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Production of Peptone From Chicken Feathers

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Abstract*— Peptones are the product of a protein hydrolysis, which serve as the main nutrient source for bacteria in a culture media. This study aimed to provide a locally feasible process for peptone production. Optimization results for Phase 1 showed that a temperature of 90°C, a digestion time of 4 hours, and a ratio of 0.06 g feathers/mL produced a yield of 50.6%. Statistical analysis showed that E. coli growth on the laboratory-produced peptone is significantly greater than the growth realized on commercial peptone and plate count agar. B. cereus growth on laboratory-produced peptone, however, was significantly lower than the growth on commercial peptone and plate count agar. Purification, drying, and characterization techniques were integrated into the existing process for Phase 2 to obtain peptones with better commercial quality. The maximum yield obtained was 30 g peptone per 100 g feathers. Freeze-dried powders from the purified hydrolysates had reduced odor and moisture as compared to the vacuum dried peptone. The vacuum-filtered batch also approximated the physical characteristics of the standard peptone hydrolysate. Performance testing for Phase 2 showed increased support for bacterial growth.

Keywords— ***Chicken Feathers, Peptone Production, Nutrient Media***

1. INTRODUCTION

Biotechnological studies and industrial processes studies use live microorganisms that are cultured and grown by a nutrient medium that supports bacterial growth. These nutrient media contain nitrogen sources necessary for bacterial growth, and usually take up a large part of the production cost for industrial processes [1].

Cultures of microorganisms in microbiological tests make use of nutrient media that must contain relatively high amounts of nitrogen. The nitrogen found in these media is usually in the form of protein, or amino acids. The presence of these nitrogen compounds, with a carbon source, vitamins, and other trace ingredients, provide the needed system of nutrients that will be able to sustain the growth of microorganisms in a culture media [2]. Scientists have used naturally occurring substances such as blood, urine, and other body fluids as the main sources of nitrogen for liquid nutrient media. The discovery of other possible nutrient sources such as milk proteins, yeast, lactose, and other sugar derivatives, and the combination of these sources with inorganic substances, has made possible the use of other forms of nutrient media. Examples of these nutrient media include beef-extract peptone broths, infusion broths (prepared from extraction of skeletal or heart muscles of animals), and blood agar (whose main ingredient is sterile blood from either rabbit, sheep, horse or human) [3].

Peptones are enzymatic digests of these animal proteins, usually obtained after alkaline hydrolysis (digestion) of a protein source. Further processing causes the product its final physical appearance: a light brown, almost white, amorphous powder [4]. The color of the peptone product may differ, depending on the protein source (meat peptones are beige in color) [5].

Studies have been conducted on the synthesis of peptone from various protein sources. These protein sources may be classified into two categories: animal-based and plant-based.

Animal-based protein sources for peptone are usually found in wastes generated by industries that produce products other than peptone. Fish-processing industries produce a large number of waste products which include viscera, head, scrap muscle and skin, and bone. Wastes from these industries are found to be rich in protein. Studies made by Safari and others [6], Poernomo & Buckle [7], and Klompong et al. [8] explore various methods on extracting valuable peptones (as protein hydrolysates) through different forms of hydrolysis (acid, alkaline, or enzymatic). Other studies have been conducted on other forms of wastes as well. Protein hydrolysates (or digested protein, a result of hydrolysis) have been produced from alkaline hydrolysis aided by enzymes on calf skin and blood wastes [9]. Peptone production from ram horn waste, which may be a good source of a fibrous protein called keratin, has been investigated as well [10].

Plant-based protein sources for peptones include soy, rice, cottonseed, wheat and peas. Several studies, although limited in number as compared to studies done on animal-based sources, have shown that peptones are readily produced by hydrolysis of sunflower seeds [10] and soya beans and African locust beans [11].

This study utilized chicken feathers as raw material for the production of peptone. Grazziotin quantified that 10% of the total mass of an average chicken is composed of feathers [12]. Furthermore, feathers are composed by at least 90% of keratin [13] and 2% fatty acids [12].

Keratin is a protein found in chicken and turkey feathers, bristles, hair, and other sources. A primary source of keratin is found in the poultry industry, which includes feathers from rendered chickens and turkey. The amino acid content of these materials is high enough to develop methods to hydrolyze keratin into digestible or other usable peptones [14].

