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Inhibition of Lipid Oxidation in Cooked Beef Patties by Hydrolyzed Potato Protein Is Related to Its Reducing and Radical Scavenging Ability†

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Protein hydrolysates were prepared by limited alcalase hydrolysis (0.5, 1, and 6 h, corresponding to degrees of hydrolysis of 0.72, 1.9, and 2.3, respectively) of heat-coagulated potato protein. The hydrolysates were characterized for peptide composition, ferric reducing/antioxidant power (FRAP), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity, and Fe^{2+}- and Cu^{2+}-chelation capacity. Hydrolyzed and intact proteins were formulated (4%, w/w) into beef patties to determine in situ antioxidant efficacy. Thiobarbituric acid-reactive substances (TBARS) and peroxide value (PV) formed in cooked and PVC-packaged patties during storage (4 °C, 0−7 days) were analyzed. Hydrolysis increased the protein solubility by 14−19-fold and produced numerous short peptides (<6 kDa). The FRAP values of the protein sample (23 μmol/g) increased markedly after hydrolysis but were similar between the three hydrolysates (597−643 μmol/g). Similarly, the ABTS radical-scavenging activity also was increased by hydrolysis and was the greatest for the 1-h hydrolysate. Hydrolysis increased the Cu^{2+}-chelation activity but decreased the Fe^{2+}-chelation ability of the protein. The production of PV in patties after 7 days of storage was lowered 44.9% and 74.5% ($P < 0.05$), and that of TBARS was reduced 40.9% and 50.3% ($P < 0.05$), by intact and hydrolyzed proteins, respectively.

KEYWORDS: Potato protein; protein hydrolysates; antioxidants; lipid oxidation; meat

INTRODUCTION

Potato protein concentrate, a byproduct of the starch industry, is used primarily as a protein source in animal feed. During the process of protein recovery from starch extraction, the heat coagulation treatment results in almost a total loss of solubility and diminishment of functional properties of proteins (*1*, *2*). Nevertheless, because potato protein has a high nutritional value (*3*) and a balanced hydrophilic/hydrophobic amino acid profile (*4*), it is of interest to convert the insoluble protein into functionally active, value-added ingredients for food applications.

Enzymic hydrolysis represents a possible means to produce functional polypeptides from potato protein. Limited peptide cleavage under controlled conditions has been employed to produce functionality-improved or bioactive peptides from a variety of low-solubility proteins (*5*). Increased solubility, water-binding capacity, emulsifying activity, and foaming capacity are common consequences of hydrolysis for plant proteins, e.g., soy and nut proteins (*6*−*8*). The functionality improvements are attributed to increased exposures of reactive amino acid side chains or hydrophobic patches and the production of peptide fragments (*9*, *10*).

In addition to their established technological functions, some hydrolyzed proteins exhibit biological activities in vitro, e.g., inhibiting angiotensin I converting enzyme (*11*), modulating mineral absorption (*12*), and suppressing microbial growth (*13*). Another important property of hydrolyzed proteins is their potential antioxidant activity, which has not received much attention until recently. For example, hydrolyzed egg albumin (*14*), casein (*15*), whey protein (*9*, *16*), soy protein (*17*), elastin (*18*), gelatin (*19*), and myofibrillar protein (*20*) have been shown to inhibit oxidation of bulk lipid or free unsaturated fatty acids in model systems.

The main component of potato protein, i.e., patatin, and a potato protein hydrolysate prepared by Amano P and pancreatin treatments were reported to exhibit antioxidant activity in vitro (*21*, *22*). However, hydrolyzed potato protein has not been used as a functional food ingredient nor has it been evaluated as a potential antioxidant for food quality preservation. The purpose of the present study was to demonstrate the in situ antioxidant activity of potato protein hydrolysates. This was accomplished through the peroxide and thiobarbituric acid assays of cooked beef patties formulated with the protein hydrolysates. Moreover, the ferric reducing/antioxidant power, the radical cation-scavenging activity, and metal ion chelating capacity of the hydrolysates were analyzed to elucidate their possible antioxidant mechanism.

