PE4.101 Antioxidant activity of bovine low value muscle and by-products 373.00

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Abstract —Cytoplasmic/sarcoplasmic proteins from bovine skeletal muscle (brisket), smooth muscle (lung) and organ tissue (liver) proteins were hydrolysed with a commercial enzyme. Antioxidant activity of respective hydrolysates at seven hydrolysis time points was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The liver hydrolysates exhibited the highest antioxidant activity compared to the other tissue types. The IC_{50} values ranged from 0.31 to 0.45 mg/ml for liver hydrolysates. Antioxidant activity of lung hydrolysates was also high with highest radical scavenging activity (IC_{50} = 0.36) at the seventh hydrolysis time point. Brisket hydrolysates for both *post-mortem* time points exhibited lower antioxidant activity compared to liver and lung with increased antioxidant activity observed over the *post-mortem* aging period. Results demonstrated that bovine liver, lung and brisket enzymatic hydrolysates possess antioxidant activities.

Keywords: antioxidant, bioactive peptides, bovine by-products, DPPH, enzymatic hydrolysis, hydrolysate

I. INTRODUCTION

In recent years it has become widely recognised that natural antioxidants are important in the prevention of human diseases and inhibition of lipid oxidation in foods. Antioxidants can protect the human body from reactive oxygen species effects and retard the progress of many chronic diseases [1]. Oxidative stress also causes lipid oxidation in foods and is the major cause of spoilage and deterioration of the nutritional quality and safety of food [2].

Synthetic antioxidants, such as BHA (buthylated hydroxyanisole) and BHT (buthylated hydroxytoluene) are currently used to prevent oxidation in foods. However, as these compounds pose potential risks *in vivo*, the use of synthetic antioxidants in foods is restricted in some countries due to potential risks related to health. As a result, research isolating and identifying natural and safe antioxidant compounds from natural sources is important.

Bioactive peptides are small peptides, usually containing between 3 and 20 amino acids residues [3]. Recently, the possibility of releasing biologically active peptides from food proteins has attracted a lot of interest [4]. In addition to their production by protein hydrolysis in food during processing and digestion in the digestive tract, bioactive peptides may also be generated by controlled protein hydrolysis [5]. Bioactive peptides from various animal and plant sources possessing antioxidant activity have been reported in milk casein, fish, eggs, soy proteins [6] [7] [8] [9].

Because of their therapeutic potential for treatment or prevention of diseases, bioactive peptides may be used as components in functional foods [10]. Meat protein hydrolysates and bioactive peptides with antioxidant activity have been found in pork, bovine, buffalo and chicken meat or by-products [11] [12] [13] [14].

Recovery of value from meat by-products is an increasing problem for the meat industry, hence there is potential to produce functional peptides from low value bovine muscle (e.g. brisket) and bovine offals (e.g. lung and liver) where the cost of the raw material is inexpensive and the amount of waste is reduced.

The objective of the present work was to investigate the antioxidant activity of enzymatic hydrolysates of bovine brisket (at two *post-mortem* time points), liver and lung hydrolysed at several time points with a commercial enzyme.

II. MATERIALS AND METHODS

Materials and chemicals

An organ tissue (liver), a smooth muscle (lung), and a skeletal muscle (brisket) were collected. The first two tissues were collected at time of slaughter; the brisket muscle was collected at two different *post-mortem* time points. All samples were obtained from the abattoir at Ashtown Food Research Centre, Dublin, Ireland and stored at -80°C until analysis. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were

purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). All other chemicals were of reagent grade or purer.

