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Antioxidant properties of pancreatic hydrolysates from various protein sources

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Abstract: Antioxidant activity of pancreatic hydrolysates of casein, spent brewer's yeast, bovine liver and tendon was evaluated in comparison to unhydrolised substrates by ABTS assay. In the result of enzymic treatment ABTS cation radical scavenging activity of spent brewer's yeast, bovine liver and bovine tendon was increased in 4.8, 3.3, and 2.3 times respectively, except casein. The IC_{50} values of ABTS cation radical scavenging for casein and it's hydrolysate were similar. The IC_{50} values of DPPH radical scavenging activity for pancreatic hydrolysates of casein, spent brewer's yeast, bovine liver and tendon were 1.8±0.1 mg/ml, 1.3±0.1 mg/ml, 1.7±0.1 mg/ml, and 6.2±0.1 mg/ml respectively. Above mentioned hydrolysates of various origin were produced at the same conditions (temperature 50°C, pH 7.5-8.9, duration 5 hours) using fresh bovine pancreatic tissue as an enzyme source. The extent of hydrolysis expressed as AN/TN (amino nitrogen/ total nitrogen) for hydrolysates had values from 0.36±0.02 to 0.46±0.04.

Keywords: casein, spent brewer's yeast, liver, tendon, enzymatic hydrolysis

INTRODUCTION

In recent years, there has been a growing interest in finding natural dietary antioxidants, moreover well-known plant derived natural antioxidants as vitamin C, polyphenols, flavonoids and carotenoids [1, 2]. Some protein hydrolysates have been reported to possess antioxidative potentials beside their nutritional value. Hydrolysates or peptides with antioxidant properties were obtained from various substrates like milk proteins [3, 4], sunflower protein [5], rice bran [6], soy protein isolate [7], spent brewer's yeast [8], bovine liver [9], kidney bean [10], fish and fishery by-products [11, 12]. These findings exhibit that food manufacturing by-products are the potential sources for the preparation of protein hydrolysates with health beneficial properties as well asprotein isolates or concentrates [1, 2, 11].

Antioxidative activity of protein hydrolysates releases through antioxidant peptides formed during enzymic hydrolysis process. Once released, the peptides have been shown to possess radical scavenging, metal ion chelation properties and the ability to inhibit lipid peroxidation [1-3]. Antioxidative activity of protein hydrolysates depends on substrate nature, enzyme specificity and hydrolysis conditions [1-3, 11].

The objective of this study was to evaluate radical scavenging activity of pancreatic hydrolysates of casein, spent brewer's yeast, bovine liver and tendon obtained in similar hydrolysis conditions and having close AN/TN values.

EXPERIMENTAL

Materials: Protein hydrolysates were prepared using acid precipitated casein, spent brewer's yeast, bovine liver and tendon as the hydrolysis substrates. Fresh bovine pancreatic tissue was used as an enzyme source. The hydrolysis of all protein substrates was conducted at the 50°C, pH 7.5-8.0 for 5 hours under the constant stirring. The procedures of preparation were described in our previous works [13-16].

1,1 diphenyl-2-picryl-hydrazyl radical (DPPH), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid(ABTS), potassium peroxydisulfate were obtained from Sigma -Aldrich (Taufkirchen, Germany). All other chemicals and solvents used were analytical grade.

Determination of total and amino nitrogen: The amount of total nitrogen in protein hydrolysates was determined using Kjeldahl semi automatic system as described by European Pharmacopoeia [17]. The amino nitrogen content was determined by formol titration method according to U.S. Pahrmacopoeia [18].

Determination of antioxidant activity in hydrolysates

DPPH radical scavenging activity: The DPPH radical scavenging activity was determined by DPPH assay, as described by Brand-Williams W., *et al.* [4]. Stock solution of DPPH was prepared daily by dissolving 40 mg of DPPH in 100 ml of 95% ethanol. The working solution of DPPH having an extinction value of 0.75-

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0.80 at 525 nm was prepared by diluting of stock solution of DPPH. To the analysed sample (1.5 ml), stock solution of DPPH (1.5 ml) is added. The mixture was vigorously mixed and incubated at room temperature in a dark place for 30 min. The absorbance of the resulting solution was measured at 517 nm. The blank was contained distilled water instead of sample. The ability to scavenge the DPPH radical was calculated as follows:

$$DPPH\ scavenging\ activity, \% = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

