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Utilization of Spent Hens as a Flavoring Base: 2. Flavoring Products from Spent Hen Meat Hydrolysate

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Abstract : The objective of this study was to develop novel products from spent hen protein hydrohysate. Protein hydrolysates have been used for flavoring purposes, as savory flavors or taste enhancers. Ground spent hen breast meat and water [1:10 % (w/w) and 1:2 (w/w)] were blended and hydrolyzed with either papain and *Aspergillus oryzae* protease [P+A, 0.5% (w/w) each of raw meat weight] or papain and *Aspergillus oryzae* protease and bromelain [P+A+B, 0.33% (w/w) each of raw meat weight] at 50°C for four hours. The enzyme activity was terminated by placing the reaction bottles in boiling water for 15 min. After cooling, either whole hydrolysates or filtrates were used for product preparations. Freeze during and evaporation methods were used in preparing hydrolysate powders or concentrated paste accordingly. Yield, moisture content, and microbiological analyses of the final products were also measured. Yields of freeze-dried powders from P+A and P+A+B hydrolysate prepared at 1:10 (meat: water) filtrates were 22.8 % (W/W) and 23.0% (W/W), respectively. Yields of freeze-dried whole hydrolysates were 26.0 and 24.8% for P+A and P+A+B, respectively. Meanwhile, freeze-dried powders from P+A and P+A+B hydrolysate prepared at 1:2 filtrates had 26.7 and 26.2% of yield, respectively, while the yields of concentrated paste from whole hydrolysates were 47.8 and 51.0% for P+A and P+A+B, accordingly. Both freeze-dried filtered hydrolysate and the freeze-dried hydrolysate products had a total aerobe plate count ranging from log 2.59 to 2.97 cfu/g. Similar range of total aerobes (log 2.74 to 2.84 cfu/g) was obtained in concentrated paste products. Neither *Salmonella* nor *E. coli* /Coliform was detected from the prepared products.

Key words: Spent hen, Papain, *Aspergillus oryzae* protease, Bromelain, Freeze-drying, hydrolysate powders, Concentrated paste, Microbiological Analyses

Introduction

Now a days 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). Potter and Hotchkiss (1995) indicated that there are over 1200 different flavoring materials used in foods, and these are typically used in trace amounts and are similar to the chemicals found in natural sources. Also included among the flavor additives are flavor enhancers or potentiators, which do not contribute flavor in the low levels but intensify the flavor of other compounds present in foods.

Protein hydrolysates are the main products derived from protein hydrolysis and have been used specifically for flavoring purposes, as savory flavors or taste enhancers for hundreds of years. Protein hydrolysates are an important ingredient used in food systems for improving texture and fortifying beverages. They can also be used in pharmaceutical products designed to supplement the nutritional requirements of patients who cannot consume, or are allergic to certain dietary proteins (Lin *et al.*, 1997). In addition, enzymatic protein hydrolysates have become popular ingredients in the food and flavor industry since they contain a large portion of peptides. All the hydrolyzed protein products could be used as an ingredient for further flavorings either by blending or reacting further with a variety of additives (Weir, 1982).

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). Because spent hen meat is not profitable, abundant spent hen carcasses will result in difficulty in their effective disposal. Spent hen meat is rich in nutrient value because of high proteins, amino acids, and 5'-ribonucleotides. These proteinaceous components impart the important roles both in nutrients and flavors in food processing. The objective of this research was to develop novel products from spent hen protein hydrolysate.

Materials and Methods

Ground Spent Hen Breast Meat Suspension : Spent layer carcasses were obtained from a commercial spent hen processing plant. The carcasses were packaged individually with polyethylene poultry bags and stored in a freezer at -18°C. Prior to the study, carcasses were thawed in a refrigerator at 2-4°C overnight, and then breast meat was separated manually from the carcasses. 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 either ten-fold or two-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 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), 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.

Preparation of Hydrolysate Products : Two types of hydrolysates, whole and filtered hydrolysate from two treatments were selected for product preparation. The first treatment involved the use of ground breast meat blended with two-fold volume of water and the second involved the use of ground breast meat with ten-fold volume of water. Ground spent hen breast meat suspensions were blended and hydrolyzed with either papain and Aspergillus oryzae protease [P+A, 0.5% (w/w) each of raw meat weight] or papain and Aspergillus oryzae protease and bromelain [P+A+B, 0.33% (w/w) each of raw meat weight] at 50°C for four hours. The enzyme activity was terminated by placing the reaction bottles in boiling water for 15 min. After hydrolyzed meat suspension with enzyme, either whole hydrolysates or filtrates were used as products. Either freeze-drying or evaporation was used in preparing hydrolysate powders or paste. For freeze-drying, hydrolysates were frozen at -18°C overnight and vacuum dried at 10 psi vacuum at -40°C for two days in a LYPH LOCK 6 (Labconco®, Kansas city, MO). For evaporation, hydrolysates were evaporated and stirred at 60-65°C for 55 min.