Preparation of cytoplasmic/sarcoplasmic enzymatic hydrolysates

Cytoplasmic/sarcoplasmic proteins were extracted from the bovine tissues using 0.02 M phosphate buffer [15], using a SPEX Prep freezer mill grinder. The extraction procedure and enzyme hydrolysis (see below) was monitored using 1D SDS polyacrylamide gel electrophoresis using the method of Laemmli [16] using 12.5% resolving gels and 4% stacking gels, with some modifications. Briefly, the running buffer contained 1.92 M glycine and 1% sodium dodecyl sulphate. The resolving gel contained Protogel (41.62%), protogel buffer (25.01%), water (32.81%), ammonium persulphate (0.5%), and N,N,N',N'-tetramethylethylenediamine (TEMED) (0.05%). A modified version of Biuret assay [17] was used for the determination of protein content of the hydrolysates. The Biuret reagent was prepared as followed: 9.0 grams of sodium potassium tartarate were dissolved in 500 ml of 0.2N NaOH. Three grams of $CuSO_4.5H_2O$ were added and dissolved with stirring. Five grams of potassium iodide were added and diluted to 1000 ml with 0.2N NaOH. Three dilutions of samples were prepared. Biuret reagent (250 µl) was added to 150 µl of diluted sample. The reaction mixtures were incubated at 37°C for 10 min and samples were read at 540 nm in UV-Cuvettes Micro (Plastibrand 7592-20) using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). Bovine serum albumin was used as standard.

The beef extracts were hydrolysed in triplicate with a commercial enzyme at seven hydrolysis time points, with a substrate to enzyme ratio of 100:1 w/w. The enzymatic hydrolysis was stopped by boiling samples at 99°C for 10 minutes. Samples were then centrifuged at 14,000 RPM for 10 minutes and the supernatants were collected and stored at -80°C until analysis.

Measurement of DPPH radical scavenging activity

DPPH radical scavenging activity was measured using a modified version of the method described by Groupy et al. [18]. Briefly, each liver and lung enzymatic hydrolysate was diluted in water at different concentrations reaching a total volume of 500 µl; brisket 1st and 2nd *post-mortem* time points hydrolysates were diluted in water at different concentrations reaching a total volume of 250 µl. Liver and lung hydrolysates in water were added to 500 µl of DPPH in methanol solution (0.604 mM); brisket 1st and 2nd *post-mortem* time points hydrolysates were added to 250 µl of DPPH in methanol solution (0.604 mM). After vortexing, the tubes were left in the dark for 30 min at room temperature after which the absorbance was measured against methanol at 515 nm using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). The control used was 50% H_2O and 50% DPPH solution. The antioxidant activity was expressed as the IC_{50} (mg/ml), where the values represented the concentration of sample required to cause a 50% reduction of the free radical DPPH. DPPH assay was carried out in triplicate for each hydrolysis time point of each hydrolysis of each tissue type. Trolox standard concentrations were used as a calibration curve.

III. RESULTS AND DISCUSSION

The antioxidant activity of the four tissue type hydrolysates was evaluated and exhibited a range of IC_{50} values from 0.32 to 1.58 mg/ml. Figures 1-4 show the scavenging effect of the bovine liver, lung and brisket hydrolysates on the DPPH radical. Different antioxidant activity trends were observed within each tissue type depending on the duration of enzymatic hydrolysis with the commercial enzyme.

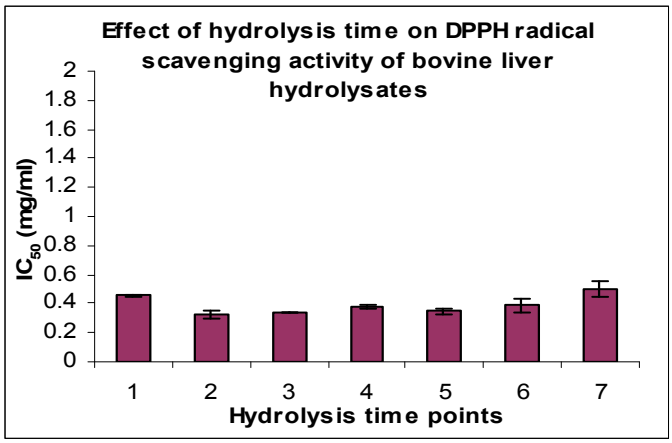

Figure 1. Antioxidant activity of liver cytoplasmic proteins hydrolysed with a commercial enzyme.
The result of the seventh hydrolysis time point is the average of the two closest replicates of the three.

In studying the antioxidant activity of the seven hydrolysis time points of cytoplasmic proteins from liver (Figure 1), the second, third and fifth showed highest antioxidant activity, showing IC_{50} values of 0.32, 0.34 and 0.35 mg/ml respectively, while the first and the seventh showed lowest IC_{50} of 0.45 and 0.50 mg/ml. The lower the IC_{50}, the higher the antioxidant capacity.