ABTS radical scavenging activity: ABTS radical scavenging activity was determined according to Buenger et al. [19]. An ABTS solution (1.7 mM×L^{-1} in water) is mixed in the ratio 5:1 with potassium peroxydisulfate solution (4.3 mM×L^{-1} in water) and incubated for 12-16 h at room temperature in darkness. Immediately prior to measurement, this stock solution is diluted with ethanol or water to an extinction value of 0.700±0.020 at 734 nm. After addition of 1.0 ml of diluted $ABTS^{\cdot+}$ solution to 10 µl sample mixed extinction at 734 nm is measured after exactly 6 min against the solvent. The cation radical scavenging activity was calculated as the same as DPPH assay.

Statistical analysis: Data were expressed as the mean±standard deviation and subjected to one-way ANOVA test using statistical software JMP. Significant differences were determined by Student's *t*-test and accepted at $p<0.05$.

RESULTS AND DISCUSSION

The ratios of amino nitrogen (AN) to total (TN) nitrogen contents (AN/TN) in commercial hydrolysates are routinely referred to the pharmaceutical and microbiological industry, and used as indicator of the extent or depth of enzymic protein degradation. The release of AN through the enzymic hydrolysis depends on the protein substrate nature, its pretreatment, applied enzyme type as well as hydrolysis conditions, including: enzyme/substrate ratios, temperature, pH, and time [20].

Crude protein content in the spent brewer's yeast reach up to 55% on a dry matter basis, whereas this mean in bovine tendon is much more higher (92%). But due to the cell wall structure or protein specificity all of two substrates are insoluble and resistant to the proteolytic digestion. Under the physical and chemical treatment influence the protein substrates are partially denaturated and peptide bonds became more accessible for the enzymic action [20, 21]. This resulted in the rise of the hydrolysis product yield and the AN/TN ratios [20].

For the determination of antioxidative activity of hydrolysates from various origin we tried to prepare hydrolysates with comparable AN/TN ratios (Table 1). Enzymic hydrolysis enables to produce hydrolysates with desired extent by varying some hydrolysis parameters [20, 21].

Casein and bovine liver are more easily degradable protein sources [9, 21] compared with spent brewer's yeast and bovine tendon. Because of we have optimized the enzyme/substrate ratios in the preparation of casein and bovine liver hydrolysates by applying fresh pancreatic tissue/substrate ratios 1:5 and 1:4 respectively. This allowed to obtain hyrolysates of casein and bovine liver with AN/TN ratios 0.42 and 0.46 respectively. Due to the pretreatment procedure for the spent brewer's yeast (three times freezing and thawing cycle) and bovine tendon (soaking in the solution with pH 14 for 10 days) we can convert insoluble material to partially soluble. Subsequent hydrolysis of two pretreated substrates was conducted using fresh pancreatic tissue/substrate ratios 1:4 as well as for bovine liver hydrolysis. The AN/TN ratios of the hydrolysates prepared from the beer and meat manufacturing by-products are comparable with the same of casein and bovine liver hydrolysates.

ABTS cation radical scavenging activity assay is commonly used for the characterization of antioxidative properties of food or food derived products [1, 2, 11]. Antioxidative activity of pancreatic hydrolysates from casein, spent brewer's yeast, bovine liver and tendon was evaluated in comparison with their unhydrolysed substrates. The results were expressed as IC_{50} concentration (protein mg/ml) where 50% of scavenging of the ABTS cation radical is obtained (Fig. 1).

Table 1. AN/TN ratios of pancreatic hydrolysates from various protein sources

Substrate	AN, %	TN, %	AN/TN
Casein	5.5 ± 0.1	13.2 ± 0.5	0.42±0.03
Spent brewer's yeast	3.9 ± 0.2	8.6 ± 0.1	0.45±0.02
Bovine liver	4.2 ± 0.3	9.2 ± 0.4	0.46±0.04
Bovine tendon	4.1± 0.2	11.5± 0.3	0.36±0.02

Values shown are the mean ±SD of triplicate determinations.

