VALUE ADDED APPLICATIONS FOR PLASMA PROTEINS FROM THE BEEF PROCESSING INDUSTRY

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Introduction

Among the by-products of the meat industry, blood is a highly proteinaceous material that is utilized in various ways. In cattle about 6% of the live weight of the animal is blood and its effective removal during slaughter is required for improved colour and keeping qualities of the carcass. According to researchers, approximately 50% of the animal's blood can be collected during bleeding in the slaughter operation and the remaining is retained in the capillary system throughout the body. Blood intended for further processing or use is collected hygienically and then an anticoagulant, usually trisodium citrate (0.2% with or without water) is added. Processing of blood includes centrifugation to separate light plasma (52-70%) from heavy erythrocytes and then chilling to 2°C, if not previously done, to minimize bacterial growth. Whole or fractionated plasma may be frozen as flakes and distributed or stored in a frozen stage. The process patented by Harimex B.V. of the Netherlands, selectively precipitates (using cryo-precipitation) fibrinogen and thrombin from the plasma fraction of blood obtained from the animal processing industry. This product is marketed as Fibrimex® and has been well utilized as a natural binder for whole muscle processing. Separation of these two proteins only, leaves behind plasma rich in albumin and globulin, which is referred as defibrinated plasma.

Various food applications for plasma proteins have been reported. Dried plasma has been used as a protein supplement in meat products (*e.g.*, sausages and puddings), and as an inhibitor for endogenous proteases in surimi type products from certain fish species. Fresh plasma has been used for preparation of textured meat analogs, and fractionated plasma proteins have been used as a functional ingredient. These proteins possess good functional properties such as gelation and emulsification. The cross-linking ability of major muscle proteins (especially myosin) by plasma proteins, and their protease inhibitory activity, which can enhance resistance to endogenous protease degradation, are well documented. Fractionation of plasma proteins into immunoglobins, fibrinogen and serum albumin for food or feed ingredients, pharmaceuticals or other technical uses is an established industry.

As with to other economical protein sources, plasma proteins can be used to prepare hydrolyzed proteins that have many valuable uses. Such uses include nutritional supplements, functional enhancers for composite foods, flavorants, or substrates for further reactions (*e.g.*, process flavour generation) or peptides with biological activities. We studied possible ways to add value to defibrinated plasma proteins in order to enhance their utilization. Our approach was to utilize the peptides obtained from breaking down the large proteins present in defibrinated bovine plasma (DBP). DBP contains nearly 80% protein (dwb) and is rich in all serum proteins except fibrinogen and thrombin. This way only the hydrolysable plasma proteins are included in the final product.

Enzyme-assisted hydrolysis of defibrinated bovine plasma proteins

Protein hydrolysis, or breaking down of the intact protein molecule into peptides and amino acids, can be achieved by acid-, base- or enzyme-catalysed hydrolytic reactions. Enzymes, which specifically catalyze only the hydrolysis of proteins, have been favored for hydrolytic modification of proteins which are intended for food use. Enzymatic hydrolysis has been widely applied for plant, dairy and other animal proteins and is a very useful tool for improving the utility of a protein which previously had a limited or single use.

Frozen, unhydrolysed DBP contained 6.13% (6.25'% N) protein, and is the predominant component of plasma dry matter. Serum albumin (66.7 kDa) and the globulins (α, β and γ: 42 to 200 kDa) were the principal proteins of the starting material. Enzyme-assisted hydrolysis was performed to obtain free amino acids and peptides of various chain lengths, because of the ease of reaction control and lack of undesired side reactions (*e.g.*, formation of monochloropropanal and dichloropropanal in chemical hydrolysis). Food-grade proteases (*e.g.*, Alcalase 2.4L®, Flavourzyme L™, Novo Nordisk North America, Franklinton, NC, USA) were able to hydrolyze thermally denatured (85°C, 15 min) plasma proteins with superior results obtained with Flavourzyme.

For a hydrolyzed protein intended for food applications, knowledge of molecular weight distribution is important since the peptide type/group and size dictate the potential taste (*e.g.*, bitter, sweet, umami) of the hydrolysate, volatile compounds generated during flavour generation reactions (*e.g.*, Maillard reaction), allergenicity, opioid or any other biological activities. Hydrolysed DBP (HDBP) is rich in free amino acids and peptides <6.5 kDa, depending on the degree of hydrolysis (% DH) of peptide bonds. In our work, it was found that free amino acid content increased with DH value, and hydrophobic amino acids were preferentially released during hydrolysis. The majority of the molecules in DBP hydrolysate were less than 1.4 kDa when DH was at maximum. At the termination of hydrolysis (43% DH, under optimum conditions), more than 60% of the molecules of the hydrolysate had a molecular mass of 1.04 kDa or less. Figure 1 shows the preparation of HDBP products for this study.

