Pressure Hydrolysis of Protein in Waste of Chicken Cartilage and Feathers in the Presence of Carbon Dioxide

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PRESSURE HYDROLYSIS OF PROTEIN IN WASTE OF CHICKEN CARTILAGE AND FEATHERS IN THE PRESENCE OF CARBON DIOXIDE

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Abstract
Proteins hydrolysis of chicken cartilage and feathers were carried out at increased temperature (till 120 °C) and in the presence of carbon dioxide (partial pressure 10 20 bar), which dissociates in water solution forming an acidic environment supporting the reaction. Carbon dioxide was easily detachable from the reaction mixture at the end of process. Bench scale tests were performed using a mixed autoclave (volume 2.5 lt, mixing time 5 hr). The resulting aqueous solution of amino acids contained in the same representation as peptides forming collagen and keratin of raw material. The reaction conversion was proportional to carbon dioxide partial pressure.

Introduction
One of the most important tasks of the Centre Biorefinery BIORAF is finding ways for obtaining valuable substances by chemical transformation of biomass, animal and vegetable origin. This task is undoubtedly important because it represents a unique biomass, a renewable raw material base. In the case of animal biomass, significant attention has been paid to the waste from agricultural, respectively. food plants - waste chicken cartilage and feathers that after acid hydrolysis to give a mixture of amino acids nutritional value. Collagen represents the main protein of the various connective tissues in animals, e.g. cartilage[1]. Producers of collagen-based food supplements containing hydrolyzed collagen sometimes claim the skin improvement and health support. For example, Schauss et al.[2] described also improved osteoarthritis-related symptoms in application of a low molecular weight dietary supplement consisting of hydrolyzed chicken sternal cartilage extract. The hard keratin forming e.g. feathers of birds represents fibrous structural protein[3]. Keratin has large amount of the sulfur-containing amino acid cysteine. Formation of disulfide bridges via intra- and inter-molecular hydrogen bonds brings rigidity and thermal stabile cross-linking of the keratin structure. Hydrolyzed keratin has become a common cosmetic ingredient. Studies have shown topical application of hydrolyzed keratin gives significant increases in skin elasticity and hydration[4]. Due to its moisturising properties, hydrolyzed keratin has also been incorporated into shampoo and conditioner. Difficult in poultry waste chicken feathers, whose liquidation is currently being dealt anaerobic fermentation biogas and mixed with other wastes from processing slaughter lines. Other methods of disposal of waste feathers - incineration, composting is problematic because the badly burning waste and composting very slowly decays. Hydrolytic cleavage of peptide bonds in the protein structure of feathers results in a mixture of amino acids. The resulting aqueous solution can be easily mixed with compost or as a component in dressings plants, thereby achieving a recycling biogenic elements in agricultural process.

Pressure hydrolysis of chicken wastes
Hydrolytic cleavage of peptide bonds in the protein structure, chicken cartilages and feathers, produces an amino acid mixture of high nutritional value. The resulting aqueous solution can be easily refined and modified thicken into valuable nutrients. For hydrolysis of collagen and keratin is however preferable to use as catalyst a mineral acid or alkali compound, since the product would be required for neutralization and thus final product is contaminated by salts.
The proposed technology eliminates the disadvantages mentioned, by applying pressure hydrolysis at elevated temperature in the presence of carbon dioxide which provides the necessary acidic environment for the successful progress of the reaction and after hydrolysis carbon dioxide is easily separable from the reaction mixture. The resulting aqueous solution contains valuable amino acids in the identical ratio as the starting collagen and keratin, respectively.
The pilot experiments were made using chicken cartilage and feathers, (Rabbit, a. s., Trhový Štěpánov). Starting materials were frozen, wrapped in icy water. The pressure mixed reactor (autoclave) with a regulated heating

was used for materials hydrolysis. Intensive mixing of reaction volume is necessary because the responsive system comprises three phases - carbon dioxide gas, a liquid aqueous medium, and a raw material in the solid phase. During the hydrolysis process, there is a gradual disappearance of solid phase and forming an aqueous solution of amino acids, optionally with a small proportion of unreacted peptides.
It is evident that a kinetic course of reaction conversion of animal waste into amino acids solutions is the faster the higher temperature and carbon dioxide pressure are used. By application of biomass to water mass ratio 1: 6 very promising results were achieved at reaction time of 5 hours, temperature interval 115-120 ° C and pressure 15-20 bar. Under these conditions, high peptides conversion to the desired amino acids was achieved (over 95%). But, choosing a higher temperature is undesirable due to possible thermal decomposition of raw materials or products, also carbon dioxide concentration in liquid reaction mixture is then reduced due to the higher water partial pressure. These facts demonstrate the following experimental data.

