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Utilization of Spent Hens as a Flavoring Base: 1. Preparation and Characteristics of Spent Hen Meat Enzymatic Hydrolysate

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Abstract: Studies were conducted to investigate the hydrolyzing efficacies of papain (P), bromelain (B), trypsin (T), and *Aspergillus oryzae* protease (A) on ground spent hen breast meat. Ground breast meat, water, and enzyme were mixed and hydrolyzed in a water bath at 50°C for four hours. The enzyme activity was terminated by placing the reaction bottles in boiling water for 15 min. Optimal pH values, enzyme concentrations, and sensory characteristics of enzymatic hydrolysates were determined for each enzyme and enzyme combinations. Results indicated that pH 5.0-7.0 was the optimal range for all treated enzymes. Papain and *Aspergillus oryzae* protease at the 1.0 % (w/w, based on weight of raw meat) level showed the highest ($P<0.05$) hydrolysis efficacy, followed by 0.5% (w/w) and 0.1% (w/w). Considering the enzyme cost factor, the hydrolysates from A, P+A, P+B, and P+A+B were selected for sensory study. Undiluted enzymatic hydrolysates showed higher ($P<0.05$) scores in chickeny, meaty, mouth feeling, bitterness, and umami sensory attributes compared with the controls but there were no ($p<0.05$) difference on all sensory attributes among those enzyme hydrolysates. Generally, P+A showed the highest acceptability in sensory attributes among treatments followed by P+A+B, A, and P+B. Considering enzyme efficacy, cost, and sensory attributes, P+A and P+A+B were recommended for spent hen meat hydrolysate preparation.

Key words: Spent hen, papain, bromelain, trypsin, *Aspergillus oryzae* protease, proteolytic activity, sensory attributes

Introduction

Spent hens are 2 to 3 years of layer's age at the end of laying cycle. The laying hens have very fragile bones which easily break during handling due to the high calcium consumption in producing eggshells (Feddes and Zuidhof, 1997). Because of the light body weight of a spent hen, the amount of edible meat that can be obtained from the carcass is small and its quality poor due to the fragile bone breakage into its flesh during processing makes spent hen meat undesirable (Mench, 2001).

Many flavor compounds found in cooked or processed foods occur as the result of reactions common to all types of foods, regardless of whether they are of animal, plant, or microbial derivation. These reactions take place when suitable reactants are present and appropriate conditions (heat, pH, light) exist (Lindsay, 1996). There are three major methods for manufacturing hydrolysates: 1) basic hydrolysis, 2) acidic hydrolysis, and 3) enzymatic hydrolysis. The sources of enzymes applied in protein hydrolysis are come from plant, animal, and microorganisms. Proteolysis occurring during ripening yields polypeptides, peptides, free amino acids, etc. The reactions involved in the generation of these compounds are catalyzed by endogenous enzymes, such as cathepsins and trypsin-like proteinases.

Enzymatic hydrolysis is one of the various methods used to modify the functional properties of muscle proteins (Hrckova et al., 2002). In enzymatic hydrolysis, reactions between selected enzyme and proteins of meat take place and many taste compounds are obtained. These taste compounds are mostly amino acids, peptides, and nucleotides which impart the characteristics of sweet, bitter, or tasteless. The types and amounts of hydrolyzed compounds formed depend on types and concentrations of proteolytic enzyme used, as well as on the pH, temperature, and time needed to conduct the procedures (deMan, 1990).

The spent hen meat is generally tough due to the collagen content and cross linkages (Bailey, 1984). Hence, the meat has limited usage in whole meat foods and has reduced market price in the US (Sams, 1990; Nurmahmudi and Sams, 1997). Moreover, abundant unprofitable spent hen meat will result in difficulty in their effective disposal. Since there is a demand in the method to utilize spent hen meat, studies were conducted to investigate the effects of papain, bromelain, trypsin, and *Aspergillus oryzae* protease in hydrolysis of spent hen breast meat at various conditions (pH and concentration) and determine the sensory characteristics of spent hen protein hydrolysate.