Potential anti-hypertensive peptides

Peptides with angiotensin I-converting enzyme inhibitory activity have been obtained from different food proteins after hydrolysis. Angiotensin I-converting enzyme (ACE) plays an important role in blood pressure regulation, and ACE inhibitors help to keep blood pressure within healthy levels when ACE is produced in excess. Peptides derived from food proteins and those that have ACE inhibitory activity are employed in designing functional foods such as antihypertensive drinks (*e.g.*, Ameel S from Calpis Co Ltd).

When DBP was hydrolyzed, the fraction containing molecules of less than 10 kDa exhibited ACE inhibiting activity. Increased activity was observed with increasing degree of hydrolysis (FIG. 2). ACE inhibiting activity of 78.9% was shown with 43% DH DBP (IC_{50} value: 1.08 mg/ml). This hydrolysate was predominantly composed of small peptides (77.7% <1040 Da).

Peptides identified from the ACE inhibiting fraction of 43% DH HDBP had sequences as given in Table 1. The sequences for the dipeptides leucine-phenylalanine (LF) and the tripeptide histidine-proline-tyrosine (HPY) are conserved within the primary structure of bovine serum albumin. There are several peptide sequences that have been identified as ACE inhibitors and those are derived from lactalbumin and lactglobulin of whey proteins. Among the peptides identified in HDBP, LF from hydrolyzed b-lactoglobulin has been reported as a potent ACE inhibitor. This shows that there is a good potential for developing plasma protein-derived peptides with ACE inhibiting activity.

Putative antioxidative peptides

Peptides obtained from food proteins may exhibit strong antioxidant activities. Antioxidants can be utilized for slowing down or suppressing oxidation reactions that are involved in the alteration of unsaturated biomolecules such as lipids, pigments and proteins. Use of antioxidants is not limited to protecting high lipid foods from flavour alteration, They can also be used for preventing nutrient losses, decreasing generation of possible reactive compounds such as peroxides, and maintaining the health benefits provided by the food. Antioxidative properties of hydrolysates were investigated because of their ability to scavenge active oxygen and lipid peroxy radicals and to chelate ferrous ions.

Antioxidant properties of HDBP (<10 kDa frac-

tion) were compared with carnosine and tocopherols, which are endogenous antioxidants found in muscle tissues. HDBP exhibited scavenging activities on hydroxyl and lipid peroxy radicals. They were able to reduce lipid oxidation in beef homogenates and had very good iron (Fe^{2+}) chelating abilities (Table 2). The combination of all these activities may provide HDBP with significant antioxidative ability in lipid rich- and even free Fe^{2+}-containing foods.

Generation of cooked meat-like aroma compounds

Endogenous peptides play a significant rôle in aroma generation by cooked meat, hydrolyzed vegetable proteins and roasted cocoa and coffee. Peptides with varying sequences could serve as precursors for the production of a variety of food aromas. Reactions between free amino groups of peptides and amino acids and reducing sugars are primarily responsible for developing aroma compounds during cooking of meat. It has also been noted that size, sequence and chemical conformation of the peptide are significant contributing factors in the production of specific aroma compounds via Maillard browning reactions.

The HDBP obtained by hydrolysis was rich in free amino groups and small peptides, which are essential for reaction flavour generation. Thermal flavour generation was carried out with D-glucose or D-ribose at 119°C (dry heat) in the presence or absence of cysteine and thiamine. Supplementary cysteine was necessary as HDBP has low levels of free S-amino acids. Under favourable conditions, free amino acids and peptides in the DBP hydrolysate developed cooked-meat-like aroma compounds due to reaction flavour generation. A set of 9 descriptors was developed by the quantitative descriptive analysis (QDA) panelists to profile the aromas of reaction mixtures prepared with glucose and ribose (FIG 3). 'Sulfurous' and 'meaty' were the dominant aroma traits, followed by 'burnt meat' and 'bouillon' notes. Both D-glucose and D-ribose reaction mixtures had cooked chicken-, liver- and pork-like notes that were of similar intensity. 'Caramel' and 'fishy' notes were detected, but had very low scores. A commercial roast beef flavour mix assessed by the same panelists had a very different QDA profile, with pronounced 'burnt meat', 'bouillon' and 'caramel' notes.

The developed flavour volatiles were isolated with pentane by simultaneous distillation extraction and then separated and identified using a gas chromatograph equipped with flame ionization, mass spectroscopic and olfactory detectors. Under GC-FID analysis, 33 peaks of volatile compounds were detected; however, only 24 were identified by GC-MS and retention indexing. There was a preponderance of sulfur containing compounds in the mixture. Addition of precursors (cysteine and thiamine) other than the reducing sugar was necessary to develop the cooked meat-like aroma. Both D-glucose and D-ribose produced more or less similar volatile profiles; only the abundance of these was different. Alkyl substituted heterocycles and *S*-compounds predominated in the tentatively identified compounds. Several of these tentatively identified alkyl substituted heterocycles and *S*-compounds have been reported in roast beef aroma. With careful manipulation of reactants, HDBP can be used to develop cooked-meat like process flavour .

