ASSESSMENT OF ANTIOXIDATIVE CAPACITY OF ANIMAL LIVER AND BILE

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Introduction

Nearly 10 million pigs are slaughtered in Taiwan each year. Only a small portion of the liver is used for human consumption, with the greater part used as animal feed or discarded. The pig bile is thrown away immediately. These practices are also particularly common in poultry slaughterhouses. Discarding those by-products is increasing the environmental load. Liver is a valuable source of numerous proteins, amino acids, minerals and vitamins. Animal bile is rich in various compounds, and is potentially a source of numerous nutrients following appropriate extraction, and thus had medicinal application in traditional Chinese therapies. Bezoar is one such traditional Chinese medicine whose curative effect has been reported (Liu, 1980). The major compound present in this medicine is bilirubin which is abundant in pig bile and is the major ingredient for the synthesis of bezoar. Large quantities of liver and bile may be obtained from slaughterhouses in Taiwan and Japan. Liver extracts and bile powders were examined in this study for their antioxidative capacity.

Materials and Methods

1. Sample preparation

(1) Liver extracts: Liver was cut into 1 cm^3 pieces, placed in boiling water for 1 min, and then water-flow cooled for removal of impurities. Following the addition of an equal volume of deionized water, the samples were placed in a 100°C water bath for 6 hours and then filtered through 2-layer swabs. Decompression was then carried out to reduce original volume by 75% (Eyela, Model N-1NW, Japan). A powder of the liver extract was obtained by freeze dehydration (FD-5N, Eyela; Pump, G-100D, ULVAC, Japan) and then stored at -20°C.

(2) Bile powders: Bile gall was obtained by needle puncture and filtered as above. This was followed by heat treatment at 100°C for 15min. The samples were converted into powder form by freeze dehydration and then stored at -20°C.

2. Antioxidation assessment

(1) Reducing capacity: This capacity was assessed according to Oyaizu (1986). 2.5 mL sample solution were added to 2.5 mL 0.2 M Na_2HPO_4 (pH 6.6) and 2.5 mL 1% $K_3Fe(CN)_6$, placed in a water bath at 50°C for 20 min and cooled in flowing water, followed by addition of 2.5 mL 10% TCA and centrifugation (3,000 rpm) for 10 minutes. 5 mL supernatant were added to 5 mL deionized water and 1 mL 0.1% $FeCl_3 \cdot 6H_2O$, and the reaction was allowed to proceed for 10 min. The absorbance was measured at 370 nm by spectrophotometer. Higher absorbance is an indication of greater reducing capacity.

(2) Fe^{2+} ion chelating capacity: 1 mL sample solution is added to 3.7 mL methanol and 0.1 mL 2 mM $FeCl_2 \cdot 4H_2O$ followed by 0.2 mL 5 mM ferrozine after 20 sec, and the reaction was allowed to proceed for 10 min. The absorbance was measured spectrophotometrically at 562 nm. Lower absorbance is an indication of greater chelating capacity.

Chelating capacity (%) = [1– (absorbance of sample at 562 nm)/ (absorbance of control at 562 nm)] × 100%

(3) Capacity for scavenging superoxide anions: 1 mL sample solution was added to 120μM phenazine methosulfate (PMS), 936 μM β-nicotinamide adenine dinucleotide (NADH) and 300μM nitro bluetetrazolium (NBT), and the reaction is allowed to proceed at room temperature for 5 min. The absorbance was immediately measured spectrophotometrically at 560 nm, followed by addition of superoxide dismutase (SOD, 5,000 units). A lower absorbance indicates greater scavenging capacity, which is expressed as [1 – (absorbance of sample at 560 nm) / (absorbance of control at 560 nm)] × 100%.

Results and Discussion

1. Reducing capacity

Figure 1 shows this ability for the various extracts and appeared greatest for PLE. The reducing capacity of PLE, PBL, CLE and CBL was significant and exceeded that of BHT ($p<0.01$), while that of PBP and CBP was not significant. Takahashi *et al.* (1995) indicated that taurine (Tau) inhibits lipid peroxidation in cells. Tau is the main component of PLE and CLE which may explain the better reducing capacity in liver extracts.