Materials and Methods

Spent Hen Meat : Spent layer carcasses were obtained from a commercial spent hen processing plant in North Mississippi. The carcasses were packaged individually with polyethylene poultry bags and stored in a freezer at -18°C. Carcasses were thawed in a refrigerator at 2-4°C overnight and breast meat were separated manually.

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Preparation of Spent Hen Hydrolysate

Preparation of Spent Hen Breast Meat Suspension : Pooled boneless and skinless breast meat were ground twice through a meat grinder plate (Kitchen Aid Model: K5SS, Troy, Ohio) with 4.2-mm-diameter holes. The ground breast meat was mixed with ten-fold volume of distilled water and blended in a Waring Blendor (Sears Insta-Blend Model#400829302, Sear Roebuck and Co., Hoffman, IL) into slurry for 30 sec. Spent hen meat suspensions were heated in boiling water for 20 min to 95°C to denature native enzymes present in the raw ground breast meat.

Enzymes Source : Bromelain (EC 3.4.22.32, from pineapple stem), trypsin (EC 3.4.21.4 from porcine pancreas), papain (EC 3.4.22.2 from papaya latex), and protease (type XXIII from *Aspergillus oryzae*) were purchased from Sigma Chemical Co. (St. Louis, MO) for hydrolyzing the ground breast meat suspensions.

Hydrolysis Procedures : The effects of enzyme concentration and substrate on hydrolysis of hen meat were investigated. For the effects of pH, meat suspensions were adjusted to pH 4, 5, 6, 7, 8, and 9 by adding either 1N HCl or 1N NaOH. Enzyme and raw meat were added at the rate of 0.1%, 0.5% and 1.0% (w/w). The enzyme-meat suspensions were placed in a 50°C water bath and incubated for four hours. During incubation, the mixtures were mixed at 30-min intervals. The enzyme activity was terminated by placing the reaction bottles in boiling water for 15 min. The hydrolysates were filtered with No. 2 paper (Whatman International Ltd., Maidstone, England) for filtrate collection (Fig. 1). The filtered hydrolysates were stored at -18°C for further analyses. Combinations of bromelain, trypsin, papain, and *Aspergillus oryzae* protease the various enzyme concentrations (Table 1) at natural pH of meat suspension were also used to hydrolyze spent hen breast meat suspension.

Table 1:Concentration of enzyme combinations

Enzyme Combination	Enzyme Concentration[1] (W/W)
Papain + Bromilain (P+B)	0.50%P+0.50%B
Papain + Protease (P+A)	0.50%P+0.50%A
Papain + Trypsin (P+T)	0.50%P+0.50%T
Bromilain + Protease (B+A)	0.50%B+0.50%A
Bromilain + Trypsin (B+T)	0.50%B+0.50%T
Protease + Trypsin (A+T)	0.50%A+0.50%T
Papain + Bromilain + Protease (P+B+A)	0.33%P+0.33%B+0.33%A
Papain + Bromilain + Trypsin (P+B+T)	0.33%P+0.33%B+0.33%T
Papain + Protease + Trypsin (P+A+T)	0.33%P+0.33%A+0.33%T
Bromilain + Protease + Trypsin (B+A+T)	0.33%B+0.33%A+0.33%T
Papain + Bromilain + Protease + Trypsin (P+B+A+T)	0.25%P+0.25%B+0.25%A+0.25%T

[1]Enzyme dose was calculated based on weight of raw meat

Hydrolysate Analyses

Proteolytic Activity : The proteolytic activity of enzymes was measured by using methods as described by Chang (1998). Briefly, 5 mL of protein hydrolysate was added into a test tube containing 1 mL of distilled water, followed by 10 mL of 0.72 N trichloroacetic acid (Fisher Scientific Co., Fair Lawn, NJ). The mixture was filtered through No. 2 filter paper. The filtrate (0.5 mL) was mixed with sodium carbonate reagent (4.5 mL) and one mL of the Folin & Ciocalteu' s phenol reagent (Sigma Chemical Co., St. Louis, MO) was mixed into the sample. After 5 min of color formation, the optical density was measured against a reagent blank at 650 nM (Gilford Stasar III Spectrophotometer, Gilford Instrument, Inc., Oberlin, OH). The proteolytic activity was obtained according to a standard curve prepared with bovine albumin (Sigma Chemical Co., St. Louis, MO).