The results of these studies show the potential of HDBP proteins for use as antihypertensive peptides and antioxidants, and for generation of aroma compounds that have significance in food applications.

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FIG 1. Products prepared from hydrolysis of DBP

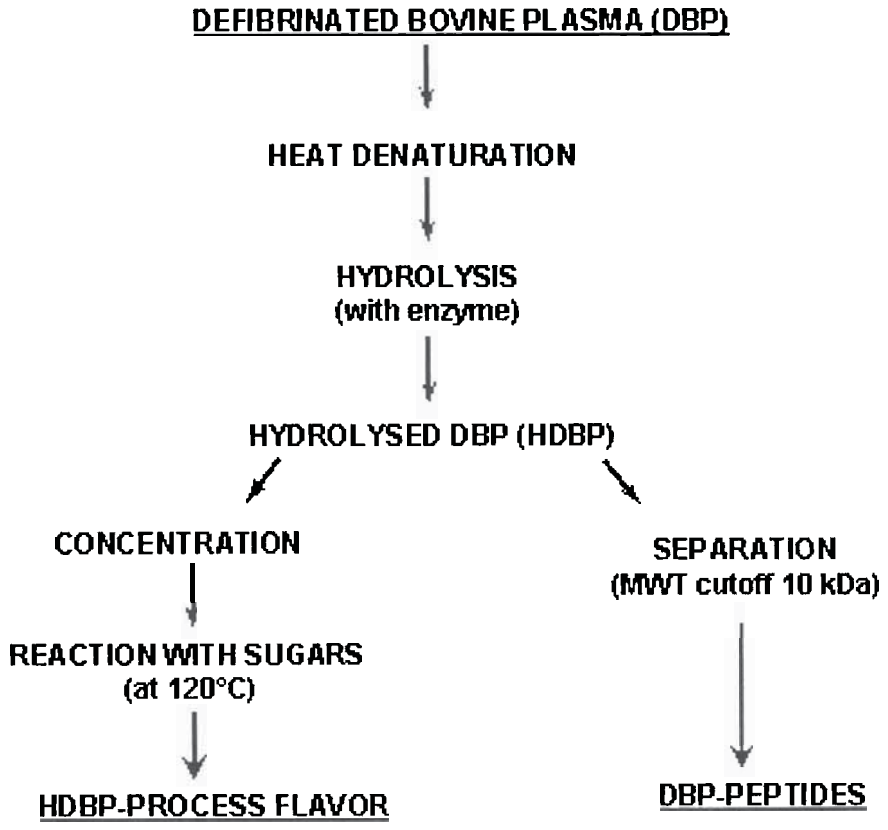

FIG 2. Percentage inhibition of ACE activity by Flavourzyme™ HDBP at different DH values (2 mg/ml samples, control had no hydrolysate or DBP and had maximum activity for ACE).

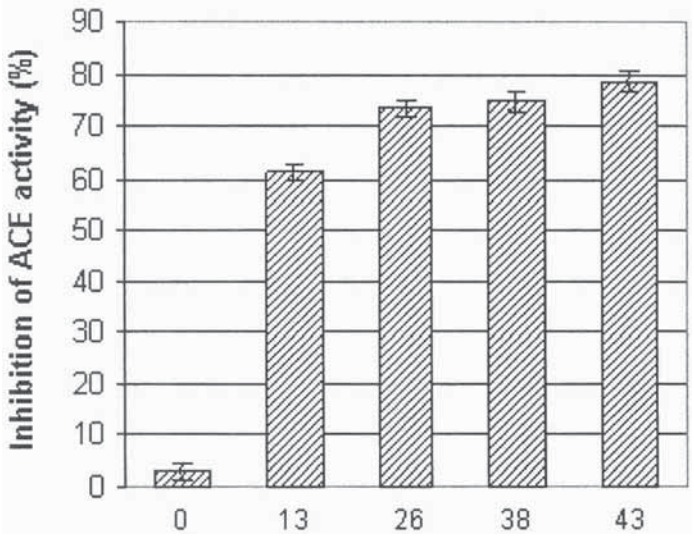

FIG 3. Radar chart for quantitative descriptive analysis. [Glucose and ribose are reaction mixtures of HDBP prepared with D-glucose and D-ribose, respectively. RBF is a commercial roast beef flavor].