Chenault and Mulidhara [14] have patented a process for peptone production from turkey feathers. Taskin et al. have shown in several studies the feasibility of chicken feather peptones as a growth substrate for bacteria [15] and fungi. In his 2011 study with Sisman et al. [16], chicken feathers were used to prepare peptones via acid hydrolysis to support the production of carotenoids. A study in 2012 with Ozkan et al. [17] concluded the effectiveness of chicken feather peptones to support exopolysaccharide production from the mushroom *Morchella esculenta*. While these studies have shown that production of peptone from chicken feathers is viable, no clear recommendations were given for its mass production on a laboratory-scale. Process steps for peptone production were proposed by studies of Chenault and Mulidhara [14] and Kurbanoglu et al. [1], but literature on optimized hydrolysis conditions of chicken feathers is lacking. Filtration as a purification step in peptone production is widely applied,

but investigations on other purification techniques is noticeably absent. This study aimed to address these concerns by providing a locally feasible process for peptone production from chicken feathers, with a set of optimized process conditions for the alkaline hydrolysis step, and recommendations on techniques that may be employed for the purification step.

Two phases have been conducted: Phase 1 determined the optimized conditions for the hydrolysis of keratin from chicken feathers and compared the performance of the laboratory-produced peptone to commercially available peptone in supporting bacterial growth. Phase 2 incorporated filtration and drying techniques into the proposed process of Phase 1, employed chemical analyses to characterize the product, and conducted additional performance testing for the produced peptone by testing its suitability for microbial growth.

2. MATERIALS AND METHODS

2.1 Phase 1

The methodology for Phase 1 is further subdivided into two phases: the Laboratory-Scale Testing followed by the Analysis and Performance Testing.

2.1.1 Laboratory-Scale Testing

The chicken feathers sourced from Bulacan, Philippines were washed to remove dirt and other foreign matter and dried to remove the residual moisture from washing. The size of these feathers was reduced using a laboratory mill. These feathers were then subjected to alkaline hydrolysis by which a predetermined amount of 0.5 wt.% NaOH was added. The hydrolysis was done at a certain temperature and for a certain period of time. The mixture was cooled and neutralized to pH 7 by dropwise addition of 10 wt.% aqueous hydrochloric acid solution. The mixture was then centrifuged at 1500 rpm for 30 minutes to remove the solids, followed by drying for an hour using the vacuum oven resulting to a powdered peptone as the final product.

Optimization operations were carried out in order to investigate the effect of three parameters (digestion time [2, 3 and 4 hours], digestion temperature [78, 88 and 98°C], and the ratio of the amount of feathers to the amount of NaOH [0.04, 0.07 or 0.1 g feathers/mL NaOH]) on the yield of peptone. A Box-Behnken Design was applied in order to generate fifteen experimental runs, done with two replicates. The yield of peptone (in grams) of the two replicates was computed using the following equation:

$$Yield = \frac{Mass\ of\ peptone\ obtained}{Mass\ of\ chicken\ feathers\ fed}$$

2.1.2 Analysis and Performance Testing

Standard methods for ash and moisture content determination were done for both chicken feathers and the produced peptone.

Two pathogens were used as test organisms (*Escherichia coli* and *Bacillus cereus*) for the performance testing of the produced peptone. These pathogens each represent two classes of bacteria: Gram-negative (for *E. coli)* and Gram-positive (*B. cereus*). The selection of these two species were based on the availability of standard cultures in the Natural Science Research Institute of UP Diliman. The laboratory-produced peptone and the commercially available peptone having one replicate each, were individually tested, with each of these organisms, and the bacterial growth (in colony forming units) was compared.

Peptone agar was prepared from the laboratory-scale produced peptone powder by dissolving 0.5 wt. % of powder with 0.1 wt. % dextrose in distilled water. The pH of the mixture was adjusted to 7.0. An agar supplement of 1.0 wt. % agar was mixed with the solution to prepare the peptone agar media. The commercially available peptone agar was produced by dissolving 2 wt. % of the powder in 0.5 wt. % saline solution. The pH of the mixture was adjusted to pH 7.2. An agar supplement of 1.5 wt. % was mixed with the solution to prepare the peptone agar media. Both solutions were placed in an autoclave for 30 minutes at 121°C. Serial dilution and spread plating were done for the performance testing. The resulting culture was examined using a colony counter.