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Table 1. Amino Acid Composition of Potato Protein

	g/16 g of N			percentage (%)		
amino acid	AVEBE data[a]	HCl-hydrolyzed PP[b]	heated PP[c]	AVEBE data[a]	HCl-hydrolyzed PP[b]	heated PP[c]
lysine	7.8	8.3	4.5	7.29	7.47	6.10
methionine	2.4	2.8	1.2	2.24	2.52	1.63
cystine	1.6	1.6	1.2	1.50	1.44	1.60
tryptophan	1.4	1.8		1.31	1.62	
threonine	6.1	5.7	3.6	5.70	5.13	4.84
valine	7.1	8.0	4.2	6.64	7.20	5.69
leucine	10.4	11.1	5.6	9.72	9.99	7.59
isoleucine	6.1	6.8	4.3	5.70	6.12	5.80
phenalylanine	6.5	6.2	4.6	6.08	5.58	6.29
tyrosine	5.7	6.1	3.8	5.33	5.49	5.15
histidine	2.2	2.4	1.3	2.06	2.16	1.73
arginine	5.0	5.5	4.2	4.67	4.95	5.69
glycine	4.8	4.9	5.0	4.49	4.41	6.79
alanine	5.0	4.7	3.5	4.67	4.23	4.77
aspartic acid	12.5	13.0	10.3	11.68	11.70	13.93
glutamic acid	11.5	11.3	9.3	10.75	10.17	12.58
proline	5.1	5.1	4.0	4.77	4.59	5.39
serine	5.8	5.8	3.3	5.42	5.22	4.44

[a] AVEBE data: AVEBE ba, Veendam, The Netherlands. [b] HCl-hydrolyzed potato protein (PP): Hughes, B. P. *Br. J. Nutr.* **1958**, *12*, 188–195. [c] Heated PP heated at 100 °C for 15 min: Wojnowska, I.; et al. *J. Food Sci.* **1981**, *47*, 167–172.

MATERIALS AND METHODS

Materials. Potato protein concentrate was obtained from AVEBE ba (Veendam, The Netherlands). The dry protein powder contained 80% protein, and the amino acid composition is shown in **Table 1**. Alcalase (endoproteinase from *Bacillus licheniformis*) was obtained from Novo Nordisk Biochem Inc. (Franklinton, NC). Ferrous sulfate ($FeSO_4 \cdot 7H_2O$), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), leucine, Trolox, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,4,6-trinitrobenzenesulfonic acid dehydrate (TNBS) were purchased from Sigma Chemical Co. (St. Louis, MO). Thiobarbituric acid (TBA) was purchased from ICN Biomedicals Inc. (Aurora, OH), and sodium dodecyl sulfate (SDS) was obtained from Bio-Rad Laboratories (Hercules, CA). All other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ) and were at least reagent grade. Vacuum-packaged fresh ground beef logs (1.36 kg each) were purchased from a local meat purveyor. The ground meat contained 20.4% fat and 17.7% protein (per product label) and was at least 10 days prior to the pullout date.

Preparation of Potato Protein Hydrolysates. Intact potato protein (4% w/v aqueous solution, pH adjusted to 8.0) was hydrolyzed with alcalase for 0.5, 1.0, and 6.0 h. The hydrolysis temperature was maintained at 50 °C, and the enzyme:protein substrate ratio was 1/100. After hydrolysis, the pH of the broths (6.2−6.7) was adjusted to 7.0 with 1 N NaOH and then heated at 80 °C for 15 min to inactivate alcalase (*9*). The hydrolysates were freeze-dried, pulverized, placed in sealed bottles, and stored at 4 °C before use.

Degree of Hydrolysis (DH). DH was determined by assaying free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) according to Alder-Nissen (*23*). The content of free amino groups in samples was expressed as leucine amino equivalents, based on the equation of leucine standard curve generated. The DH of hydrolyzed potato protein was calculated by using the following equation:

$$\% \text{ DH} = [(h_s - h_o)/(h_t - h_o)] \times 100\%$$

Here h_s and h_o represent respectively the amino concentrations of hydrolyzed and nonhydrolyzed potato protein and h_t represents the total amino concentration of potato protein as measured by completely hydrolyzing the potato protein with 6 N HCl. The nonhydrolyzed protein solution containing no enzyme was set as 0% DH.

Protein Solubility. Intact and hydrolyzed potato proteins were suspended (4%) in distilled water, and the pH was adjusted to 7.0 with 0.1 N NaOH/HCl. The suspensions were centrifuged at 1800*g* for 15 min, and the protein concentration of the supernatant was measured by the Biuret method (*24*). Protein solubility (%) was defined as the protein concentration in the supernatant divided by the protein concentration of the original suspension then multiplying by 100.

Electrophoresis. Nonhydrolyzed potato protein and its hydrolysates were characterized by SDS−polyacrylamide gel electrophoresis (SDS−PAGE) as described by Laemmli (*25*) with slight modifications. The protein samples were suspended in the SDS−PAGE sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.125 M Tris-HCl, pH 6.8), heated at 100 °C for 3 min, and then centrifuged with a Centrific benchtop centrifuge (Fisher Scientific, Fair Lawn, NJ) at 1800*g* for 10 min to remove particulates. Electrophoresis was done with a 15% acrylamide resolving gel and a 3% acrylamide stacking gel, using a Mini-PROTEAN 3 Cell electrophoresis system (Bio-Rad Laboratories, Hercules, CA). Aliquots of 20 μL of the supernatants were loaded per well on the gel. A protein standard, consisting of a myosin heavy chain (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa) (Bio-Rad Laboratories, Hercules, CA), was also run. Molecular weights (MW) of sample protein bands were estimated from the regression line of protein migration distances versus logarithm MW of the proteins.