Analyses

Yield : The percentage of yield was determined by calculating product weight and raw material weight.

$$\% \text{ Yield} = \frac{\text{Product wt (gm)}}{\text{Raw ground meat wt (gm)}} \times 100$$

Moisture : Two grams of sample were placed into an aluminum dish (Fisher Scientific Co., Fairlawn, NJ) and dispersed evenly across the dish. The sample dishes were placed in an oven and dried at 120-130°C for 16-18 hours (AOAC, 1999). The dishes were reweighed, and the moisture content was calculated.

Microbiological analyses : Total aerobes, *Salmonella*, *E. coli.*, and *Coliforms* of final hydrolysate products were enumerated and analyzed.

Total aerobes : One gram of chicken hydrolysate products was aseptically placed into a tube of 9 mL of 1% sterile peptone solution (Difco, Detroit, MI). The tube was agitated for 60 sec. One mL of the appropriate dilution was utilized with a pour plate method. Plate counts agar (Difco, Detroit, MI) was used as the growth medium. Plates were incubated at 30°C for 24 hr. Results were reported as log colony forming unit/g (cfu/g).

Salmonella : Dilutions performed for the *Salmonella sp.* test were utilized with the spread plate method using 0.1 mL of the appropriate dilution. Brilliant green agar (Difco, Detroit, MI) was used as the growth medium. Plates were incubated at 30°C for 24 hr. Results were reported as log colony forming unit/g (cfu/g).

E. coli./Coliforms : 3M *E. coli/Coliform* Petri-film™ (3M, St. Paul, MN) was used for the *E.coli* or *coliforms* count. One mL of the appropriate dilution was spread on 3M Petri-film™. Plates were incubated at 30°C for 24 hr. Results were reported as log colony forming unit/g (cfu/g).

Statistical Analyses : The data were statistically analyzed by using a completely randomized design (Steel and Torrie, 1980) with at least four replications per treatment. All data was 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.

Results and Discussion

Spent Hen Hydrolysate Products : Two types of freeze-dried spent hen hydrolysate products were prepared, one product with filtrate from filtered hydrolysate and the other with hydrolysate (Fig. 1and 2). The yields of freeze-dried powders from P+A and P+A+B filtrates were 22.8 % (W/W) and 23.0% (W/W), respectively (Table 1). These freeze-dried products also contained 13.2 and 14.3% of moisture, respectively (Table 1). The filtrates were prepared by hydrolyzing breast meat suspensions with a meat to water ratio of 1:10. When the whole hydrolysates

were freeze-dried, the yields were 26.0 % (W/W) and 24.8% (W/W) for P+A and P+A+B, respectively, and the moisture contents of these products were 17.0 and 15.8%, respectively (Table 1).
For hydrolysates prepared from meat suspensions with a meat to water ratio of 1:2, both freeze-dried products and concentrated paste products were prepared (Fig. 3 and 4). Yields of freeze-dried hydrolysates from P+A and P+A+B were 26.7 and 26.2%, respectively. These products also contained 14.2 and 15.8% of moisture, respectively (Table 1). The yields of concentrated paste products from P+A and P+A+B hydrolysates were much higher than those of the freeze-dried products and were 47.8 and 51.0%, accordingly
Table 1: The moisture contents of those concentrated paste products were 54.2 and 53.5%, respectively

Table 1. Yield (%) and moisture (%) of whole and filtered hydrolysate (10g of meat:100 mL of water) products, and whole hydrolysate (60g of meat:120 mL of water) products

Parameter	Ratio of meat and water			
	10g of meat : 100 mL of water		60g of meat : 120 mL of water	
	Paste	Powder	Paste	Powder
Yield (%)				
Whol				
Papain + Protease (P+A)	*	26.0a	47.8a	26.7a
Papain + Protease + Bromelain (P+A+B)	*	24.8a	51.0a	26.2a
Filtered				
Papain + Protease	*	22.8a	#	#
Papain + Protease + Bromelain	*	23.0a	#	#
Moisture (%)				
Whole				
Papain + Protease	-	17.0a	54.2a	14.2a
Papain + Protease + Bromelain	-	15.8a	53.5a	15.8a
Filtered				
Papain + Protease	-	13.2a	-	-
Papain + Protease + Bromelain	-	14.3a	-	-

Each value represents the mean of three replications.
No differences were observed between the groups within a row of each parameter ($P>0.05$).
* Too dilute for paste product processing.
# Too thick to be filtered.

