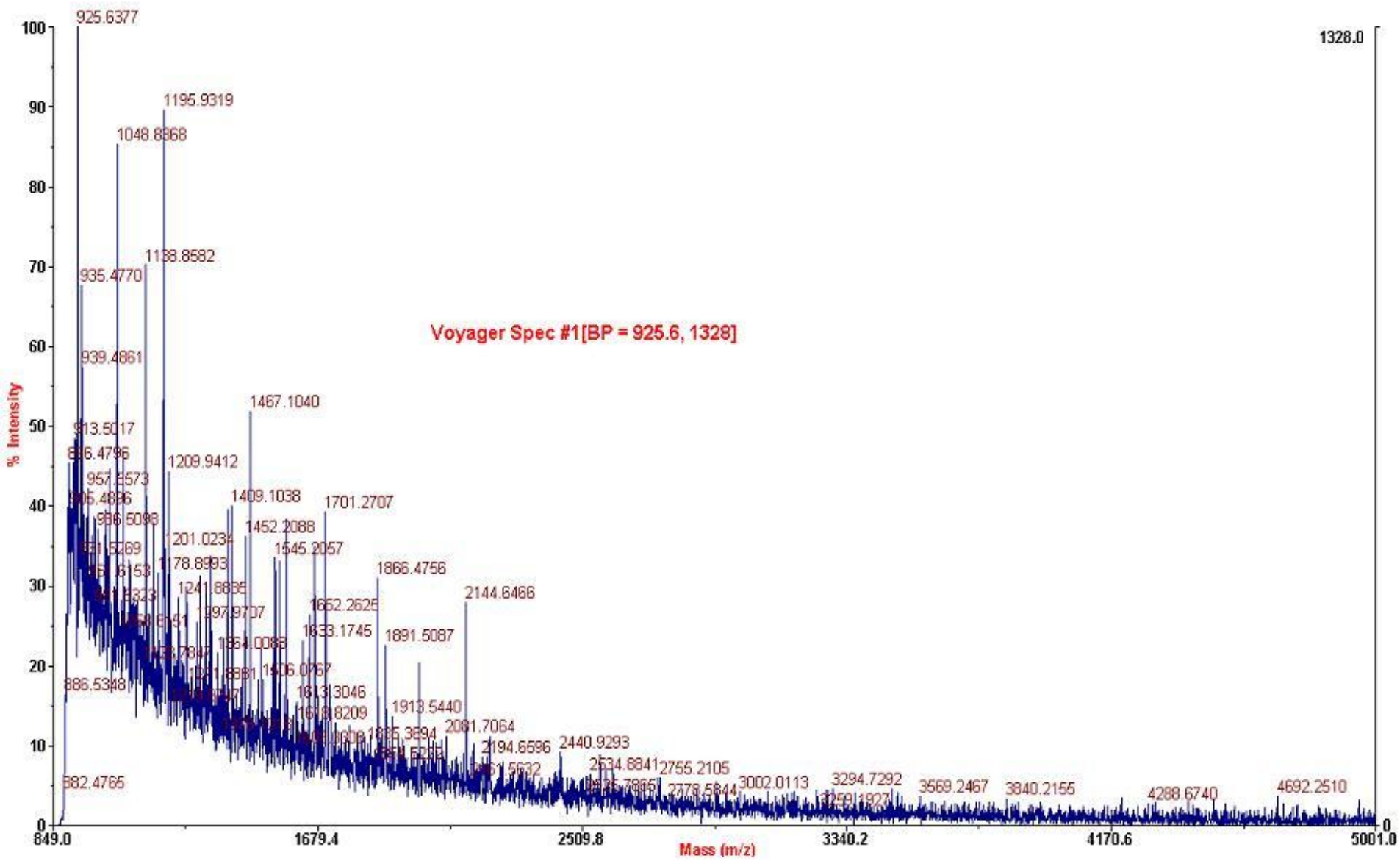

Figure 2. The molecular weight distribution of PSB was measured by a mass spectrograph; PSB was obtained by fermentation of swine blood with A32, micro-filtration, ultra-filtration, and spray-drying.