Reduction Potential. The ability of hydrolyzed potato protein to act as a reducing compound was assessed by means of the ferric reducing/antioxidant power (FRAP) assay (*26*). This method is based on the principle that the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) at low pH can form a colored ferrous−tripyridyltriazine complex that absorbs maximally at 593 nm. Working FRAP reagent was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution. A 1000 μmol/L $FeSO_4 \cdot 7H_2O$ aqueous solution was used for calibration. Absorbance readings were taken every 15 s up to 5 min, and the FRAP values, expressed as μmol/g of sample, were calculated (*26*).

Radical-Scavenging Activity (RSA). The analysis was performed using an ABTS decolorization assay (*27*). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stocking solution (7 mM) with 2.45 mM (final concentration) of potassium persulfate. The mixture was left in the dark at room temperature for 12−16 h before use. The $ABTS^{\bullet+}$ solution was diluted with 0.2 M sodium phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Radical-scavenging activity (RSA) of protein samples was measured by mixing 10 μL of the samples with 990 μL of diluted $ABTS^{\bullet+}$ solution, and the absorbance reading was taken at 1 min intervals for a total of 10 min. A standard RSA curve was also prepared by reacting 10 μL of a series of concentrations of Trolox (50, 100, 250, 500, 1000 μM) with 990 μL of diluted $ABTS^{\bullet+}$ solution. The degree of RSA of the protein samples

was calculated on the basis of the Trolox standard curve and was expressed as Trolox equivalent antioxidant capacity (TEAC, μM).

Metal Ion-Chelating Activity. The ability of potato protein hydrolysate to chelate the prooxidative transitional metal ions Cu^{2+} and Fe^{2+} was investigated according to respectively Saiga et al. (*20*) and Guo et al. (*28*) with slight modifications. For the Cu^{2+} chelation experiment, 1 mL of $CuSO_4$ was mixed with 1 mL of pyridine and 20 μL of pyrocatechol violet. After the addition of 1 mL of 4% potato protein solutions, the disappearance of the blue color, due to dissociation of Cu^{2+} (by polypeptides), was monitored by determining the absorbance at 632 nm. For the Fe^{2+} chelation experiment, 1 mL of 20 μM $FeCl_2$ was mixed with 2 mL of 50 μM ferrozine, which produces a chromaphore that absorbs strongly at 562 nm. After the addition of 0.5 mL of 4% protein solutions, the color change, due to dissociation of Fe^{2+}, was measured spectrophotometrically at 562 nm. The Cu^{2+}- and Fe^{2+}-chelating activity by potato protein or hydrolysates were calculated as [1 − (sample solution absorbance/blank solution absorbance)] × 100.

Preparation of Meat Patties. This experiment was replicated twice each with a new batch of ground beef with identical proximate composition. In each replication, three beef pattie formulations, all with 1.5% (w/w) added NaCl, were prepared: (1) control (with no potato protein); (2) with 4% nonhydrolyzed potato protein; (3) with 4% 1-h hydrolyzed potato protein. The 1-h hydrolysate was selected because it seemed to produce the greatest antioxidant effects overall on the basis of the reduction potential, radical-scavenging, and metal-chelating data (described later). For each formulation treatment, the mixture was prepared by blending for 5 min with Kitchen Aid mixer (St. Joseph, MI). Duplicate patties of 50 g each were shaped by hand into an approximately 8 cm (diameter) × 1 cm (thickness) geometry. The patties were cooked on an open electric broiler (Toastswell Co., St. Louis, MO) with a heated metal plate (300 °C) by flipping every 3 min until the patties' internal temperature reached 73 °C (measured with a thermocouple). Each pattie was weighed before and after cooking to measure cooking loss. After being cooled to room temperature, patties were placed in styrene foam trays, overwrapped in an oxygen permeable poly(vinyl chloride) film, and stored at 4 °C for 0, 1, 3, and 7 days.