Sensory Evaluation : Prior to the test, spent hen hydrolysates were thawed in a refrigerator at 2-4°C overnight. For each treatment, the hydrolysate was diluted four times with distilled water and the diluted hydrolysate was warmed to 50°C in a water bath before evaluation by panelists. A six-member sensory panel was selected from faculty and students who have prior sensory evaluation experience. A quantitative descriptive analysis technique was used for aromatic, feeling factor, and taste analyses (Stone and Sidel, 1993). Four samples of hydrolysates were served each time.

Statistical Analyses : The data were statistically analyzed by using a completely randomized design (Steel and Torrie, 1980) with at least four replications. All data were analyzed using the General Linear Models procedure. The least significant difference test was used for separating means among treatments when mean differences were

significant ($P<0.05$) (Freud and Wilson, 1997). The Statistical Analysis System software package V.8.2 (SAS, 2001) was used for the statistical analysis.

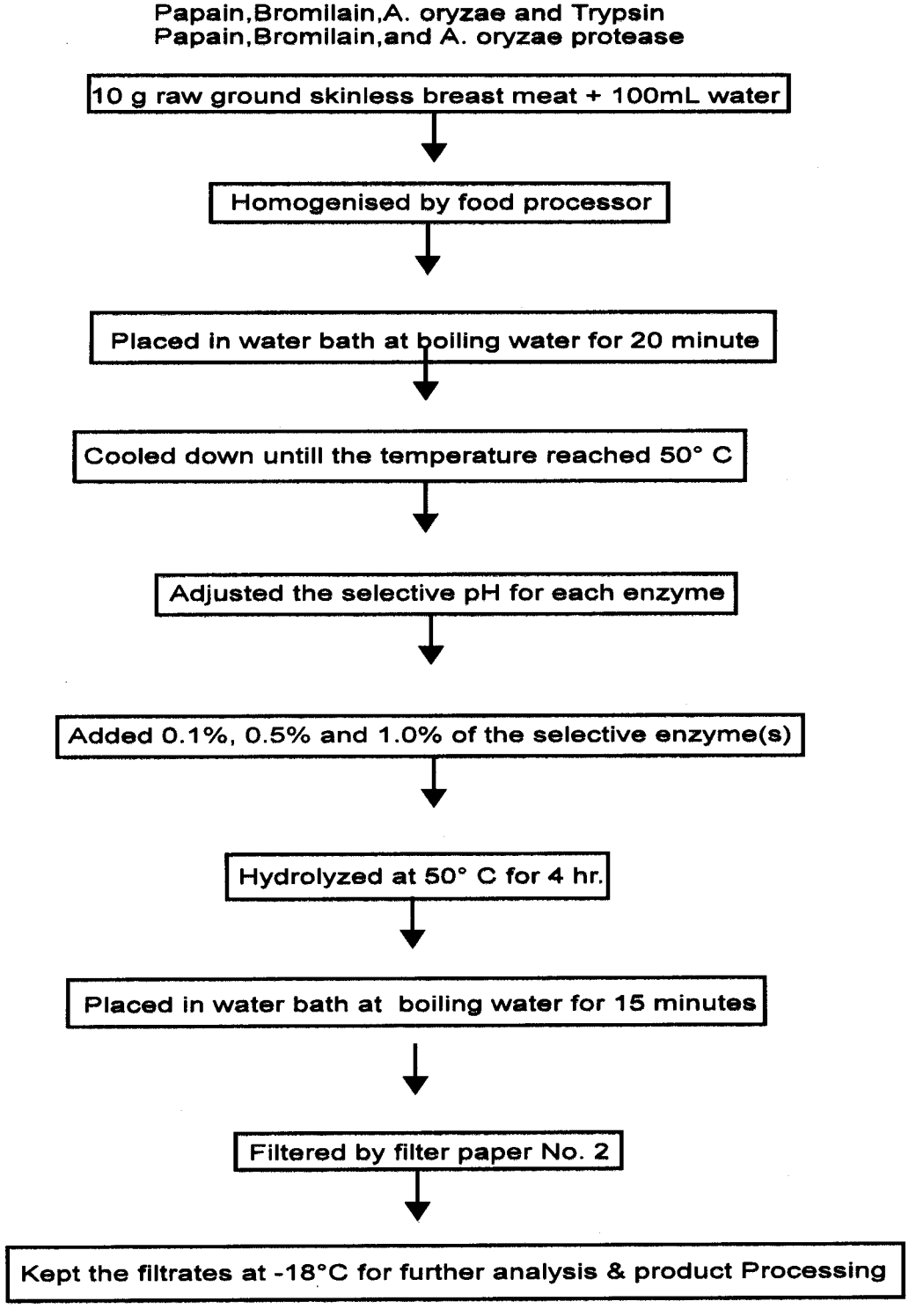

Fig. 1:Hydrolysis flow chartof ground skinless spent her breast meat suspension

Results and Discussion

Optimal Spent Hen Meat Enzymatic Hydrolysis Conditions

Optimal pH : Generally, results indicated that optimal pH ranges for spent hen meat suspension enzyme hydrolysis were 6.0-7.0 for papain, 6.0-7.0 for bromelain, 6.0 for *Aspergillus oryzae* protease, and 6.0 for trypsin (Table 2). Liaset et al. (2000) reported that the factors affecting enzymatic hydrolysis were the nature of the substrates, the type of enzymes, the enzyme-to-substrate ratio, and the concentration of substrates. In addition, each enzyme can only react on its specific peptide bonds (Webster et al., 1982). Sen et al. (1962) studied the papain hydrolysis of fresh water fish and discovered that the release of free amino acids was greater at pH 7 than at pH 5. pH 7.0 was optimal for papain, bromelain, trypsin, and ficin in hydrolyzing fish protein (Hale, 1969). In general, the enzymes from fungal sources are effective in slightly acid conditions (Saunder, 1995). Our study showed similar optimal pH conditions for papain, bromelain, *Aspergillus oryzae* protease, and trypsin on spent hen meat hydrolysis, which is around pH 6 to 7. Hence, the natural pH condition of meat suspension around pH 6.2 was chosen for further hydrolysis studies.

Table 2: The proteolytic activity of blended breaset meat suspension as affected by different enzymes at various pH

Enzyme (1%,W/W)[1]	pH	ProteinConcentration(mgalbumin/mL)
Papain		
	4	13.92c
	5	22.40ab
	6	26.96a
	7	27.26a
	8	17.99bc
	9	20.15bc
Bromilain		
	4	21.23c
	5	29.77ab
	6	32.48a
	7	32.58a
	8	25.39bc
	9	24.70bc
Aspergillus Oryzae Protease		
	4	22.55c
	5	33.97ab
	6	35.06a
	7	32.72ab
	8	28.44bc
	9	25.45bc
Trypsin		
	4	3.68e
	5	7.58d
	6	16.60a
	7	12.95bc
	8	10.41dc
	9	14.94ab

Each value represents the mean of four perlications,
a-e, Mean in a column is an enzyme group followed by unlike letters differ significantly ($P<0.05$)
[1]Enzyme usage was calculated based on weight of raw meat

Optimal Enzyme Concentration : As expected, one percent (w/w) of papain, bromelain, trypsin, and *Aspergillus oryzae* protease showed the highest ($P<0.05$) hydrolysis efficacy, followed by 0.5% (w/w) and 0.1% (w/w) (Table 3). The percentage of enzyme was based on the raw meat weight in the suspensions. For bromelain and trypsin, the effects of enzyme concentrations on hydrolysis efficacy did not show as great as those of the papain and *Aspergillus oryzae* protease (Table 3). Rebeca et al. (1991) reported that the increase of protease concentration and duration in fish protein hydrolysis were associated with an increase in soluble nitrogen. When hydrolyzing rapeseed meal with pepsin, papain, trypsin, ficin, and hemicellulase at various concentrations (range of 20-100 mg/g protein), Mahajan and Dua (1998) found that the degree of hydrolysis increased significantly up to 60 mg/g of protein with increasing concentrations of all enzymes.