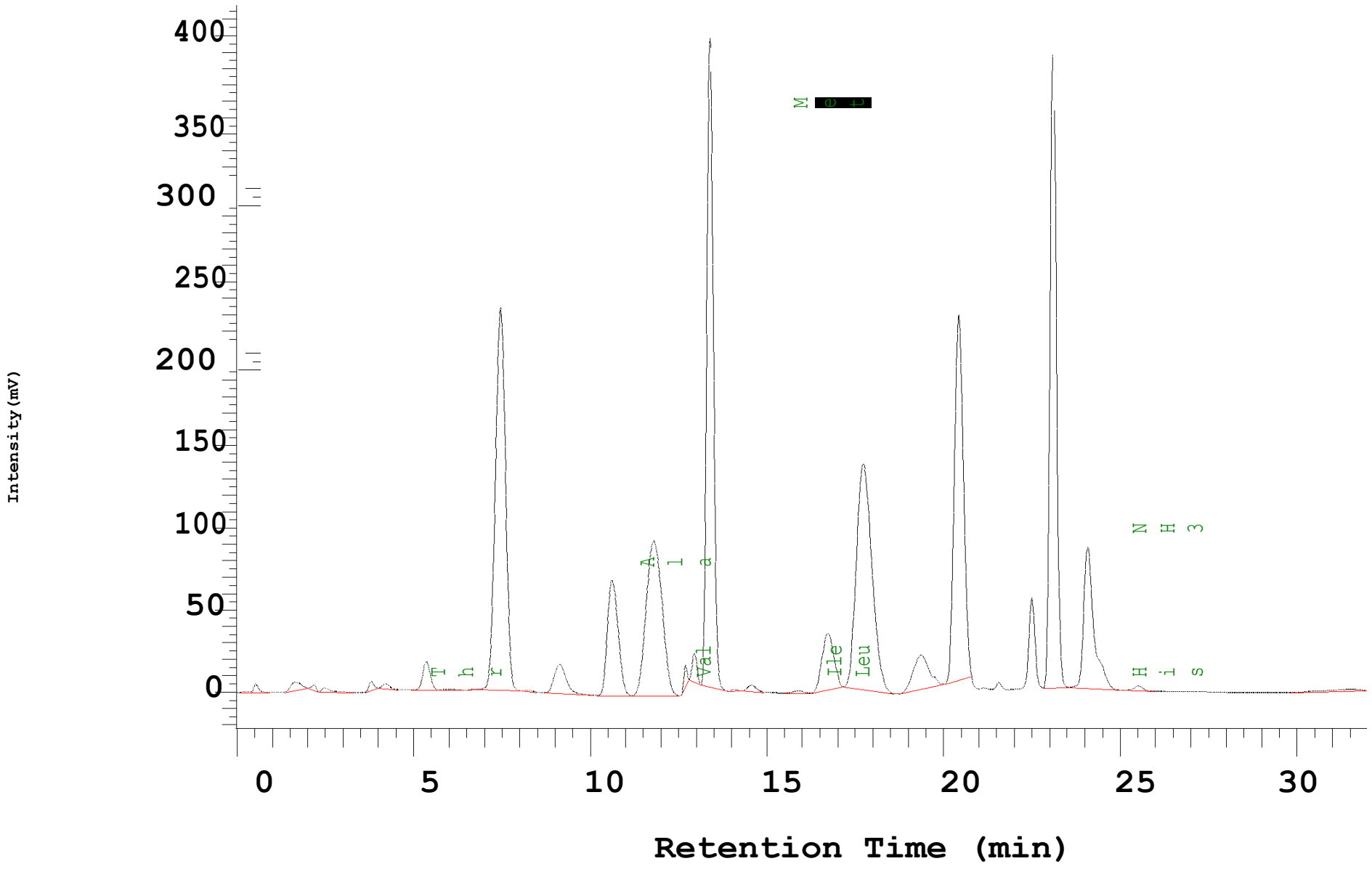

Figure 3. The amino acid composition of PSB was measured by an automatic amino acid analyzer L-8800. PSB was obtained by fermentation of swine blood with A32, micro-filtration, ultra-filtration, and spray-drying.