Peroxide Values. Peroxide values of cooked beef patties were measured according to the AOCS standard procedure (*29*). Specifically, patties were finely chopped by blending in a micro Waring blender for exactly 30 s. A 5.0-g sample was then mixed with 30 mL of acetic acid−chloroform solution (3:2). The slurry was gently swirled to extract lipid, and 0.5 mL saturated potassium iodine solution was then added. After reaction for 1 min with occasional shaking, 30 mL of distilled H_2O and 0.5 mL of 0.1% starch solution were added. The mixed solution was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until the intense blue color disappeared. A control blank (without meat sample) was also analyzed alongside. The peroxide value (PV) was calculated as

$$\text{PV (mequiv/kg)} = [(S - B) \times 1000 \times N]/W$$

where S and B are the volume (mL) of sodium thiosulfate solution consumed by the sample and by the blank, respectively, N is the concentration (N) of sodium thiosulfate solution, and W is the sample weight (g).

Thiobarbituric Acid-Reactive Substances (TBARS). Lipid oxidation was evaluated by TBARS according to Sinnhuber and Yu (*30*) with slight modifications. Meat patties from each treatment were individually chopped by blending in a micro Waring blender for exactly 30 s. An accurately weighed finely chopped meat sample (ca. 0.4 g) was placed in a 25 mL screw cap test tube, and 3 drops of antioxidant solution (BHA), 3 mL of TBA solution, and 17 mL of TCA-HCl solution were subsequently added. The mixture was vortexed, flushed with nitrogen gas, and then heated in boiling water (100 °C) for 30 min. After being cooled to room temperature, a 5 mL aliquot of the suspension was mixed by vortexing with 5 mL of chloroform for 1 min, followed by centrifugation with a Centrific benchtop centrifuge (Fisher Scientific, Fair Lawn, NJ) at 1800*g* for 10 min. The upper phase (aqueous) was centrifuged again for 10 min under the same condition, and the absorbance of the supernatant was read at 532 nm. The TBARS value, expressed as mg of malonaldehyde/kg of muscle sample, was calculated by using the following equation:

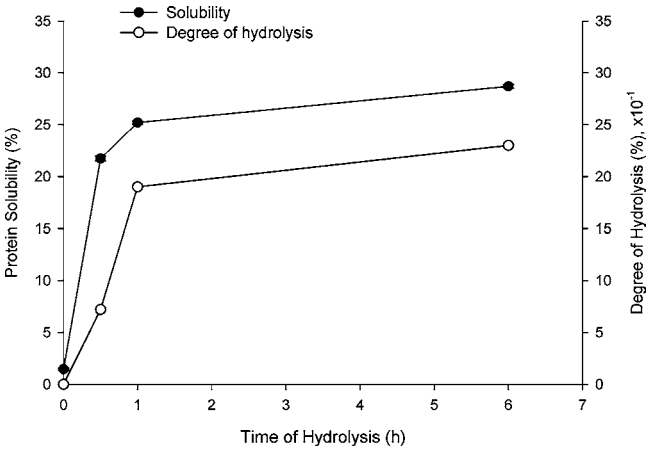

Figure 1. Solubility and degree of hydrolysis of hydrolyzed potato protein. The sizes of error bars are within the symbols.

$$\text{TBARS (mg/kg)} = (A_{532}/W_s) \times 9.48$$

Here A_{532} is the absorbance (532 nm) of the assay solution, W_s is the meat sample weight (g), and "9.48" is a constant derived from the dilution factor and the molar extinction coefficient (152 000 M^{-1} cm^{-1}) of the red TBA reaction product.

Statistical Analysis. All antioxidant assays were carried out in triplicate unless indicated otherwise. The in situ evaluation of antioxidative hydrolysates with cooked beef patties (PV, TBARS) was replicated two times with different batches of raw meat. Data were subjected to analysis of variance (ANOVA). When treatment effects were found significant ($P < 0.05$), the differences between means were identified by Tukey's test.

RESULTS

Hydrolysis. As expected, the degree of hydrolysis of potato protein increased with the alcalase incubation time, showing 0.72, 1.9, and 2.3% after 0.5, 1, and 6 h, respectively (**Figure 1**). The degree of hydrolysis corresponded to the protein solubility change, which also increased with hydrolysis time (**Figure 1**). The additional charged groups (i.e., NH_3^+ and COO^-) produced by peptide cleavage not only promoted protein−water interactions but also enhanced electrostatic repulsion between peptides, thereby increasing the protein solubility.

Electrophoretic analysis revealed a major band (I) at 42 kDa in nonhydrolyzed potato protein (0 h), which matched the MW of patatin reported in the literature (*21*, *31*) (**Figure 2**). Several other minor bands (18−25 kDa) were also present (band II), and they were presumably protease inhibitors (*31*). The patatin band almost completely disappeared after 0.5 h of the alcalase treatment, and concomitantly, a stack of small peptides (band III), with MW of 3−5 kDa, emerged. These oligopeptides, which were ostensibly dissociated patatin or patatin fragments, were degraded by alcalase upon further incubation, which was evidenced by their attenuated band intensity and a reduced bandwidth after 6 h.

