Poultry By-products as a Potential Source of Nutrients

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Abstract

Large amounts of bones and trimmings are generated as by-products of the poultry industry in mechanical deboning of chicken. These by-products are potential sources of valuable components such as proteins and phosphorus. Enzymatic hydrolysis of animal by-products can be used to separate lipids and produce protein hydrolysates resulting in the formation of insoluble sediment rich in phosphorus and nitrogen. In this study, the sediment from the enzymatic hydrolysis of by-product from mechanical deboning of chicken is hydrothermally treated to evaluate the possibility for nutrient recycling. Hydrothermal processing was performed at elevated temperature of 150°C and 220°C using water as the reaction medium with varying pH and time. The elemental composition of the liquid and solid fractions was measured after the treatments. With higher pH, the majority of phosphorus was distributed to the solid fraction while with pH 1 the majority of phosphorus was found in the liquid fraction. Nitrogen was concentrated in the liquid fraction in all processing conditions. The major part of the carbon was distributed to the liquid fraction.

Keywords: Hydrothermal treatment; Poultry; Chicken; Nutrient; Phosphorus; Nitrogen

Introduction

Phosphorus is an essential nutrient for all living organisms and the global food production is highly dependent on the use of phosphorus fertilizers. The world’s population is projected to rise 30-50% by the year 2050 which could implicate a similar increase in the global phosphorus demand [1]. At the same time the phosphate rock reserves, the main phosphorus source currently, are becoming increasingly scarce. To reduce the dependency on the non-renewable phosphate rock, identification of alternative phosphorus sources and development of efficient technologies for phosphorus recovery from currently unexploited sources are required. Besides the sewage sludge from wastewater treatment, by-products and wastes from animal production and meat processing industries are potential sources of phosphorus compounds. For example, the global broiler meat production is estimated to reach 89.5 million tons in 2017 [2]. The rapid growth of poultry production has led to a massive generation of food-processing by-products like bones, viscera, feet, head, blood and feathers. It has been estimated that bones and trimmings constitute about 7-8% to the broiler live weight [3] resulting to the 12.5 million tons of potentially valuable phosphorus-rich by-product in global scale. In addition to dumping, landfilling and combusting exercised in different parts of the world currently, processes such as rendering and composting as well as chemical-, microbial- and thermal treatments have been developed and used to convert animal wastes into livestock feed and organic fertilizers [3,4]. However, the use of meat industry by-products for livestock feeding collapsed after the outbreak of the Bovine Spongiform Encephalopathy (BSE) and currently research on suitable processing technologies for converting animal production by-products to value added products is ongoing [4].

Enzymatic hydrolysis of animal by-products has been widely used to produce lipids and fractions with high amount of amino-acids and a broad spectrum of food ingredients including flavor enhancers and compounds having functional, bioactive and therapeutic properties [4]. In addition to enzymatic treatment, chemical, thermal and microbial treatments have also been used to hydrolyse these by-products. During the production of protein hydrolysates three fractions are generated, lipids, the soluble fraction containing the proteins and the insoluble sediment [5,6]. Several studies have concentrated on the utilization of the soluble fraction while the sediment is seen as a non-desirable fraction and often discarded [4]. Enzymatic hydrolysis of by-product from mechanical deboning of chicken results in a sediment containing considerable amount of bones of rigid and heterogeneous structure. Hydrothermal processing can be seen as a potential option to treat this kind of material.

In hydrothermal processing raw material is heated at elevated temperature and pressure using water as a reaction medium to produce a range of valuable products [7-9]. Processing between 100 and 180°C is usually referred as hot water extraction and it results in the extraction of inorganics and hydrolysis of carbohydrates [10]. When the temperature is increased to the range of 180-250°C, the organic raw material starts to carbonize and the process is called hydrothermal carbonization (HTC) [8]. In hydrothermal liquefaction (HTL) at 280-370°C biocrude oil-like component is produced with increasing amount of gas [9]. Benefits of using hydrothermal processing for animal wastes are the destruction of pathogens typically present in such raw materials [10] and the fact that wet raw materials can be used without energy intense drying step. In this study, the insoluble sediment-rich in phosphorus and nitrogen-from the enzymatic hydrolysis of by-product from mechanical deboning of chicken is hydrothermally treated with varying process conditions (temperature, time, pH) in order to evaluate the possibility for nutrient recovery (Figure 1).
Materials and Methods