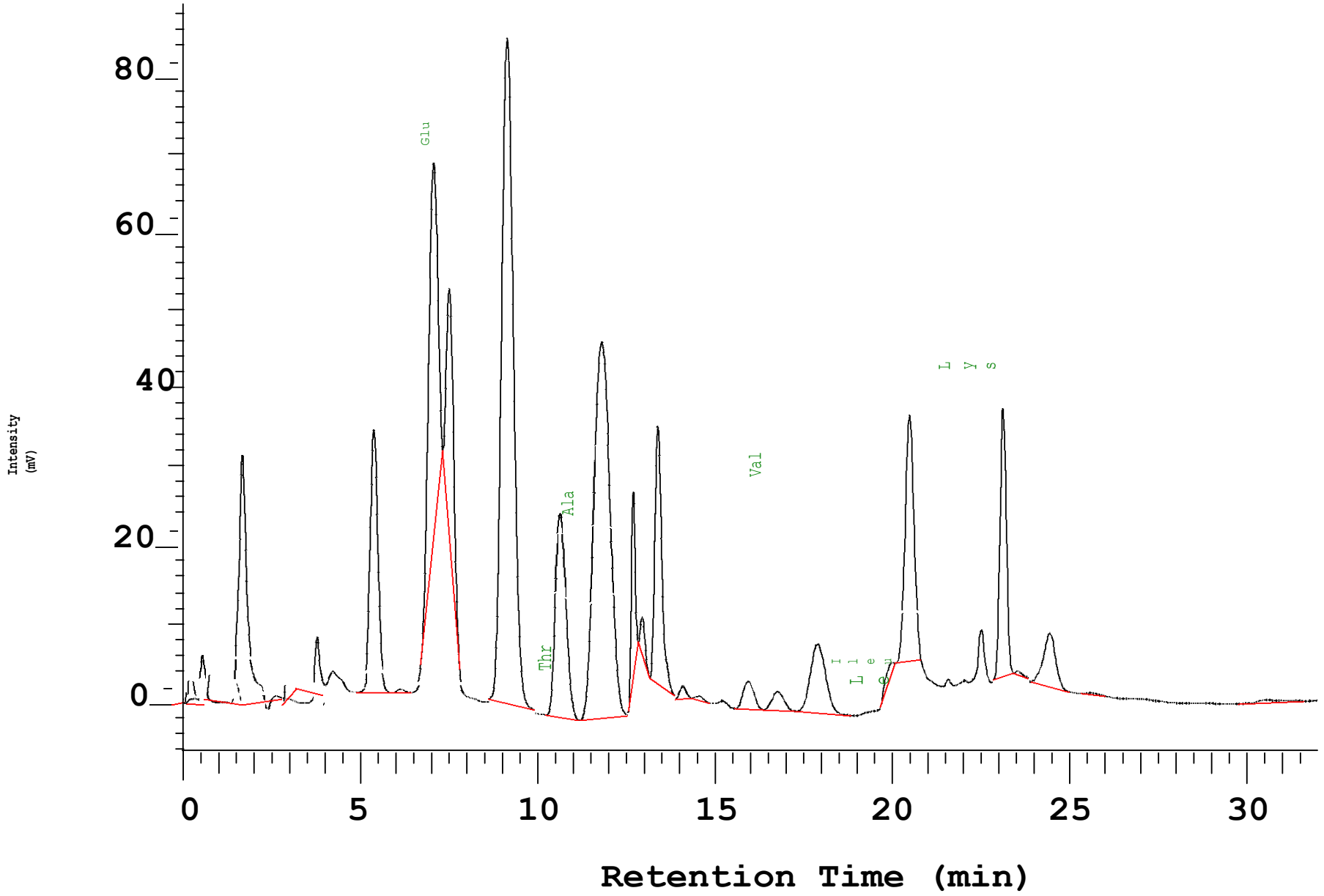

Figure 4. The amino acid composition of swine blood was measured by an automatic amino acid analyzer L-8800. Swine blood was spray-dried.