Table 2: Total aerobes, Salmonella, and E. coli./Coliform results of the products

Product	Treatment	Total aerobes (log cfu/g)	Salmonella (log cfu/g)	E. coli./Coliform (log cfu/g)
Powder[1]				
Whole (10g of meat : 100 mL of water)	Papain + Protease (P+A)	2.59	ND[2]	ND
	Papain + Protease + Bromelain (P+A+B)	2.62	ND	ND
Whole (60g of meat : 120 mL of water)	Papain + Protease	2.82	ND	ND
	Papain + Protease + Bromelain	2.97	ND	ND
Filtered (10g of meat : 100 mL of water)	Papain + Protease	2.60	ND	ND
	Papain + Protease + Bromelain	2.67	ND	ND
Paste[1]				
Whole (60g of meat : 120 mL of water)	Papain + Protease	2.74	ND	ND
	Papain + Protease + Bromelain	2.84	ND	ND

[1] Each value represents the mean of three replications.
[2] ND-Non Detected.
No differences were observed between the groups within a row of each parameter ($P>0.05$).

Liceaga-Gesualdo and Li-Chan (1999) reported that the yield of freeze-drying herring hydrolysate was 6.6%. The typical yields of other freeze-dried fish protein hydrolysates were 10 to 15% based on the fresh fish substrate (Quaglia and Obban, 1990; Rebeca *et al.*, 1991). However, few reports, if any, related to the product yields of

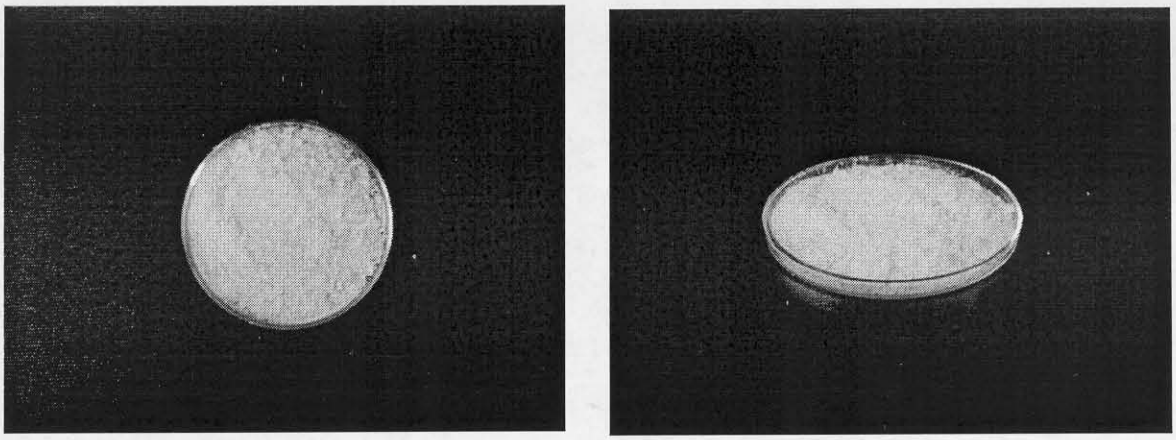

Fig. 1: Freeze-dried products from 1:10 (meat/water ratio) filtered spent hen breast meat suspension

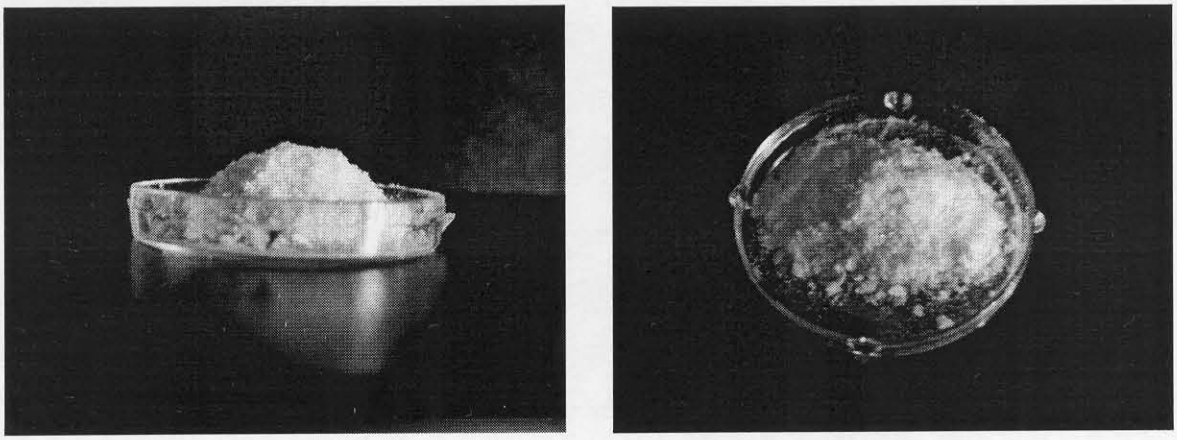

Fig. 2: Freeze-dried products from 1:10 (meat/water ratio) whole spent hen breast meat suspension