2.2 Phase 2

The methodology for Phase 2 is further subdivided into three phases: (1) Peptone Production, Purification, and Drying, (2) Characterization and Analysis, and (3) Performance Testing.

2.2.1 Peptone Production, Purification, and Drying

The feathers were thoroughly washed to remove dirt, impurities, and other unwanted matter present. A strainer was necessary to avoid the loss of feathers as well as the clogging of the drainage system. The dried feathers were cut so as to separate the actual feather material from the hard feather stalks and also to avoid any subsequent blinding of the mill's sieve. The cut feathers were ground into smaller pieces using the laboratory's hammer mill, which comes with a sieve size of 1 mm. The ground feathers were placed in clean and dry containers to prevent any contamination.

Approximately 20 grams of the ground feathers were weighed in the analytical balance and transferred into the beakers. Based on the optimum 0.06 g feathers to ml base ratio, the prescribed volume of 0.5 wt. % NaOH is added. The feathers were thoroughly mixed with the base before subjecting them to heat. The hydrolysis was done for four hours at a temperature of 98°C in a pre-heated hot plate and was constantly stirred either with the help of a magnetic stirrer or manually using glass stirring rods. After the four-hour mark, the digested mixtures were cooled to 58°C in a water bath. Then, the pH of the mixtures was adjusted to 7 by the dropwise addition of 10 wt. % HCl.

The neutralized mixtures were transferred into 15 ml covered tubes and centrifuged at 1500 rpm for 30 minutes to remove the solids and precipitates. These solids were then collected, dissolved in water, and disposed. To ensure that the large solid particles are completely removed from the liquid hydrolysate, centrifugation is performed twice. The supernatant is then divided into two portions, which undergoes the succeeding purification steps.

The first half of the centrifuged hydrolysates is mixed with 20 grams of activated carbon powder. The mixture is left to standby for at least 15 minutes to let the activated carbon particles settle at the bottom. The solution is decanted and centrifuged at 2400 rpm for 25 minutes twice. The supernatant is filtered using ordinary filter paper with a pore size of about 11 μm.

The remaining portion from the centrifugation step is filtered using a vacuum filter covered with Whatman 40 filter paper with a pore size of 20 μm prior to vacuum filtration with a polyethersulfone membrane to remove large particles that may clog the membrane.

Corning 431097 0.22μm PES vacuum filter is used to approximate an ultrafiltration setup. The pore size of the membrane is at 0.22 μm. Since the peptone and peptide materials are estimated to be at 0.01 to 0.10 μm, this membrane size was assumed large enough to let the desired components to pass through, leaving behind the larger particles unfiltered from the preceding centrifugation steps.

Freeze drying was done thru the assistance of the staff of the Seaweed Chemistry Building of UP Diliman. Samples were prepared in plastic cups, frozen, and sent to the equipment for drying.

2.2.2 Characterization and Analysis

The common parameters used for characterizing protein hydrolysates include: ash and moisture content, nitrogen content, elemental content, and ion content. The choice of method and equipment depend mainly on availability and cost.

2.2.2.1 Moisture and Ash Content Determination

Pre-weighed crucibles (pre-heated until constant weight is reached) were filled with 1 gram of feathers (for feather analysis) and 0.5 grams of peptone (for peptone analysis). The crucibles with the sample were placed in an oven at 105°C for 30 minutes. The crucibles were weighed, and the process was repeated until constant weight is achieved.

The same samples for moisture content determination were used for ash content determination. The furnace is preheated to 575°C. The samples were placed inside the furnace at the time it reached the desired temperature, and allowed to combust for two hours. The crucibles were cooled and placed in a desiccator until these are ready for weighing. The weight of the residue left corresponds to the ash content of the sample.

2.2.2.2 Chloride Ion Analysis

In an analytical balance, 6.35 g of $AgNO_3$ was weighed. The crystals were then dissolved in 250 ml deionized water and the solution was stored in a 250 ml volumetric flask. On the other hand, 1.82 grams of KSCN were dissolved in 500 ml deionized water and was stored in a 500 ml volumetric flask. Six molars of HNO_3 was prepared by diluting 38 ml of 15.8 M HNO_3 with a suitable amount of deionized water in a 100 ml volumteric flask. From the 6 M HNO_3, a 250 ml of 0.01 M HNO_3 was prepared by diluting 0.42 ml of the 6 M HNO_3 with a suitable amount of deionized water in a 250 ml volumetric flask. The Fe^{3+} indicator was prepared by dissolving 10 g of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ crystals in 100 ml deionized water. The solution was acidified by adding 1 ml of 15.8 M HNO_3.