Reduction Potential. The FRAP screening assay showed a time-dependent production of the reducing power ($Fe^{3+} \rightarrow Fe^{2+}$) of the potato protein hydrolysates (**Figure 3**). This phenomenon, which is characteristic of the assay, has been previously reported on phenolic antioxidants (*26*). While the increase in the FRAP value was highly significant ($P < 0.05$) following hydrolysis, the FRAP was insensitive to the hydrolysis time. The 1-h

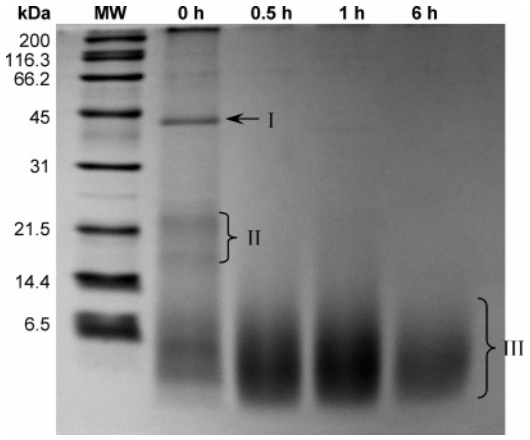

Figure 2. SDS–PAGE of intact (nonhydrolyzed) and hydrolyzed potato protein. I = patatin, II = trypsin inhibitors, and III = small peptides.

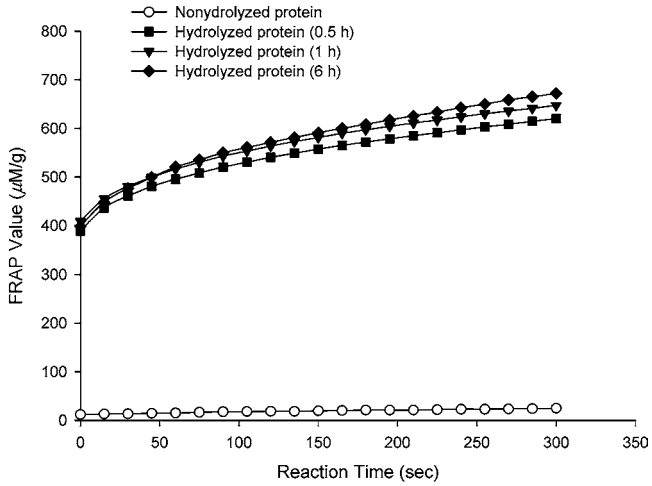

Figure 3. Effect of hydrolysis time on the FRAP value of potato protein (4%, w/v).

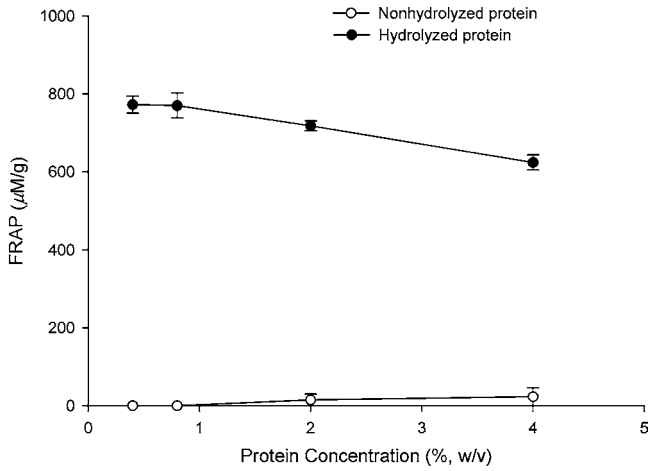

Figure 4. FRAP values of potato protein hydrolysates at various protein concentrations. The values were obtained from a 4-min reaction time of the 1-h protein hydrolysate.

hydrolysate was further analyzed at different protein concentrations to establish its efficacy. For example, the FRAP values obtained from the 4-min (240s) assay decreased slightly ($P < 0.05$) with the protein concentration but remained constant ($P > 0.05$) for the intact protein sample (**Figure 4**).