Enzymatic treatment

By-product (bones and trimmings) from mechanical deboning of chicken was obtained from Nortura SA (Norway) and treated enzymatically as described by Tveit et al. [6]. The minced chicken by-products were incubated at 50°C with Protamex® enzyme (Novozymes), inactivated by microwave heating (<90°C) and centrifuged to fractionate oil, emulsion, protein hydrolysate and sediments. The insoluble sediments taken after 30, 60 and 120 minutes of hydrolysis were combined, mixed and freeze-dried.

Hydrothermal treatment

The sediments from the enzymatic hydrolyses were processed by varying the pH (pH 6, pH 2, pH 1), temperature (150°C, 220°C) and residence time (15 min, 3 h) (Table 1). The samples (90 g) were suspended to water and the pH was adjusted to the desired level with 4 M sulphuric acid. The dry matter content of the suspensions in all the experiments was 11 wt%. The suspensions were placed in 500 ml autoclaves which were placed in an electrically heated hot air oven. After the hydrothermal treatments reaction mixtures were centrifuged for 20 min (4500 rpm) and liquid fraction was collected separately. A small amount of tar-like oily component was formed on the surface of the liquid fractions. The solid fraction was collected from the bottom of centrifuge tubes, lyophilized and vacuum-dried at 70°C before analyses.

Chemical analyses

Elemental analysis of solid samples (raw material, residue from hydrothermal treatment) for carbon, hydrogen, nitrogen, and oxygen, and liquid and oily component for carbon were determined using using FLASH 2000 EA series analyzer (Thermo Fischer Scientific). The total nitrogen content of liquid samples was analyzed by Kjeldahl digestion method. The amounts of phosphorus, calcium, and potassium in solid were analyzed by using Agilent 7500 ce ICP-MS. The amount of oily component was so small that phosphorus, calcium and potassium content could not be analysed. Phosphate (SFS 3025, modified), total phosphorus (SFS 3026, modified), and ammonium nitrogen (SFS 5505, modified) of liquid samples were analyzed spectrophotometrically. The ash content was determined gravimetrically after burning the samples at 550°C for 23 h.

Results and Discussion

The composition of the sediment from the enzymatic hydrolysis before and after hydrothermal processing is presented in Table 1. The sediment is known to contain a high amount of bone and protein, which explained the high levels of calcium, phosphorus and nitrogen in the original sample, approximately 9.4 wt%, 4.5 wt% and 8.5 wt%, respectively. The ash content of the sediment was 37 wt% on a dry basis. The amount of potassium was minor, 0.35 wt%. The elemental composition (C, H, N, O, P) of the sediment was very typical relative to similar residues [11].

The images of the sediment before and after the hydrothermal treatments are shown in Figures 2a and 2b, respectively. It can be seen that the original sediment contained visible bone pieces (Figure 2a).
which were more or less degraded in the hydrothermal treatments, resulting in solid fractions having 1.5-11 wt% of phosphorus (Figures 2b and 3a). The effect of processing conditions was clear with the experiments. Apart from the experiment performed under pH 1, the solid fractions still contained very small but visible white particles whose amounts were dependent on the pH. The main source of phosphorus in the solid fractions was undoubtedly the existing bone fragments, with a smaller contribution from the organic matter.