Peptone samples for testing were prepared by mixing 0.5 g of each sample in 100 ml deionized water.

Ten ml of the prepared $AgNO_3$ were combined with 30 ml deionized water and 3 ml of 6 M HNO_3. One ml of the ferric indicator was added to the solution. This solution was titrated with the prepared KSCN solution. The endpoint of the titration was signaled by the faint brown color of the analyte.

Ten ml of the prepared peptone samples was combined with 10 ml the prepared $AgNO_3$ solution, 10 ml deionized water, and 3 ml of 6 M HNO_3 in a 100 ml beaker. The solution was mixed and was covered with a watch glass. This was then heated in an electric heater for 30 minutes or until the supernatant is clear. The solutions were filtered by using a filter paper. The precipitate, container, and watch glass were washed with the prepared 0.01 M HNO_3, and the washings were added to the supernatant.

One ml of the ferric indicator was added to the supernatant. The supernatant was titrated with the standardized KSCN solution until the analyte reaches a faint brown color.

The entire procedure was done for three trials for each of the Normal, Vacuum Filtered, and Activated Carbon treated peptone.

2.2.3 Performance Testing

The same procedure implemented in Phase 1 for performance testing was also implemented for Phase 2

3. RESULTS AND DISCUSSION

3.1 Results of Phase 1

The parameters that were optimized, and the corresponding high-, middle-, and low-level values are shown in Table 1. These values were based on on parametric studies done by Chenault and Mulidhara [14] on peptone production from turkey feathers.

Table 1. High-, medium-, and low-level values of parameters for optimization

Parameter	High	Medium	Low
Digestion Time (hours)	2	3	4
Digestion Temperature (°C)	78	88	98
Ratio of Feathers to NaOH (g feathers/mL NaOH)	0.04	0.07	0.1

The highest percent yield of powdered peptone was obtained from Run 6 in which ground feathers were hydrolyzed at 98°C for 4 hours using 0.07 grams feathers/mL NaOH (36.558 ± 0.101 %). The lowest percent yield was produced by Run 4, hydrolyzed at 78°C for 2 hours using 0.07 grams feathers/mL NaOH (0.682 ± 0.010 %) (Table 2).

Table 2. Yield (grams) and percent yield (%) of peptone powder produced from hydrolyses of chicken feathers

Temperature (°C)	Time (h)	Ratio of feathers to NaOH (g /mL)	Mean Yield	Mean Percent Yield
78	**3**	**0.1**	0.5888 ± 0.0044	5.888 ± 0.044
98	**3**	**0.04**	2.5360 ± 0.1500	25.360 ± 1.500
88	**4**	**0.1**	1.5497 ± 0.0684	15.497 ± 0.681
78	**2**	**0.07**	0.0682 ± 0.0010	0.682 ± 0.010
88	**3**	**0.07**	3.3053 ± 0.0597	33.053 ± 0.597
98	**4**	**0.07**	3.6558 ± 0.0101	36.558 ± 0.101
78	**4**	**0.07**	2.7370 ± 0.1454	27.370 ± 1.454
88	**3**	**0.07**	1.3324 ± 0.0910	13.324 ± 0.910
78	**3**	**0.04**	0.3894 ± 0.1137	3.894 ± 1.137
88	**4**	**0.04**	2.5334 ± 0.1253	25.334 ± 1.253
98	**2**	**0.07**	2.4127 ± 0.0660	24.127 ± 0.660
88	**2**	**0.04**	3.5666 ± 0.1099	35.666 ± 1.099
88	**3**	**0.07**	3.3704 ± 0.0743	33.704 ± 0.743
98	**3**	**0.1**	0.3652 ± 0.0643	3.652 ± 0.643
88	**2**	**0.1**	0.8363 ± 0.0287	8.363 ± 0.287

**Combination of parameters done by Design Expert software, in accordance with a Box-Behnken design of experiment*

Quadratic models were used to describe the yield of the two replicates. The empirical models derived for Yield 1 and Yield 2, in terms of coded factors A (digestion temperature), B (digestion time), and C (ratio of feathers to NaOH), are shown in Equations 1 and 2 (Yield 1 for the first replicate, and Yield 2 for the second replicate), respectively.