Table 3:The proteolytic activity of blended breast meat suspension as affected by different enzymes with various concentrations

Enzyme	Enzyme Concentration[1] (W/W)	Protien Concentration (mg albumin/mL)
Papain		
	0.1%	17.77c
	0.5%	24.59b
	1.0%	31.66a
Bromilain		
	0.1%	15.40c
	0.5%	25.97b
	1.0%	33.29a
Aspergillus Oryzae Protease		
	0.1%	15.40c
	0.5%	25.97b
	1.0%	35.01a
Trypsin		
	0.1%	9.91b
	0.5%	12.95ab
	1.0%	16.60a

Each value represents the mean of four perlications,
a-e, Mean in a column is an enzyme group followed by unlike letters differ significantly ($P<0.05$)
[1]Enzyme usage was calculated based on weight of raw meat

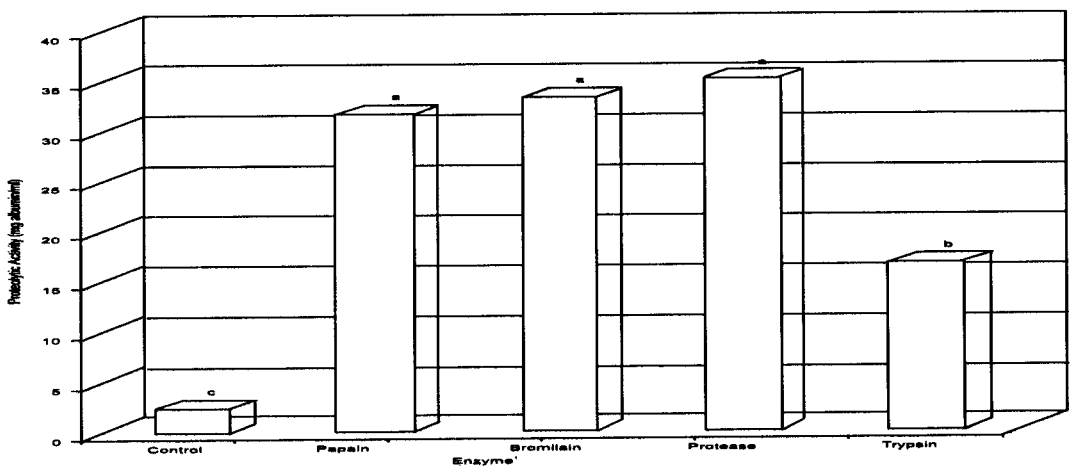

Fig. 2: The proteolytic activity of blended breast meatsuspension as affected by different enzymes [1]One persent(w/w, based on weight of raw meat) of enzyme was used

Effects of Enzyme Combinations : While a lower ($P<0.05$) proteolytic activity reading was recorded for trypsin, no differences ($P>0.05$) in protein proteolytic activity were observed among 1% papain, bromelain, and *Aspergillus oryzae* protease (Fig. 2). Papain, bromelain, pepsin and ficin are most widely applied to hydrolyze poultry and fish products (Hale, 1969; Hevia et al, 1976).
Data from enzyme combination studies have indicated that reducing individual enzyme concentration reduced proteolytic activity. The proteolytic activities of various enzyme combinations are summarized in Figure 3. For example, the combination of 0.5% *Aspergillus oryzae* protease and 0.5% bromelain had lower proteolytic activity than either 1% *Aspergillus oryzae* protease or 1% bromelain. Mimouni et al (1999) found that the hydrolysis of gluten by the combination of pepsin and alcalase showed a higher nitrogen solubility index than individual pepsin. Pedersen et al. (1996) studied organoleptic properties of meat hydrolysates and concluded that the use of neutral and alkaline protease combinations provided more satisfactory hydrolysates than those from individual enzymes.