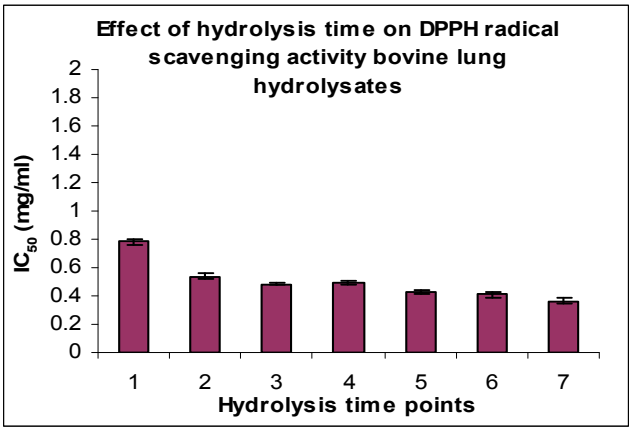

Figure 2. Antioxidant activity of lung sarcoplasmic proteins hydrolysed with a commercial enzyme.

The DPPH radical scavenging activity of bovine sarcoplasmic protein hydrolysates of lung (Figure 2) increased with the increasing of hydrolysis time showing an IC_{50} (mg/ml) value for the first hydrolysis time point of 0.78, and an IC_{50} (mg/ml) value for the seventh hydrolysis time point of 0.36. The hydrolysates produced by hydrolysing lung tissue with a commercial enzyme showed the highest scavenging effect on DPPH at the fifth, sixth and seventh hydrolysis time points with IC_{50} values of 0.42, 0.41 and 0.36 mg/ml respectively.

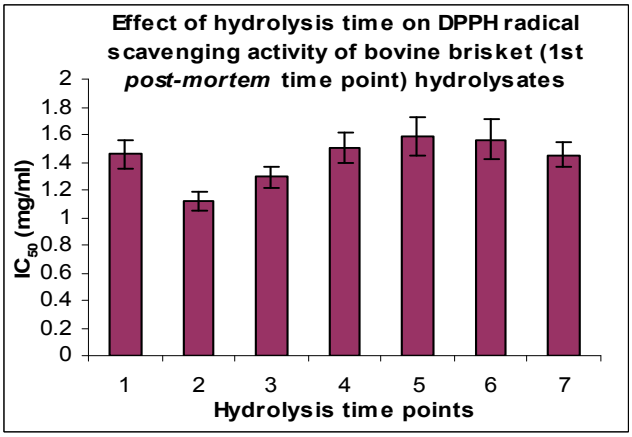

Figure 3. Antioxidant activity of brisket (1st post-mortem time point) sarcoplasmic proteins hydrolysed with a commercial enzyme.

The antioxidant activity of the seven hydrolysis time points of brisket (1st *post-mortem* time point) (Figure 3) was lower than those exhibited for liver and lung tissue types (brisket 1st *post-mortem* time point IC_{50} mg/ml values: between 1.12 and 1.58; liver: IC_{50} mg/ml values comprised between 0.31 and 0.49; lung: IC_{50} mg/ml values between 0.36 and 0.53). The IC_{50} (mg/ml) values found for brisket (1st *post-mortem* time point) were about four fold higher than the values found for bovine liver, and about three fold higher than the values found for bovine lung hydrolysates. The highest radical scavenging activity in bovine brisket 1st *post-mortem* hydrolysates was found at the first, second, third and seventh hydrolysis time points, with the IC_{50} values of 1.45, 1.12, 1.28, and 1.45 respectively.

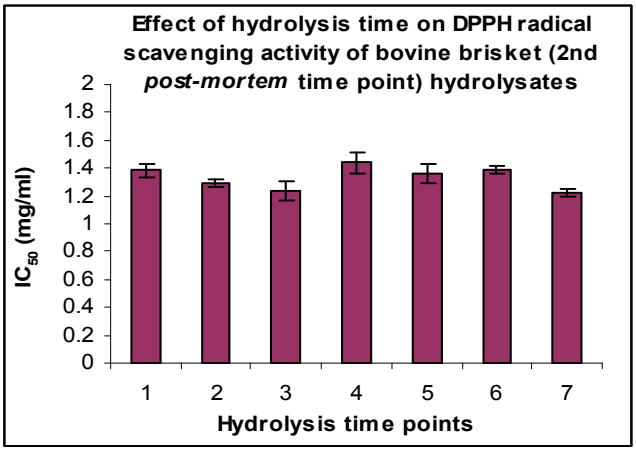

Figure 4. Antioxidant activity of brisket (2nd post-mortem time point) sarcoplasmic proteins hydrolysed with a commercial enzyme.
The result of the seventh hydrolysis time point is the average of the two closest replicates of the three.