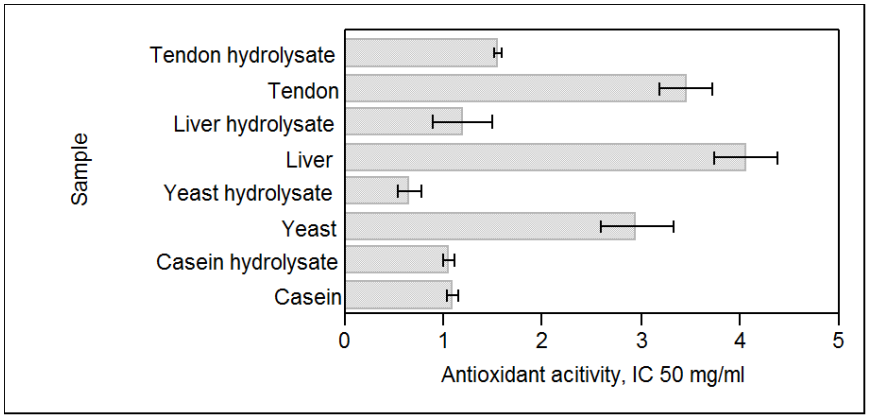

Fig. 1. IC_{50} values of ABTS cation radical scavenging activities of the pancreatic hydrolysates from casein, spent brewer's yeast, bovine liver and tendon in comparison with unhydrolised substrates

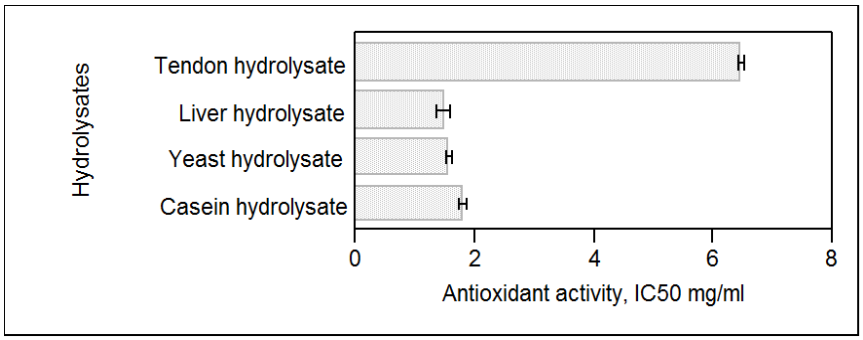

Fig. 2. IC_{50} values of DPPH radical scavenging activities of the pancreatic hydrolysates from casein, spent brewer's yeast, bovine liver and tendon

In the result of hydrolysis using fresh pancreatic tissue the ABTS cation radical scavenging activity of spent brewer's yeast, bovine liver and tendon was increased in 3.6, 3.2, and 2.1 times respectively, except casein. The IC_{50} values of ABTS cation radical scavenging activity for casein and its hydrolysate has no difference. The hydrolysates of casein, spent brewer's yeast and bovine liver had higher ability to scavenge ABTS cation radical in comparison with tendon collagen hydrolysate (p<0.05). Herewith spent brewer's yeast hydrolysate had significantly higher ABTS cation radical scavenging activity than all other obtained hydrolysates (p<0.05). The DPPH assay had revealed the same pattern of activity, however the ability of spent brewer's yeast to scavenge DPPH radical had no difference (p>0.05). The results of DPPH assay are shown in Figure 2.

Spent brewer's yeast treated with commercially available enzyme complex preparation Flavourzyme has exhibited intense radical scavenging activities in both ABTS and DPPH assays [8]. The IC_{50} values of this hydrolysate in ABTS and DPPH assays were 0.9 mg/ml and 1.9 mg/ml respectively. As shown in Figure 2 the hydrolysate prepared from spent brewer's yeast using fresh pancreatic tissue as an enzyme source had the IC_{50} values comparable with the same (0.6±0.1 mg/ml and 1.33±0.05 mg/ml). Jung E.Y., *et al*. demonstrated that intense radical scavenging activity of yeast hydrolysate was correlated with the higher concentration of cyclic dipeptide Cyclo-His-Pro and influenced on glucose oral tolerance [8].

CONCLUSIONS

The spent brewer's yeast, bovine liver and tendon collagen are the promising raw material for the preparation of enzymic hydrolysates as well as milk casein. In comparison to casein and other semipurified protein sources the utilization of the by-products or wastes from beer and meat manufacturing in the production of protein hydrolysates will have lower cost. Furthermore it will help to promote their efficient recovery and producing highly valuable products as well to design products with health benefits including antioxidant properties.

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