Sensory Characteristics of Spent Hen Hydrolysate : Flavor profiles of hydrolysate were different for each type of enzyme and enzyme combination. Generally, hydrolysate from A and P+B had higher scores in the chickeny attribute than other treatments (Table 4). Higher meaty intensities in A, P+A+T, A+T, P+B+T, and P+B were judged by panelists. In mouth filling, A, P+A+T, P+A, and P+A+B had higher scores rather than others. For

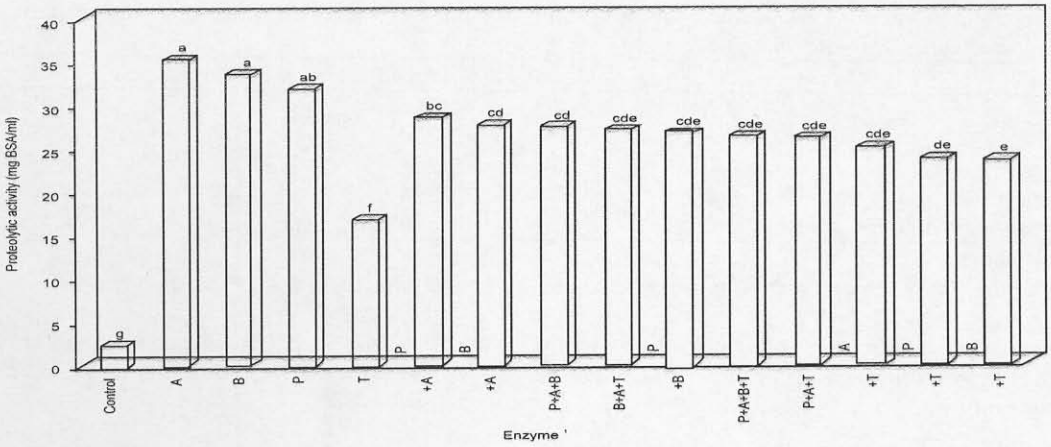

Fig. 3: The proteolyticactivity of blended breast meat suspension as affected by individual enzyme and combinations [1]P-Papain, A-Aspergillus oryzae protease, B-Bromilain, T-Trypsin

the umami attribute, the highest score ($P<0.05$) was recorded in hydrolysate form A. However, panelists regarded hydrolysates from B+A and bromelain as the most bitter among treatments.

For enzyme-hydrolyzed spent hen meat, the content of free amino acids, sweet and bitter amino acids, and nucleotides varied, depending on the types of enzymes used. Sucan et al. (2002) mentioned that the qualitative and quantitative aroma profiles of meat-based flavors varied by meat type, enzyme type, and thermal processing conditions.

The reaction of the mixture of amino acids, sulfur compounds, and reducing sugar provided the species-specific flavor of chicken (Farmer, 1999). About 500 volatile aroma compounds have been identified in chicken meat (Rose, 1997). The meaty flavor in chicken broth has been attributed to 2-Methyl-3-furanthiol (Sucan et al, 2002). Weir (1982) indicated that 5' -ribonucleotides and MSG provide the umami sensation in protein hydrolysates. The bitter taste of protein hydrolysate is related to the hydrophobicity, the large number of amino acids, and the amino acid derivatives. The amino acids released during the protein hydrolysis, such as histidine, tryptophan, isleucine, and phenylalanine result in a bitter flavor (Silva, et al, 1999). Hydrolysis of chicken meat by using bromelain tends to produce a bitter taste (Surowka and Fik, 1994). Hence, considering the hydrolyzing degree, acceptability of sensory attributes and enzyme cost, A, P+A, P+B, and P+A+B were chosen for further sensory evaluation.