2. Capacity for Fe^{2+} ion chelation

Metal ions can promote lipid peroxidation and some may induce free radical production. Fe^{2+} ions strongly promote oxidation which in turn may enhance this production. However, this ability is lost subsequent to the chelation of these ions. Chelating ability may thus serve to enhance that of antioxidation, as evident from Figure 2. All extracts were found to have more than 50% chelating ability. Pierno *et al.* (1998) consider Tau to chelate Fe^{2+} ions in cells, and bilirubin has this ability to the same extent. Chelating ability may possibly be due to Tau and bilirubin.

(3) Scavenging ability of superoxide anions

Superoxide anions may possess reducing and oxidation capacity simultaneously, and under certain conditions may act as a single oxide, hydroxyl radical or hydrogen peroxide. Superoxide dismutase (SOD) cleans superoxide anions by the dismutase reaction. Many substrates having the same ability are said to be SOD-like and remove superoxide anions by a different mechanism. Figure 3 shows the scavenging ability of superoxide anions under various conditions, with PLE and CLE showing greater ability to scavenge superoxide anions, the extent differing significantly ($P<0.01$). PBP and CBP appear not to possess this ability. Liver extracts were noted in this study to contain more Tau than bile powder. This possibly may explain why liver extracts possess the ability to scavenge superoxide anions.

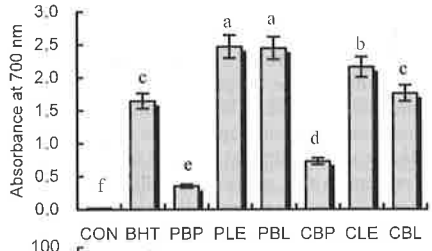

Figure 1: Reducing capacity of liver and bile extracts.
Bars indicate mean ± S.D. Different superscript letters (a-f) indicate significant differences ($P < 0.01$).
Deionized water (CON), butylated hydroxytoluene (BHT), pig bile powder (PBP) ; pig liver extract (PLE), pig bile and liver extract (PBL), chicken bile powder (CBP), chicken liver extract (CLE), chicken bile and liver extract (CBL), n=3.

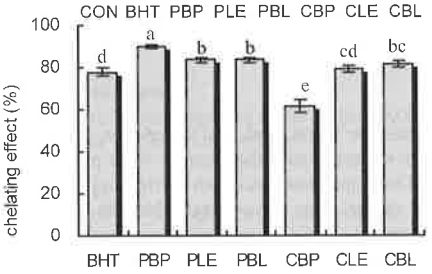

Figure 2: The Fe^{2+} ion chelating capacity of liver and bile extracts.
Bars indicate means ± S.D. Different superscript letters (a-e) indicate significant differences ($P < 0.01$).
Butylated hydroxytoluene (BHT), pig bile powder (PBP), pig liver extract (PLE), pig bile and liver extract (PBL), chicken bile powder (CBP), chicken liver extract (CLE), chicken bile and liver extract (CBL), n=3.

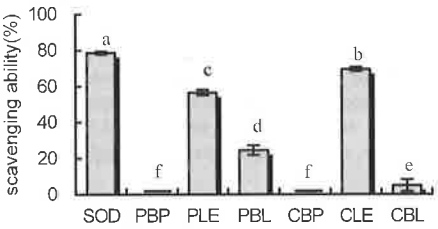

Figure 3: Superoxide anion scavenging capacity of various liver and bile extracts.
Bars indicate means ± S.D. Different superscript letters (a-f) indicate significant differences ($P<0.01$).
Superoxide dismutase (SOD), pig bile powder (PBP); pig liver extract (PLE), pig bile and liver extract (PBL), chicken bile powder (CBP), chicken liver extract (CLE), chicken bile and liver extract (CBL), n=3.

Conclusions

Pig liver and bile extracts were found to have greater capacity for reduction. Chicken and pig liver had greater capacity for scavenging superoxide anions. For pig bile extract, Fe^{2+} ion chelation capacity was greatest. These extracts are thus shown to have significantly potential as nutrients and healthful constituents in food production.

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