$$Yield\ 1 = -86.3703 + 1.74197\boldsymbol{A} + 0.46526\boldsymbol{B} + 256.19381\boldsymbol{C} - 0.039185\boldsymbol{AB} - 1.99425\boldsymbol{AC} + 13.40083\boldsymbol{BC} - 0.00807692\boldsymbol{A^2} + 0.42101\boldsymbol{B^2} - 1026.79630\boldsymbol{C^2} \quad (1)$$

$$Yield\ 2 = -83.69301 + 1.69859\boldsymbol{A} + 0.46402\boldsymbol{B} + 236.16559\boldsymbol{C} - 0.032102\boldsymbol{AB} - 1.95600\boldsymbol{AC} + 15.70833\boldsymbol{BC} - 0.007948792\boldsymbol{A^2} + 0.27972\boldsymbol{B^2} - 969.22685\boldsymbol{C^2} \quad (2)$$

The closer the R^2 value to unity, the better the model as this will provide a smaller difference between the predicted value of the response quadratic model and the actual value from the experiment. The R^2 values for Equations (1) and (2) are 0.7607 and 0.7595. These values indicate the inability of the models to provide a predicted response value close to the experimental value.

Analysis of Variance (ANOVA) for the response surface quadratic models (1) and (2) was carried out in order to further determine whether the models derived are both valid and significant. Both results ($p=0.2754$, $\alpha=0.05$ for Yield 1; $p=0.2779$ for Yield 2) indicate that the models derived are not significant. Temperature (A), time (B), and ratio of feathers to NaOH (C), and their corresponding combinations (AB, AC, BC, A_2, B_2, C_2) do not exhibit significant effects on the predicted response of Yield 1. This same trend is also exhibited by ANOVA results obtained from the predicted response model of Yield 2.

Using the Design Expert software, the final optimum conditions were obtained by setting all the variable values within the specified range of their lower and upper limits, while maximizing the values for both yield responses. Table 3 shows the optimized conditions (Temperature = 90.16°C, Time = 4 hours, Ratio = 0.06 g feathers/mL NaOH) that were applied for mass production (using 70 grams of chicken feathers), and the obtained yield after the operation. The moisture and ash content analysis on chicken feathers and lab-produced peptone are shown in Table 4.

Table 3. Yield and percent yield of peptone after mass production

Temperature	Time	Ratio	Yield	Percent Yield
90.16	4.00	0.06	35.5329 grams	50.655

Table 4. Moisture and ash content analysis on chicken feathers and laboratory-produced peptone

Sample	Laboratory Analysis		Industry Standard/ Literature Specification	
	Moisture Content, %	*Ash Content, %*	*Moisture Content, %*	*Ash Content, %*
Chicken Feathers	7.22 ± 0.53	8.52 ± 0.31	6.07	6.62
Peptone	4.47 ± 0.01	18.01 ± 0.48	≤ 6.0	≤ 28.0 [a] ≤ 15.0 [b]

[a] *from Standard Meat Peptone Composition,* [b] *from Standard Casein Peptone Composition*

Bacteriological testing of the laboratory-produced peptone, based from the optimum conditions provided by Phase 1, was done on *Escherichia coli* (Gram-negative) and *Bacillus cereus* (Gram-positive). Each plate of culture media, streaked with the test microorganism, was replicated four times.

Table 5 summarizes the results of this phase. Laboratory-produced peptone yielded the highest bacterial count for *E. coli* (86.8 ± 8.871 CFU/mL) while commercial peptone yielded the lowest count (65.6 ± 5.550 CFU/mL). Laboratory-produced peptone provided the lowest count for *B. cereus* (20.2 ± 3.701 CFU/mL), while both commercial peptone and plate count agar produced relatively similar counts (Commercial = 120.8 ± 3.271 CFU/mL; Plate Count = 138 ± 43.818 CFU/mL).