The white particles were not analysed separately but taken into account the high amount of bone pieces in the sediment, they most probably consisted of undissolved bone as well as precipitated calcium compounds. Based on the calcium content of the original sediment, 79 to 129% of the calcium input was found in the solid fraction in all processing conditions, which indicated low dissolution and possible precipitation [12]. The values exceeding 100% are most likely caused by inaccuracies in the used analytical methods due to challenging analysable matrix. Bone is a heterogeneous composite material that consists approximately 65% of hydroxyapatite, HAp (generally Ca$_5$(PO$_4$)$_3$(OH)) and 35% of proteins [13,14]. The solubility of calcium (ortho) phosphate compounds increases as the pH decreases and the speed of the dissolution depends on the basicity of the salt [14]. HAp in bones is a basic compound and its dissolution in acidic conditions increases the pH of the solution through the release of hydroxide ions, which can be seen as a pH increase in all the hydrothermal treatments (Table 1). There are multiple models describing the dissolution of HAp (e.g., [15]). In general, the bone HAp dissolution is a multistep process where the surface dissolution is followed by diffusion of calcium and phosphorus into the bulk solution. Depending on the pH and the acid, the dissolved species of phosphorus and calcium can then in some extent be absorbed back to the surface or form various insoluble salts or complexes [15,16]. Calcium has also been suggested to form semi-permeable calcium-rich layers on the apatite surfaces in acidic buffers in the pH range 3.7-6.9 [15].

Distribution of phosphorus between solid and liquid fractions in hydrothermal treatments is shown in Figure 3a. Altogether 102 to 131% of the original phosphorus input was detected in these fractions after hydrothermal treatments. The majority of phosphorus was distributed in the solid fraction in pH close to neutral (pH 6). It is reported that in neutral conditions, the majority of the phosphorus is typically retained in the solid fraction in similar conditions when hydrothermal carbonization process was used for manure [16], wheat straw [17] and sewage sludge [18]. In the case of microalgae, however, all the phosphorus was distributed in the liquid fraction in near neutral pH range at the temperature of 200°C [19]. It has been suggested, that the amount of inorganics influence the distribution of phosphorus during the hydrothermal treatment [10,17] and the amount of multivalent cations (e.g., Mg and Ca) in microalgae was too small for the formation of insoluble phosphates that could be incorporated in solid fraction [17]. Our results for enzymatically treated poultry by-products showed that in pH 2 most of the phosphorus was still located in the solid fraction while in pH 1 the majority of phosphorus was found in the liquid fraction in the form of phosphate-ions (PO$_4^{3-}$-P) (Table 2). This observation showed that the behaviour of the sediment during the hydrothermal treatment in acidic conditions was similar compared to swine manure [10].

<table>
<thead>
<tr>
<th>Processing conditions</th>
<th>PO$_4^{3-}$-P/tot P (%)</th>
<th>Total P/tot P (%)</th>
<th>NH$_4^+$-N/tot N (%)</th>
<th>Total N/tot N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 2, 15 min</td>
<td>87</td>
<td>2651</td>
<td>500</td>
<td>6800</td>
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<tr>
<td>pH 2, 3 h</td>
<td>97</td>
<td>2344</td>
<td>700</td>
<td>7500</td>
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<tr>
<td>220°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6, 15 min</td>
<td>95</td>
<td>398</td>
<td>2100</td>
<td>10000</td>
</tr>
<tr>
<td>pH 2, 15 min</td>
<td>100</td>
<td>1323</td>
<td>1700</td>
<td>8600</td>
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<tr>
<td>pH 2, 3 h</td>
<td>98</td>
<td>796</td>
<td>2600</td>
<td>8000</td>
</tr>
<tr>
<td>pH 1, 3 h</td>
<td>98</td>
<td>5453</td>
<td>2700</td>
<td>8700</td>
</tr>
</tbody>
</table>