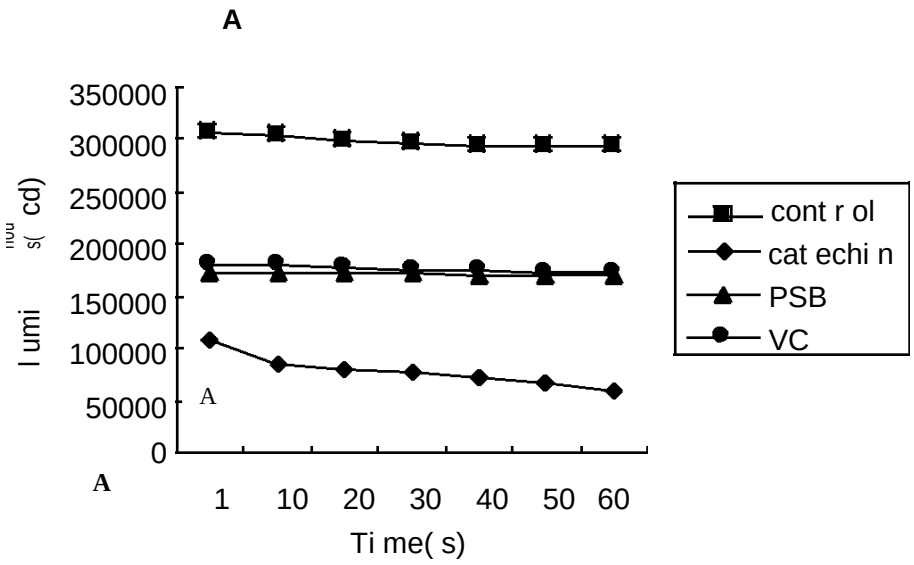

Figure 5A Scavenging of H_2O_2 by the various substrates used.

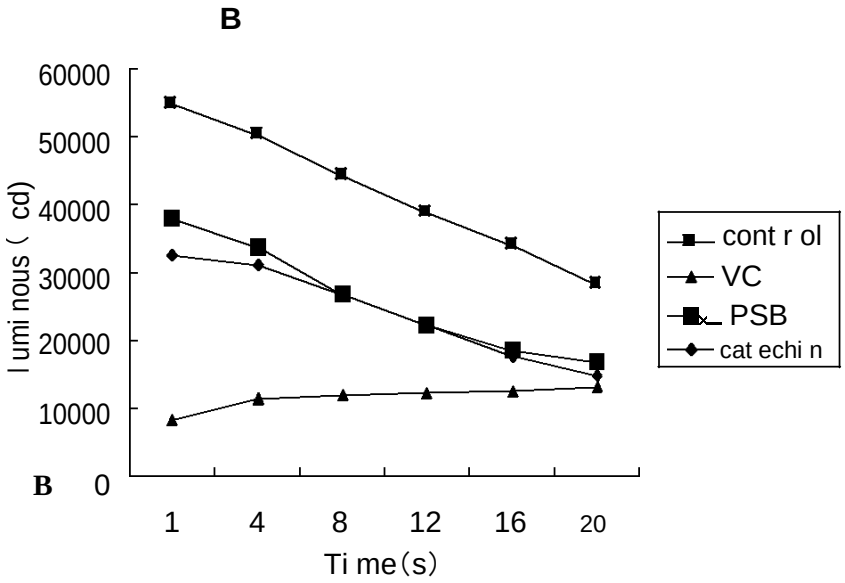

Figure 5B. Scavenging of $O_2^{\cdot -}$ by the various substrates used.