Table 5. Mean colony forming units (cfu/ml) found in bacterial inocula of *E. coli* and *B. cereus*

Bacterial Inoculum	Mean Colony Forming Units (CFU/mL)		
	Laboratory-Produced Peptone	**Commercial Peptone**	**Plate Count Agar (Standard)**
Escherichia coli	86.8 ± 8.871	65.6 ± 5.550	76.4 ± 7.092
Bacillus cereus	20.2 ± 3.701	120.8 ± 3.271	138.0 ± 43.818

Statistical analyses (One-Way Analysis of Variance and Fisher's Least Significant Difference Post-Hoc Test) showed that the mean colony forming units for *E. coli* on laboratory-produced peptone is significantly greater than those on commercial and plate count agar. Furthermore, the mean colony forming units produced by *E. coli* on plate count agar is significantly greater than those on commercial peptone.

Statistical analyses also showed that the mean colony forming units for *B. cereus* on plate count agar is statistically equal to those produced on commercial peptone. Furthermore, the mean colony forming units produced by *B. cereus* on laboratory-produced peptone is significantly lesser than those on commercial peptone and plate count agar.

3.2 Results of Phase 2

The yields presented in Table 6 are based on the amount of powders received with respect to the amount of hydrolysate sent to the freeze dryer. Vacuum filtered peptone exhibited the largest yield.

Table 6. Yields of peptone (%) based on different purification techniques performed

Purification Technique	Yield
Normal	21.944%
Activated Carbon Treated	26.542%
Vacuum Filtered	50.575%

The moisture content of the chicken feathers and the resulting powders were determined via constant weighing. The chicken feathers underwent six runs of drying and weighing before a constant weight was achieved. Results are shown in Table 7.

Table 7. Ash and moisture content determination

Sample	Moisture Content	Ash Content
Feathers	14.867%	2.433%
Activated Carbon Treated I	12.65%	24.304%
Activated Carbon Treated II	13.681%	24.213%
Vacuum Filtered	12.951%	19.758%
Normal I	8.920%	24.482%
Normal II	14.159%	22.772%

Volhard titration was done to determine the chloride, both free and from sodium chloride, present in the peptone samples**.** Table 8 presents the amount of chloride for each of the peptone samples.

Table 8. Chloride content of peptone samples

Sample	% Cl
Normal	0.804295
Vacuum Filtered	0.670198
Activated Carbon Treated	0.688077

Table 9 presents the results of the bacterial growth test conducted in the NSRI-MRSL. The same set of microorganisms were used for Phase 2 performance testing. Three types of culture media were prepared: normal product peptone, activated-carbon-treated peptone, and vacuum-filtered peptone. Among the three, the normal product performed the best for *E. coli* but did not support bacterial growth for *B. cereus*. The activated-carbon-treated peptone supported the highest growth for *B. cereus*, reaching 16.2% colony growth efficiency. Vacuum-filtered product performed well for *E. coli*, but failed to support good growth for *B. cereus*.

Table 9. Colony Growth Efficiency

Test Agar Medium	Colony Growth Efficiency for *E. coli*	Colony Growth Efficiency for *B. Cereus*
Untreated product	87.9%	0%
Activated-carbon-treated product	82.2%	16.2%
Vacuum-filtered product	82.2%	1.5%

3.3 Discussion of Results

Chenault and Muralidhara [14] has indicated several combinations of temperature, digestion time, and amount of NaOH necessary to dissolve and hydrolyze the chicken feathers. The reaction for hydrolysis occurs typically at around 90-95°C, with a pressure of around 0-15 psig. Furthermore, the time in which the reaction proceeds in order to produce a desired mixture of peptones is a function of the temperature, pressure, nature of the alkaline material used, and its concentration [14]. Results and further statistical analysis of the yield collected after the runs show that none of the pre-determined parameters (temperature, time, and ratio) have a significant effect on the total yield of peptone from the process. This discrepancy may be accounted for by the absence of necessary purification steps in Phase 1. While centrifugation was applied after the neutralization step, no other purification method was employed.

BD Biosciences, one of the leading producers of peptone and other culture media reagents, as well as the aforementioned patent, have included several purification steps in their suggested processes. Ultrafiltration allows for the separation of the lighter permeates from the solution (salt, sugar, other lighter compounds). This leaves a heavier concentrate, consisting of mainly proteins [18]. Electrodialysis may remove lighter proteins from the mixture, creating a permeate solution consisting of smaller peptones. Activated carbon treatment may be done to deodorize the slurry, and remove color bodies in the solution [14]. Activated carbon treatment and vacuum filtration were consequently implemented in Phase 2.