Trypsin is expensive and had a lower ($P<0.05$) proteolytic activity than papain, bromelain, and protease. Therefore, A, P+A, P+B, and P+A+B hydrolysis were chosen for further sensory evaluation (Table 5). No differences ($P>0.05$) in sensory attributes among undiluted enzymatic hydrolysates were observed, but all of them were higher ($P<0.05$) in score than the control (Table 5). Generally, P+A showed the highest acceptability in

Table 4:Average mean values for chickney, meaty, mouth feeling, biterness and umani sensory attributes of dilutic enzymatic hydrolysates

Enzyme	Cickney	Meaty	Mouth Feeling	Biterness	Umani
Control	2.26d	2.11c	1.67c	0.75d	1.30cd
Papain(P)	4.76bc	2.72bc	3.28b	1.75bcd	1.15d
Bromilain(B)	4072bc	3.67abc	4.49ab	2.71ab	1.39cd
Protease(A)	7.71a	5.08a	5.83a	2.46bc	4.02a
Trpysin(T)	4.40c	2.78bc	4.10b	1.9bc	2.48b
Papain + Bromilain(P + B)	6.25ab	3.82ab	4.10b	2.48bc	2.36b
Papain + Protease(P + A)	6.08b	3.77ab	4.67ab	2.59bc	2.14bc
Papain + Trypsin(P + T)	5.09bc	2.90bc	4.05b	1.52cd	1.58bcd
Bromilain + Protease(B + A)	5.33bc	3.42bc	3.97b	3.80a	2.45b
Bromilain + Trypsin(B + T)	4.38c	2.73bc	3.82b	2.35bc	2.17bc
Protease + Trypsin(A + T)	5.88bc	4.15ab	4.00b	1.95bc	2.01bcd
Papain + Protease + Trypsin(P + A + T)	5.50bc	4.19ab	4.78ab	2.59bc	1.99bcd
Papain + Bromolain + Trypsin(P + B + T)	5.46bc	3.94ab	4.06b	2.51bc	2.45b
Papain + Protease + Bromilain(P + A + B)	5.85bc	3.40bc	4.52ab	2.00bc	1.82bcd
Protease + Bromilain + Trypsin(A + B + T)	5.34bc	2.94bc	3.73b	2.10bc	1.65bcd
Papain + Protease + Bromilain + Trypsin(P + A + B + T)	5.75bc	3.52abc	4.40ab	1.92bc	2.01bcd

Each value represents the mean of 12 replications,

abcd, Mean within column not followed by the same letters differ ($P<0.05$)

[1]-On a 10 point scale with 1 being none and "10" being strong

Table 5: Average mean values for chickney, meaty, mouth feeling, biterness and umani sensory attributes of undilutic enzymatic hydrolysates

Enzyme	Chickney[1]	Meaty[1]	Mouth Feeling[1]	Biterness[1]	Umami[1]
Control	3.24b	2.17b	2.54b	2.11b	1.95b
Protease(A)	6.12a	5.45a	5.25a	3.79a	4.29a
Papain + Protease(P + A)	6.98a	5.78a	6.20a	4.92a	4.75a
Papain + Bromilain(P + B)	5.40a	4.67a	5.33a	4.67a	4.38a
Papain + Protease + Bromilain(P + A + B)	6.09a	4.75a	5.88a	4.29a	4.42a

[1]Each value represents the mean of 12 replications
a-b Means in a column in a sensory attribute group followed by unlike letters differ significaqntly($P<0.05$)

sensory attributes among treatments and control followed by P + A + B, A, and P + B. Again, considering enzyme efficacy, acceptability of sensory attributes, and enzyme cost, P + A and P + A + B should be the two candidates for product manufacture.

Conclusion

Optimal pH ranges for spent hen meat suspension enzyme hydrolysis were 6.0-7.0 for papain, 6.0-7.0 for bromelain, 6.0 for protease, and 6.0 for trypsin. Among the 3 test levels, 1% (w/w) of papain, bromelain, trypsin, and protease showed the highest hydrolysis efficacy, followed by 0.5% (w/w) and 0.1% (w/w). No differences ($P>0.05$) in protein proteolytic activity was observed among 1% papain, bromelain, and *Aspergillus oryzae* protease; meanwhile a lower ($P<0.05$) proteolytic activity reading was recorded for trypsin. The sensory evaluation data showed that P + A had the highest acceptability in sensory attributes among treatments and control followed by P + A + B, A, and P + B. According to enzyme efficacy, acceptability of sensory attributes, and enzyme cost, P + A and P + A + B could be chosen for product manufacture.

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