Radical-Scavenging Activity. The $ABTS^{\bullet+}$ radical cation scavenging assay is applicable to both lipophilic and hydrophilic compounds and has been widely used for the assessment of

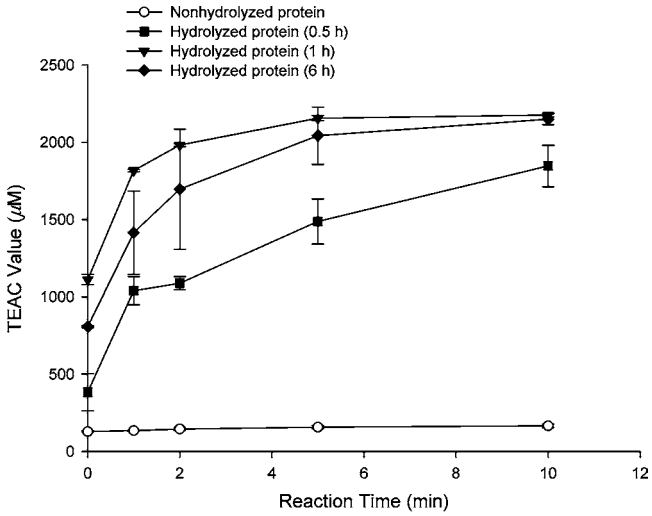

Figure 5. Effect of hydrolysis time on the radical scavenging activity of potato protein (4%, w/v). The activity was expressed as Trolox equivalent antioxidant capacity (TEAC).

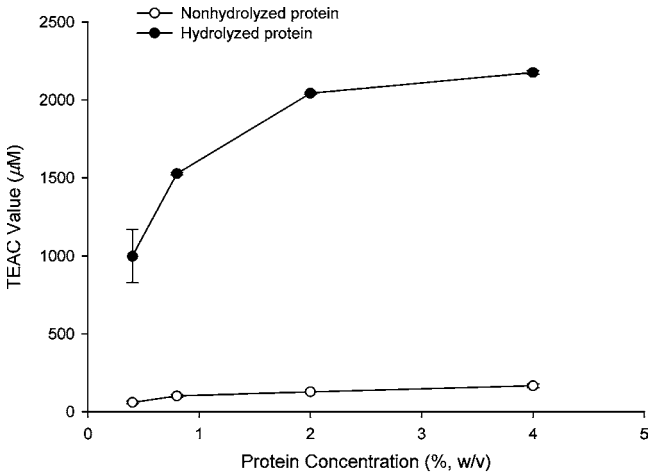

Figure 6. TEAC values of potato protein hydrolysates at various protein concentrations. The values were obtained from a 10-min reaction time of the 1-h protein hydrolysate.

antioxidant activity (*32*). The radical-scavenging activity of potato protein, expressed as Trolox equivalent antioxidant capacity (TEAC), increased drastically after hydrolysis (**Figure 5**). As with the FRAP analysis, reaction of peptides with the $ABTS^{\bullet+}$ radical was time dependent, requiring at least 5 min to reach a plateau. The 1-h hydrolysis produced the greatest increase in the TEAC value. Therefore, this hydrolysate was further assayed for protein concentration effect. As displayed in **Figure 6**, the radical-quenching capacity increased with increasing protein concentrations until a 2% concentration was reached.

Metal-Chelating Activity. Nonhydrolyzed potato protein was capable of quenching both copper and ferrous ions. Hydrolysis changed the chelating power. The relative Cu^{2+}-chelation activity of the protein sample increased from 24.8% (nonhydrolyzed) to 43.0% after 6 h of hydrolysis. However, hydrolysis weakened the Fe^{2+}-chelating effect, showing a 2-fold reduction after 6 h of hydrolysis (**Figure 7**).

Cooking Loss of Meat Patties. Both nonhydrolyzed and hydrolyzed (1 h) protein samples tended to reduce the cooking loss of patties (**Table 2**). However, only the hydrolyzed protein had the significant effect ($P < 0.05$). The result was in good agreement with previous findings on hydrolyzed soy and whey

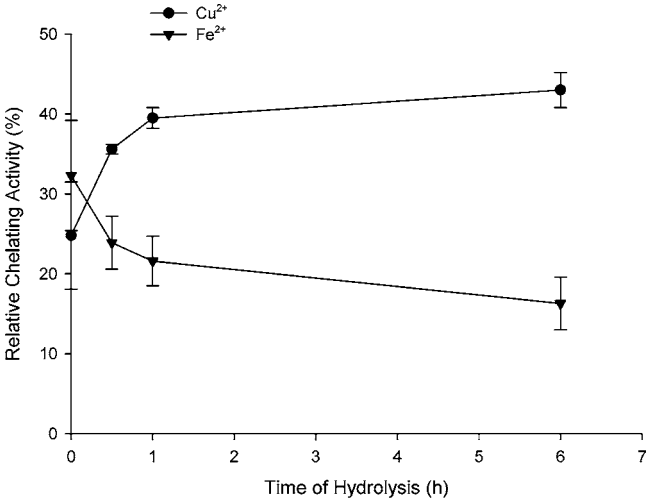

Figure 7. Copper (Cu^{2+}) and iron (Fe^{2+}) chelating activity of hydrolyzed potato protein.