Uzeh et al. [11] applied repeated centrifugation and subsequent freeze-drying in order to obtain powdered peptone from soya and African locust beans. Kurbanoglu and Kurbanoglu [1] opted to use filtration through a filter paper and rotary evaporation to obtain the peptone from ram horn waste. Parrado et al. [10] used resuspension in water and repeated centrifugation to increase the yield of peptone. These steps may be employed in the process developed for the production of peptone from chicken feathers in order to improve the yield, as well as the appearance of the final product. Observations made on the culture media report a murky appearance of the laboratory-produced peptone. Such appearance is opposite of what is produced by the commercial peptone, which yielded a yellowish transparent liquid. The presence of foreign particles was also noted in these observations. The lack of additional purification steps brought about the presence of these solids, and the opaque characteristic of the liquid media. Freeze-drying was subsequently implemented in Phase 2 to determine any observable change in the product characteristic.

Colony forming units (CFU) are a measure of the number of viable bacterial cells that grow in a sample per milliliter [19]. The presence of bacterial colonies on the surface of an agar medium may be expressed in terms of CFU/mL liquid. Thus, CFU/mL indicate the bacterial growth property of a certain agar medium. The higher the CFU/mL count, the higher the bacterial growth observed.

The produced peptone for Phase 1 exhibited higher *E. coli* growth than the commercial peptone and the plate count agar. However, the laboratory-produced peptone exhibited lower bacterial growth than both the commercial peptone and the plate count agar for *B. cereus*.

The difference in the amount of bacterial growth of *E. coli* and *B. cereus* in the laboratory-produced peptone may be understood in the light of their differences in their nutritional requirements. *E. coli* is known to be not selective in its nutritional requirements; it can synthesize all 20 amino acids that it needs for growth. This ability allows *E. coli* to proliferate even in the harshest conditions, as it can metabolically transform any form of glucose into all of the macromolecular components of the cell. Studies have shown, however, that *B. cereus* is known for its fastidious (complex) nutritional requirements. Gram-positive and spore-forming bacteria such as *B. cereus* require the presence of calcium as part of their nutrition in order to synthesize cell walls and spores [20]. Furthermore, White [21] has shown that *B. cereus* requires the addition of amino acids in its medium to ensure optimal growth, unlike *E. coli* which can readily synthesize the amino acids that it needs from glucose only.

The laboratory-produced peptone media has no calcium content, as it only required dextrose, sodium chloride, disodium phosphate, and agar for its composition. This may have attributed to the poor bacterial growth exhibited by *B. cereus* on the laboratory-produced peptone media.

That *E. coli* exhibited the best bacterial growth for the laboratory-produced peptone was also reported by Uzeh et al. [11] for their peptone produced from soya and African locust beans.

Phase 2 recovery of the other powders was small because of the inability of the equipment to completely freeze-dry all of the products.

The production process is prone to unavoidable material loss. In the raw feather procurement, some of the feathers drained down with the water in every washing. Feathers were also lost during air drying since the air carries the light feathers with it. Those which are too small, different in color and appearance were thrown out. During the milling step, more materials were lost as feathers escaped through the spaces between the mill's parts.

Activated-carbon-treated peptone underwent two more rounds of centrifugation. More solids are lost along with the activated carbon. However, there is impurity gain since not all the activated carbon particles are removed. Minimal losses occur during the filtration step since the filtration of the solids left is done via regular filter papers only. Losses in the vacuum filtration are attributed to losses that occur during washing, which is necessitated when blinding of the filter occurs.

The vacuum filtered hydrolysate had a golden yellow translucent color with no trace of solids. The solution gradually got darker as more products are obtained. The activated carbon treated hydrolysate had a grayish green color with dispersed activated carbon particles. The normal hydrolysate was opaque and had a creamy yellow to beige color. All hydrolysates still exhibit the foul odor associated with the feathers.

Vacuum filtration with a polyethersulfone membrane produced a clearer hydrolysate that approximates the appearance of a commercial peptone. The 0.22 μm pore size of the membrane provided better separation of the peptone from the impurities. The membrane material has low binding with

respect to proteins meaning the proteins would pass through the membrane easily. The product obtained had a creamy yellow color resembling that of proteose peptone and finer particles with a more uniform particle size distribution. The distinct poultry farm odor of the product was removed upon drying the hydrolysates. While this type of filtration gave the product with the closest appearance to the commercial peptone, the product would have to be obtained after long hours of operation and frequent occurrences of clogged membrane.