Hydrolysis of chicken cartilage

For hydrolysis of 300 ml frozen cartilage was used. Chopped wet waste chicken cartilage of two different origin (minced and sliced forms) were fed along with 1000 ml of water into the autoclave volume of 2.5 lt. Experiments 1 and 2 were carried out at temperature 105 °C and pressure 1.5 MPa.
Elemental composition of dried product is given in Table I. and show a good reproducibility of elemental analysis. The process results did not depend on disintegration of cartilage prior to hydrolysis. A similar conclusion can be drawn when comparing amino acid content in the product from experiments 1 and 2, as shown in Figure 1. It is also confirm good reproducibility of cartilage hydrolysis.

Table I
Elemental analysis of dry product (wt. %)

Element	#1-Minced chicken cartilage	#2-Sliced chicken cartilage
Carbon	40.1	40.3
Hydrogen	5.61	5.58
Nitrogen	12.0	12.1
Sulfur	2.60	2.32
Oxygen (imputation)	39.7	39.7

A detailed picture of the resulting product gave HPLC-MS analysis of the hydrolysate, see Fig. 1. (UCT Prague, FFBT). From the results follows that the hydrolyzate contains also undeterminated components (unreacted collagen, glykosoaminoglykany, etc.) because total quantity of amino acids is significantly less comparing to processed batch cartilages. The most significantly represented in the hydrolyzate represents asparagine, the content of the order exceeds the proportion of other amino acids.
Likewise, the chromatographic analysis of the hydrolyzate is well reproducible. The hydrolysis product can be recommended to perform application tests both to stimulate growth of plants, algae, as well as nutritional tests for livestock and possibly after further evaluation as an ingredient for food supplements, etc.

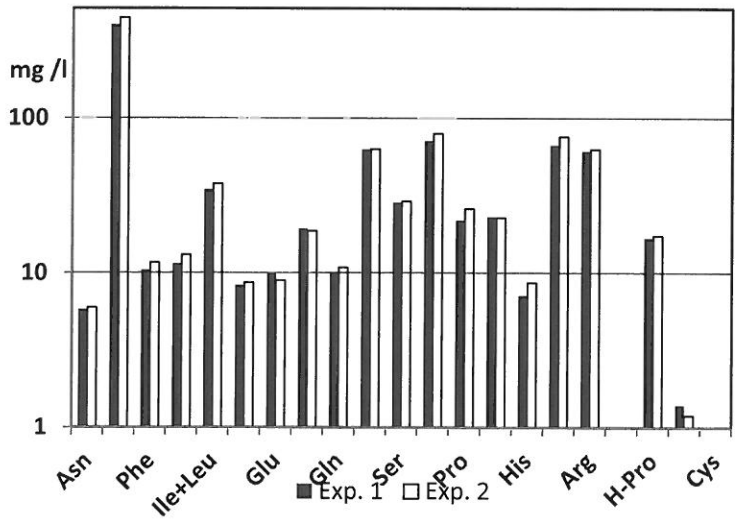

Figure 1. Comparison of amino acids content in liquid hydrolysate; experiments 1 and 2

Hydrolysis of chicken feathers

For hydrolysis of waste chicken feather was chosen the same procedure as in the case of cartilage. In experiments 300 ml of wet waste chicken feathers (dry matter 25%) was used and poured over 1000 ml of water. The weight of dry solids feathers was 12.5 g. This mixture was submitted to a stainless steel autoclave 2.5 liter. The autoclave was filled with cold carbon dioxide and its pressure in experiments 1 and 2 was maintained at 11 and 21 bar, respectively. After heating the mixture (2 hr) to reaction temperature (105-112 °C) under stirring frequency of 1 Hz the feathers were hydrolyzated for 5 houres and autoclave was cooled down over night to ambient temperature (12 hr). The reaction products were brown colored without any solid rests. The very important parameter which significantly influences the kinetics of the hydrolysis process is the carbon dioxide pressure. Its higher concentration control lower pH value of reaction mixture which support the hydrolysis process. Thus, there was observed a direct proportionality between reaction conversion and carbon monoxide partial pressure applied during hydrolysis process, see Fig. 2.