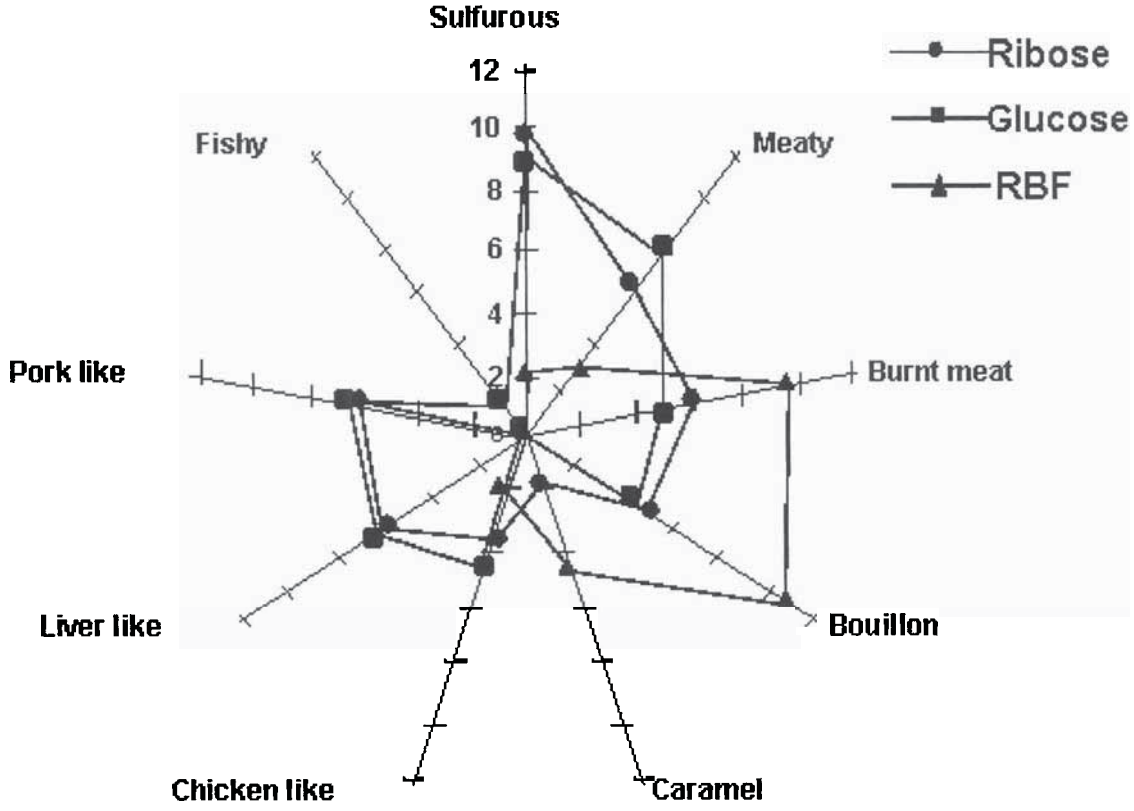

Table 1. Sequence and molecular mass of peptides identified from ACE inhibitory fraction of 43% DH DBP hydrolysate and their matching peptide fragment in bovine serum albumin (BSA).

Peptide	Estimated molecular mass[a]	Matching peptide fragment in BSA[b]
H(L/I) [histidine-leucine/isoleucine]	269	
SPY [serine-proline-tyrosine]	366	
YPH [tyrosine-proline-histidine]	416	-
HPY[histidine-proline-tyrosine]	416	f(145-147)
(L/I)F [leucine/isoleucine-phenylalanine]	279	f(69- 70) & f(503-504)[c]
HPGH [histidine-proline-glycine-histidine]	428	
GYP [glycine-tyrosine-proline]	336	

[a] MH^+, m/z by using MALDI-TOF-MS and LC-MS-MS

[b] Bovine serum albumin

[c] Only LF sequence matched

Table 2. Antioxidant activity of HDBP.

Sample[a]	Scavenging of peroxy radicals[b]	Inhibition of oxidation in beef homogenate[c]	Scavenging of hydroxyl radicals[d]	Iron chelation[e]
Unhydrolysed	28.0	27.6	10.0	60.0
Hydrolysed				
13% DH	60.2	28.8	48.0	95.0
26% DH	43.0	40.0	68.0	90.0
43% DH	48.5	45.0	52.0	100
Carnosine	45.8	59.0	84.0	-
α-Tocopherol	70.0	68.0	90.5	-

[a] DBP and HDBP 10 mg/ml, carnosine and α-tocopherol 2mg/ml

[b] Percentage inhibition of linoleic acid hydroperoxide formation in linoleic acid emulsion (40°C, 3 h)

[c] Percentage inhibition of thiobarbituric acid reactive substances development in beef homogenate (37°C, 3h)

[d] Extent of hydroxyl radical scavenging (as percentage) in deoxyribose-ascorbic acid model system