Applied Bio-systems Voyager System 4307 are not precise, since they are influenced by the instrument itself. Thus, the optimum range for the molecular mass was chosen to be from 850 to 5000 Da. PSB with a different molecular weight distribution was also obtained in the ionization process, and the peak value of some peptides could not be scanned. Therefore, the PSB samples contained some pieces of peptides.

PSB could clearly scavenge $O_2^{\cdot -}$, H_2O_2 and ·OH with IC 50 values of 3.42, 1.27, and 0.66 mg·mL-1. PSB is rich in hydrogen, and can provide a hydrogen proton, which can reduce highly oxidized oxygen radical. Thus, it can terminate the chain-reaction of oxygen radical and can scavenge or inhibit oxygen radical. The hydroxy free radical is the most active and dangerous among numerous oxygen radicals (Rong 2001). PSB had the strongest hydroxy free radical-scavenging activity, which suggests that PSB may be useful as an oxidation inhibitor.

In addition, the oxygen radical-scavenging activities of catechin and VC in this study were the same as those reported by Hu (2004), but there was a difference in the IC 50 values. This discrepancy may have been due to the fact that the catechin and VC reagents were produced by different companies and the duration of recording the illumination curve also differed.

At present, the use of chemical antioxidant additives like butylated hydroxyanisole (BAH) and butylated hydroxytoluene (BHT) has been limited due to considerations regarding food safety. The use of some natural antioxidants such as VE and herbal extracts has also been limited due to their high cost, their effects on food flavor and color. Furthermore, oxidative stress can strongly

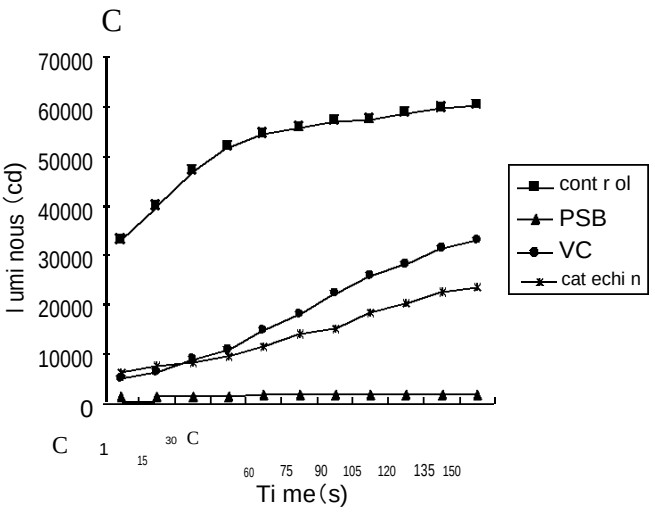

Figure 5C. Scavenging of ·OH by the various substrates used.

Table 1. Radical-scavenging activities of different antioxidants.

Antioxidants	H_2O_2	$O_2^{\cdot-}$	·OH
PSB	1.27±0.13[a]	3.42±0.23[a]	0.66±0.14[a]
Catechin	0.45±0.09[b]	1.19±0.14[b]	14.23±2.11[b]
VC	1.31±0.11[a]	0.45±0.06[c]	16.74±2.60[c]

[abc] Values with different lowercase letters are significantly different ($P < 0.05$). Values are given in IC_{50}(mg· ml^{-1}).

influence animal's growth. Thus, the identification of minimally toxic and yet highly effective natural antioxidants is important for maintaining health, promoting animal growth and enhancing animal performance.

PSB may be produced by the fermentation of swine blood with strain A32. The production cost is very low, regardless of whether it is to be used as an antioxidant additive in food or feed. At present, there have been few reports on PSB, and this promises to be a fruitful field of research.

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