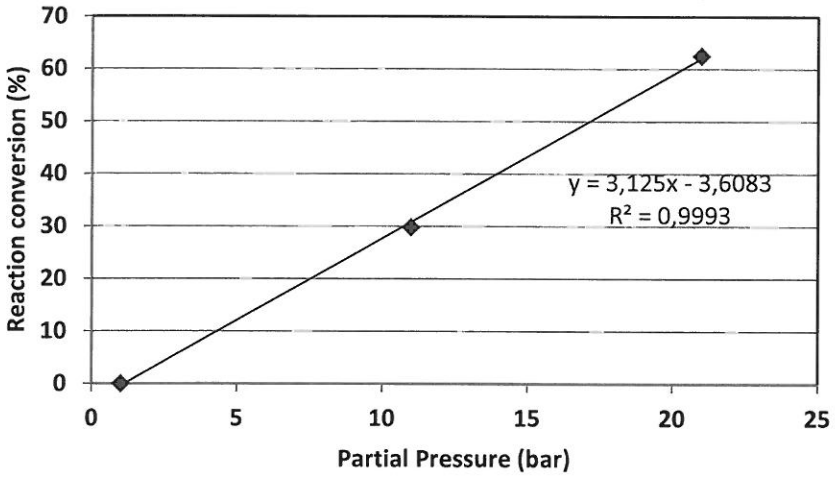

Figure 2: Effect of carbon dioxide pressure on reaction conversion of feather hydrolysis

A detailed product composition was investigated by HPLC/MS analysis (UCT – FFBT Prague). Figure 3 demonstrates a representation of amino acids in hydrolysate. The content of unreacted protein was determined by subsequent product hydrolysis in presence of hydrochloric acid. The reaction conversion was 29.8 % and 62.5 % in experiments 1 and 2, respectively. It is evident the hydrolyzate does not contain all the essential amino acids in the proportions that the human body requires.

From this analysis it was possible to further specify a representation of the individual elements and amino acids in a protein - carbon, hydrogen, nitrogen, sulfur and oxygen (determined by imputation up to 100%), which was compared with the elemental analysis of the dried residue of the hydrolyzate (3.6 and 4.8 g / 100 g hydrolyzate; Experiments 1 and 2, resp.). There was found very good agreement elemental representation in the hydrolyzate and its residue, as shown in Table II.

The liquid product was also analyzed by the classical elemental analysis for sulfur content in hydrolyzate of 0.118 g / lt and nitrogen 2.02 g / lt; the ratio corresponds very well to the content of these elements in the feathers (nitrogen, 13.69% and sulfur 0.80 % respectively). The data for nitrogen is also in relative good agreement with the mean content of nitrogen in the amino acid mixtures in both experiments, see Table II. Content of the other elements, except sulfur, in hydrolyzate corresponds very well to mean elemental composition of amino acids mixture determined by HPLC/MS method.

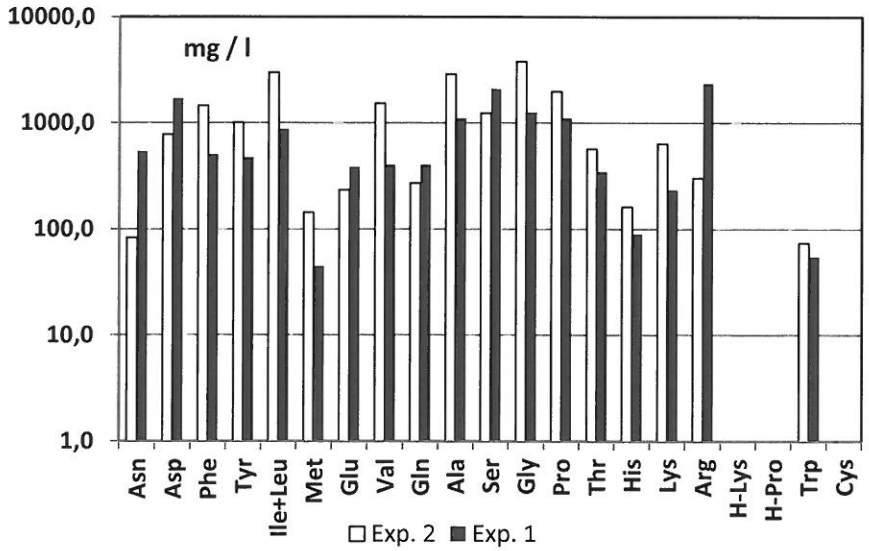

Figure 3: Comparison of the amino acids concentration in hydrolyzate; Experiments 1 and 2

Table II

Comparison of the elemental composition of dry feathers hydrolysis product and mean elemental composition of amino acids in hydrolyzate (Experiments 1 and 2)

Element	Dry residue (% wt.)	Amino acids (% wt.)
Carbon	46.7	44.0
Hydrogen	7.64	7.56
Nitrogen	12.0	14.0
Sulfur	1.18	2.73
Oxygen (imputation)	32.5	31.7

Conclusion

The hydrolysis product is perspective one for various applications, first, for dampening the composted agricultural waste, next could be useful as nutritional additives to livestock feed and finally as a nutrient supporting growing algae for biotechnology applications. For any culture, or nutritional usage of hydrolyzate as ingredient in feed mixtures have to be necessary to perform additional relevant field tests.

Acknowledgement

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