Table 2. Cooking Loss (%) of Ground Beef Patties with Potato Proteins and Their Hydrolysates

treatment[a]	wt loss (%)	% reducn over control
control	33.7[b]	
nonhydrolyzed protein	32.3[b]	4.2
hydrolyzed protein	28.9[c]	14.2[d]

[a] Protein addition level = 4%, w/w; hydrolyzed protein = 1 h of hydrolysis. [b,c] Different letters in this column indicate significant ($P < 0.05$) differences between means. [d] Indicates significant reduction ($P < 0.05$).

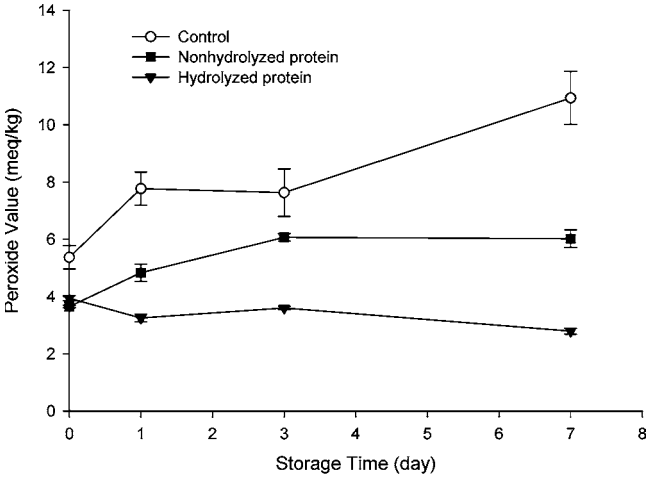

Figure 8. Inhibition of peroxide formation in cooked beef patties by potato proteins (4%, w/v) during refrigerated storage.

proteins applied to ground meat (*10*, *33*). Because hydrolysis dissociated potato protein into subunits and produced additional polar and charged groups, it allows stronger protein−water interactions and a greater water-holding capacity of the protein matrix in meat.

Inhibition of Lipid Oxidation in Meat Patties. The inhibitory effect of potato protein on cooked beef patties was remarkable. Control patties after cooking had a PV value of 5.4 mequiv/kg, and with the presence of intact or hydrolyzed potato proteins, the peroxide level was lowered by 27% (**Figure 8**). Over the 7-day storage period, the PV value in protein-free control patties was doubled; with intact potato protein, the PV production remained largely suppressed ($P < 0.05$), and with hydrolyzed protein, the PV formation was completely inhibited.

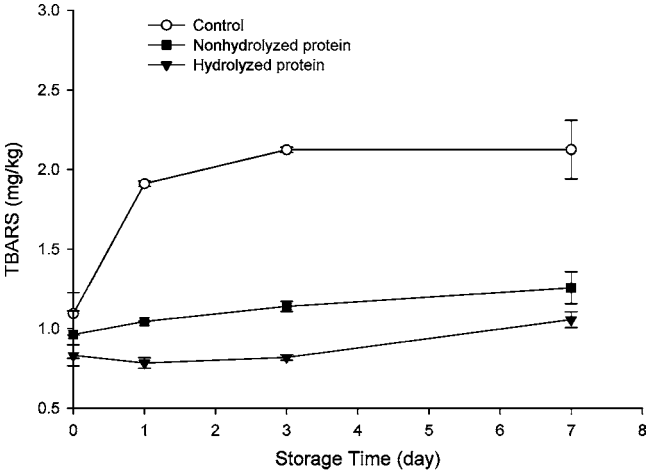

Figure 9. Inhibition of TBARS formation in cooked beef patties by potato proteins (4%, w/v) during refrigerated storage.

The peroxide contents of patties treated with nonhydrolyzed protein (6.0 mequiv/kg) and hydrolyzed protein (2.8 mequiv/kg) at the end of storage, when compared with that of control patties (10.9 mequiv/kg), represented 44.9% and 74.5% oxidation inhibitions ($P < 0.05$).

Because peroxides newly formed were unstable and degraded quickly to form secondary oxidation products (*34*), malonaldehyde-equivalent carbonyl compounds (TBARS) were analyzed as well. As depicted in **Figure 9**, both nonhydrolyzed and hydrolyzed potato proteins inhibited TBARS formation in patties during storage. Compared to the control sample at the end of storage (7 days), the production of TBARS was lowered by 40.9% and 50.3% ($P < 0.05$) by nonhydrolyzed and hydrolyzed protein samples, respectively. The additional inhibitory effect due to Alcalase hydrolysis was also significant ($P < 0.05$).