The radical scavenging activity of bovine brisket hydrolysates at the 2nd *post-mortem* time point (Figure 4) was lower than those showed for bovine liver and lung. Interestingly all the brisket 2nd *post-mortem* hydrolysis time points showed higher antioxidant activity than the first, the fourth, the fifth, the sixth and the seventh hydrolysis time points of the activity found for bovine brisket 1st *post-mortem* hydrolysates. Only the second hydrolysis time point of brisket 1st *post-mortem* showed higher antioxidant activity than brisket 2nd *post-mortem* time point hydrolysates. The IC_{50} (mg/ml) values of brisket 2nd *post-mortem* hydrolysates were between 1.22 and 1.43, and the best antioxidant activity was recorded at the second, third and seventh hydrolysis time points with IC_{50} values of 1.29, 1.23 and 1.22 respectively.

In between the two brisket sample hydrolysates the second hydrolysis time point of the brisket 1st *post-mortem* time point showed the highest antioxidant activity of all the hydrolysis time points of brisket 1st and 2nd *post-mortem* time points.

In meat, processes such as rigor developing and ageing are known to influence the properties of the structural components. It is well known that in the *post-mortem* period proteolysis of myofibrillar and myofibrillar-associated proteins occurs [19]. The enzymatic changes in *post-mortem* muscle, which affect the water soluble fractions, include the increase of free amino acids and peptides through proteolysis [20].

Post-mortem degradation is in fact a result of the sequential action of the proteolytic enzymes. It is

suggested that the early *post-mortem* changes are due to calpain that initiates disruption and destabilisation of the myofibrillar structures, allowing the proteasome and cathepsines to act on the partially degraded proteins [21]. In particular, the role of calpains in the *post-mortem* tenderisation of meat is responsible for to 95 % of all the proteotically induced post-mortem tenderisation [22].

The proteolitic action during the *post-mortem* period could have led to the formation of protein fragments, polypeptides or peptides and may explain the fact that all the brisket 2nd *post-mortem* time point hydrolysates showed higher antioxidant activity than the first, fourth, fifth, sixth and the seventh brisket 1st *post-mortem* time point hydrolysates. On the other hand some protein fractions present in the second brisket 1st *post-mortem* time point hydrolysates, showing high antioxidant activity, could have been broken down during ageing which may have decreased the antioxidant activity in the second hydrolysis time points of brisket 2nd *post-mortem* time point.

In studying and interpreting the antioxidant activity of all the four tissue types, it is important to mention the protein concentration of each of them. The protein concentration of the liver cytoplasmic extracts was about 15 µg/µl, of lung sarcoplasmic extracts 9 µg/µl, of brisket 1st *post-mortem* sarcoplasmic extracts 6 µg/µl, and of brisket 2nd *post-mortem* sarcoplasmic extracts about 5 µg/µl. The higher antioxidant activity was reported for liver, followed by lung, and then the brisket tissue types. The high protein contents of liver and lung extracts could have been responsible for the formation of a greater quantity of protein fractions and/or peptides during the hydrolysis and, these protein fragments could have been responsible of the higher antioxidant activity.

IV. CONCLUSION

The seven hydrolysis fractions of bovine liver, lung and brisket (at two *post-mortem* time points), produced using a commercial enzyme, exhibited antioxidant activity. Antioxidant activity of the enzymatic hydrolysates was in the order: liver > lung > brisket 2nd *post-mortem* time point > brisket 1st *post-mortem* time point. The antioxidant activity varied depending on the tissue type and the duration of hydrolysis. These results suggested that tissue hydrolysates from bovine low value muscle and offal proteins might be useful as food additives or pharmaceutical agents. Further work is being carried out on fractions with the highest antioxidant activity which will be purified, characterised and their activity will be studied further.

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