Activated carbon treatment had no significant perceivable effect other than the dispersed black particles it left behind in the solution.

Vacuum-dried peptone was hard to recover from the vessel where it is dried. Dissolution for the culture media preparation is also difficult because of the sticky gel-like properties of the vacuum-dried peptone. The freeze-dried peptone came in powder form and provided ease of transfer from one vessel to another. The powder was also easier to dissolve in water compared with the vacuum-dried peptone.

The freeze-dried peptone had moisture which is about twice of the standard content of commercial peptones. With its hygroscopic nature, the product may have accumulated unwanted moisture between the time of acquisition and moisture determination. A high moisture can be a problem since moisture promotes bacterial growth and degradation. The ash content of the peptone produced is comparable to that of the standard meat peptone but is too high for the standard casein peptone. High ash content is favorable since ash contains the minerals such as calcium and phosphorus needed by the microorganisms.

Peptone samples that were further purified by either vacuum filtration or treatment with activated carbon had lower chloride content than the centrifuged only peptone. The chloride ions present in the peptone samples are present as NaCl. The chloride ion has an ionic radius of 0.181 nm. However, when sodium chloride is in its aqueous form, both the sodium and chloride ions are surrounded with water molecules and chloride ion is heavily solvated by water molecules with an average of six water molecules surrounding each ion [22]. The chloride ion may be removed by the vacuum filter, with pore size of 0.22 μm, by either of two reasons: (1) the solvated chloride ions cannot pass through the vacuum filter, or (2) since the chloride ions is solvated by the water molecules, the solvated chloride ions may have adhered to the surface of the filter.

Compared to the chloride values of the commercially available peptone, as seen in Table 10, the chloride content of the laboratory produced peptone from chicken feathers were lower. This may be due to the difference in the extraction method (alkaline hydrolysis was used for this study). If the commercially produced peptone is produced by acidic hydrolysis with HCl, the acid can be a source of the chloride ions. Also, it could be possible that ion exchange resins were used to decrease the unwanted ions in the peptone and chloride ions were used to replace the unwanted ions.

Table 10. Chloride content of commercially available peptone

Sample	% Cl
Bacto peptone	1.7
Proteose peptone	4.9
Plant peptones	0.2 – 1.0

Compared with the results of Phase 1, the peptone powders produced in Phase 2 have produced higher bacterial growth for *E. coli*.

4. CONCLUSIONS AND RECOMMENDATIONS

This study has shown that a laboratory process involving the alkaline hydrolysis of chicken feathers at specific conditions (Temperature = 90.16°C; Time = 4 hours; Ratio= 0.06 gram feathers/mL NaOH) has produced peptone. Furthermore, this laboratory-produced peptone exhibited the ability to grow *Escherichia coli* and *Bacillus cereus.*

Hydrolysate produced underwent two rounds of centrifugation and were divided for succeeding purification steps. Activated carbon treatment was done on half of the hydrolysates. Activated carbon treated hydrolysate yielded a product with no other perceivable changes than the dark appearance caused by the presence of activated carbon. Though it did not resemble any of the commercial peptones available, the activated carbon treated peptone was able to culture the gram positive bacteria, bacillus cereus. The other powder products were not able to culture the said bacteria but generally the peptone produced had better colony growth than those produced by the previous study. The untreated peptone had the highest colony growth efficiency among all the peptones produced.

The chloride content of the peptone produced was lower than commercial peptones. Activated carbon may have adsorbed some of the chloride ions. For the case of the vacuum-filtered peptone, chloride ions may have been filtered out of the solution since they are highly solvated in their aqueous form.

The moisture content of the powders was higher than the industry specified moisture content of peptones. The additional water content of the peptone powders may be attributed to the incomplete drying of the hydrolysates due to time constraints.

Ash content of the powders were comparable to the ash content of standard meat peptone but were higher than those for the casein peptone.

The appearance of the produced peptone was improved by vacuum-filtration, and approximates the appearance of a commercial proteose peptone.

Further chemical analyses on chicken feathers and the laboratory-produced peptone are also recommended in order to determine the major constituents involved in protein hydrolysis, as well as bacterial nutrition. Studies on growth kinetics of *E. coli* and *B. cereus* and characterization on their respective nutritional requirements may shed light on the behavior of the microorganisms at different culture media. Finally, additional studies on the possibility of isolating peptones from other waste materials may be done.

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