Table 2: The amount of phosphate PO$_4^{3-}$ and ammonium NH$_4^+$ in liquid fraction as well as their percentage share from the total P and N, respectively.
According to the results, the influence of pH was more significant on the migration of phosphorus than temperature and treatment time. For sewage sludge it has also been shown [18] that temperature and processing time did not significantly influence the migration of phosphorus even though higher temperature did enhance the solubility of nitrogen. The solid fraction from pH 6 contained the largest amount of small bone particles due to the poor dissolution of the bone under higher pH, which explained the lowest soluble phosphorus concentration of the liquid fractions (Figure 3a). The result obtained using pH 1 was also clear-cut as the pH was low enough to dissolve the bones (no visible particles and the darkest color of all resulting solids) and the phosphorus was transferred to the liquid fraction. Interestingly, the amount of soluble phosphorus in pH 2 in 220°C was lower than in 150°C. Also, the longer reaction time produced less soluble phosphorus in both temperatures. One possible explanation for these phenomena is the end-point pH (Table 1), which was higher under 220°C than in 150°C, indicating higher dissolution of HAp and release of hydroxide ions. The dissolved calcium and phosphorus may therefore formed larger amount of insoluble (acidic) calcium phosphate compounds as the pH increased, thereby keeping the phosphorus in the solid fraction. The end-point pH values may have also been suitable for calcium-rich layer formation with the used conditions, preventing further dissolution of the bone material. The high concentrations of calcium in the solid fraction support these conclusions although the difference to pH 1 is not very high. These interpretations are, however, tentative as the original sediment was treated as such, without further homogenization. Homogenized sediment with similarly sized bone fragments probably would have produced more comparative dissolution behavior for all samples.

Distribution of nitrogen between solid and liquid fractions is shown in Figure 3b. Altogether 92 to 122% of the original nitrogen input was detected in these fractions after hydrothermal treatments. Solid fraction also included oily fraction forming up to 4% from the original nitrogen input. The majority of nitrogen was concentrated in the liquid fraction. The origin of nitrogen is the organic residue and probably also the proteins remaining inside the bone fragments in the sediments after the enzymatic treatment. The nitrate and nitrite concentrations were very low (below detection limits) which supports the statement of the nitrogen origin being mainly organic [20]. As proteins hydrolyse, the released amino acids first dissolve in water and then further hydrolyse into ammonium nitrogen. Increasing temperature and time promoted the hydrolysis resulting in higher ammonium concentrations [21] which was clearly seen in the results. The amount of ammonium nitrogen (NH$_4^+$-N) from the total nitrogen in liquid fraction varied between 7 and 9% in 150°C and between 21 to 33% in 220°C (Table 2). The results are also consistent with the study of Kruse et al. [21] who found increasing ammonium concentrations after hydrothermal carbonization of carrot green as the temperature was raised from 180 to 220°C, although the used pH was much higher. They also found temperature to be a more significant factor for the ammonium increase than time, which again is consistent with our results.

The majority of potassium (79-96%) was distributed to the liquid fraction being negligible in the solid fraction (0.04-0.11%). Potassium, released from the organic matter of the sediment, remained in the solution phase, as potassium compounds are mostly water-soluble. Furthermore, under acidic conditions, the remaining solid surfaces are generally positively charged, thereby unattainable for cation adsorption.

Up to 61% of the original carbon was migrated to the liquid fraction with the higher temperature resulting in a solid fraction with rather low carbon content between 10-23% (Table 1, Figure 4). At lower processing temperature of 150°C, the carbon contents of the solid fractions were slightly higher. Solid yield varied between 42 and 48% at 220°C and between 62 and 65% at 150°C. Tar-like oily fraction formed at 220°C contained 71% of carbon and formed 0 to 21% of the original carbon input. Overall 75 to 98% of the original carbon input was detected in solid, liquid and oily fractions with the lowest rates at the higher temperature. The rest of the carbon was gas, mass losses and/or analytical errors. The amount of produced gas was not measured in this study, but the trend of gas production was very similar to the previous studies showing a gas increase with the increase of the processing temperature and time [22].

![Figure 4: Distribution of carbon between solid and liquid fractions during hydrothermal processing.](image)

**Conclusion**

Phosphorus solubility was mainly pH dependent whereas nitrogen extraction from the sediment was mostly affected by temperature. Decreasing the process pH increased the amount of P extracted whereas increasing temperature and time favoured nitrogen extraction. A significant amount of organic-N was transferred to the liquid fraction. The behaviour of potassium was independent of processing conditions and highly soluble. The extraction of carbon was mainly temperature dependent with higher temperature increasing the amount of C in the liquid fraction, thereby decreasing the solid yield. Calcium dissolution from the bones was again pH dependent with higher extraction under lower pH. The results show that hydrothermal
treatment is a viable solution for processing poultry by-products to recover nutrients.

Acknowledgment

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References