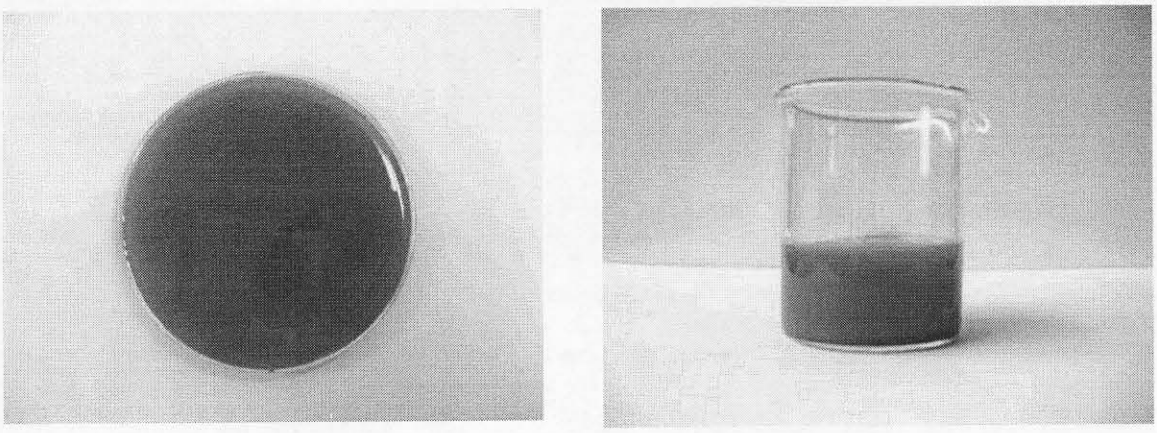

Fig. 3: Concentrated paste products from 1:2 (meat/water ratio) whole hydrolyze spent hen breast meat suspension

spent hen hydrolysate are available. Yield is one of the beneficial factors to producers; a higher yield means higher revenue for the manufacturer.

Microbiological Profiles of Spent Hen Hydrolysate Products : Both freeze-dried filtered hydrolysate products and the freeze-dried hydrolysate products had a total aerobe plate count ranging from log 2.59 cfu/g to 2.97 cfu/g. The total aerobes from concentrated paste products were similar and ranged from log 2.74 to 2.84 cfu/g (Table Neither Salmonella nor *E. coli./Coliforms* was detected from any types of spent hen hydrolysate products that were

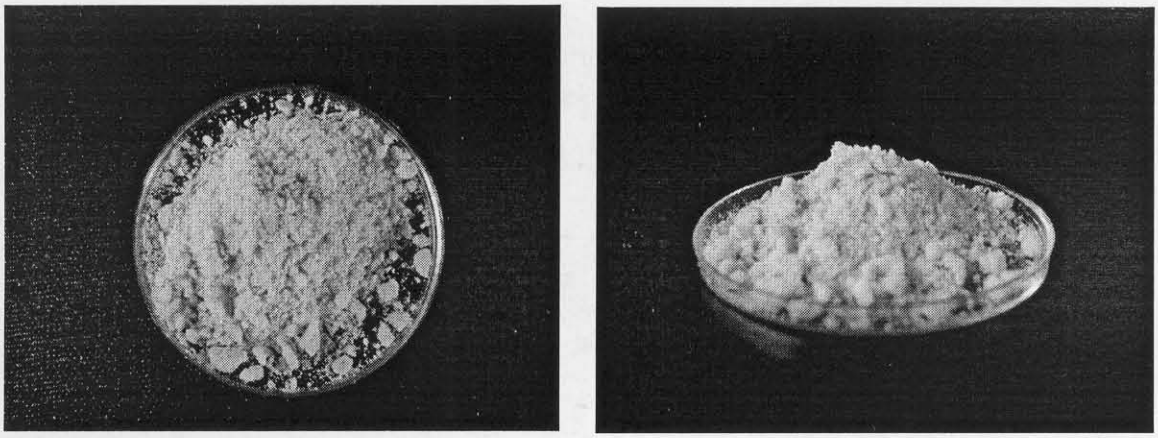

Fig. 4: Freeze-dried products from 1:2 (meat/water ratio) whole hydrolyze spent hen breast meat

prepared. Data supported the result of Suroska and Fik (1994), who indicated the absence of microbiological pathogens in the dried chicken head hydrolysates. Based on our knowledge, no regulation or limitation of microbiology in dried meat hydrolysate products was published by legislated organizations such as USDA or FDA. Therefore, the products should be safe for the consumers.

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