DISCUSSION

The strong reducing power of hydrolyzed potato protein (HPP) may be attributed to the increased availability of hydrogen ions (protons and electrons) due to peptide cleavages. Donation of protons could occur through specific side-chain groups or through the specific peptide structure. Likewise, the increased radical scavenging ability of HPP probably resulted from structural changes leading to more effective radical quenching and stabilization. The increase in protein solubility (to 22−29%, $P < 0.05$) following alcalase hydrolysis would suggest an increased concentration of reactive (mobile) peptides. Yet, the pronounced antioxidant effect of nonhydrolyzed potato protein (NHPP), even in its minimal soluble state (1.5%), as shown by all the three antioxidant indexes, seemed to indicate that reactions of NHPP with oxidizing peroxides or free radicals likely occurred on the surface of proteins or protein aggregates. Patatin is the main constituent in nonhydrolyzed potato protein. Its scavenging capability of hydroxyl radical in vitro has previously been reported (*21*).

The amino acid sequence of peptides was likely an important factor that contributed to the overall antioxidant activity of HPP in aqueous solutions and in cooked beef patties. Short chain potato peptides with characteristic amino acid compositions or specific sequences presumably reacted with oxidizing agents to form stable compounds, thereby enhancing the oxidative stability of beef patties during cooking and storage. The preponderance of small peptides (<6 kDa) in HPP indicated that hydrolytic components with antioxidant activity were mostly small molecules which were probably preferred targets of

radicals. The inverse relationship between the reducing power and the protein concentration suggested that diffusivity of peptides could be a limiting factor for the antioxidative efficacy of HPP. Furthermore, certain particular free amino acids, which were released during hydrolysis, probably were involved in the reduced lipid oxidation in cooked patties. It has been reported that tryptophan and cysteine residues, which are present in potato protein and can be released in the process of hydrolysis, acted as strong antioxidants (*35*). Certain other amino acids, particularly methionine, histidine, and lysine, which are fairly abundant in potato protein, have also been shown to inhibit lipid oxidation in model systems (*36*, *37*).

The ability to sequester metal prooxidants is a common attribute of many short peptides, such as carnosine, fragments of myofibrillar proteins, and synthetic oligopeptides (*20*, *38*). The enhanced Cu^{2+} chelation by HPP ($P < 0.05$), when compared with NHPP, likely resulted from stronger electrostatic interaction due to an increased concentration of carboxylic groups (COO^-). A greater affinity of peptides in HPP for Cu^{2+} can also result due to increased exposures of certain particular amino acids (e.g., histidine) or patches which were occluded in intact proteins. On the other hand, the weakened Fe^{2+}-chelating capability ($P < 0.05$) of potato protein following hydrolysis would indicate a different peptide−metal binding mechanism. It seemed that hydrolysis disrupted to the iron-binding structure in intact potato protein. Different protein-binding properties have been reported for Cu^{2+} and Fe^{2+} in site-specific, metal-catalyzed oxidation of proteins (*39*).

Although subjected to debate, the heme or heme complexes, e.g., metmyoglobin and hemin, are considered stronger prooxidants than nonheme or free iron in the initiation of lipid peroxidation in cooked meat (*40*, *41*). It was shown that the prooxidant activity of metmyoglobin increased sharply when the metmyoglobin solution was heated to 60−70 °C, and this was attributed to the exposure of catalytic heme groups due to protein denaturation (*40*). Because the release of iron from the heme complex by cooking was negligible (*40*) and the concentration of free iron in meat was low (e.g., 0.2−2.5 μg/g for poultry muscles) (*42*), the prooxidant activity of heme would likely exceed that of free iron in cooked meat. Therefore, the strong suppression of lipid oxidation (both PV and TBARS, $P < 0.05$) by hydrolyzed potato protein in cooked beef patties, even though its iron chelation capability was diminished, must have resulted from other mechanisms, i.e., an improved reducing and radical scavenging capability or copper-sequestrating activity. It was also plausible that short peptides derived from potato proteins may interact with heme iron or heme proteins in producing the overall strong antioxidative power exhibited by the protein hydrolysate.

Conclusions. Alcalase-hydrolyzed potato protein was able to improve the oxidative stability of cooked ground beef during refrigerated storage. The antioxidant activity was most likely related to the reducing and radical scavenging ability of peptides and/or free amino acids released and possibly also other unknown factors. The role of metal ion chelation in the overall antioxidative phenomenon was inconclusive. Notwithstanding, the antioxidant effect, together with the enhanced functionality (e.g., solubility and water-binding ability), would allow hydrolyzed potato protein to be utilized as value-added food ingredients in processed muscle foods.

ABBREVIATIONS USED

NPP = nonhydrolyzed potato protein; HPP = hydrolyzed potato protein; TBARS = thiobarbituric acid reactive substances; FRAP = ferric reducing/antioxidant power; PV = peroxide value; ABTS = 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

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