Kinetics of Keratin Acidic Hydrolysis in Batch Autoclave.

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KINETICS OF KERATIN ACIDIC HYDROLYSIS IN BATCH AUTOCLAVE

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Abstract

The contribution brings novel data on the non-traditional process presented during previous ICCT 2015 conference[1]. According to the patent application[2] the hydrolysis of waste chicken feathers was performed in a reaction mixture containing water and a soluble acid catalyst with the pKa value lower than 4, in a stirred batch reactor for 0.5 to 10 hours, in the temperature range from 90 to 130°C and at the pertinent vapour pressure of reaction mixture. Kinetics of preparing a mixture of proteins and amino acids by hydrolysis of a waste material containing chicken feathers was investigated in this study.

Introduction

Hydrolytic splitting of peptide bond in a keratin protein structure in waste feathers results in a mixture of low molecular weight peptides and amino acids. This aqueous solution can be easily mixed with compost or added as a component in a plant's dressing. Thus, it enables recycling of biogenic elements in an agricultural process. The hydrolysate has a promising use for plant's protective sprays against stress, caused by e.g. increased intensity of sun exposure, lack of moisture, *etc.*

Proteins hydrolysis is accelerated in an acid or alkaline environment and by elevated temperature. Use of inorganic or mineral acids is disadvantageous policy, because resulting hydrolysate prior its application requires a neutral adjustment of pH value. Hydrolysis can be processed in the presence of organic acids and/or using carbon dioxide dissolving in water and creating the necessary slight acidic environment by their dissociation. Its concentration in water can be increased using a higher pressure in the reactor.

Simultaneous proteins and fat hydrolysis of chicken feathers were carried out at increased temperature and in the presence of carbon dioxide (partial pressure 1 - 2 MPa) in this study. Bench scale tests were performed using a mixed autoclave (volume 2.5 lt., typical reaction time 5 hr). This procedure was described in the patent application[2]. Knowledge of kinetic aspects of the feather hydrolysis and process parameters operation window are inevitable for a process scale up.

Experiment

Low molecular proteins and amino-acids formation by keratin hydrolysis was investigated in this study. Experiments were carried out using fried chicken feathers from Rabbit Co. Trhový Štěpánov. This waste material (typically 300 ml, weight 75 g) was put together with 1 litter tap water into pressure autoclaves, volume 2.5 and 2.0 litters, respectively. In some tests dried and cut feather was used, too. In series of experiments the effect of hydrolysis time, temperature (or corresponding reaction mixture vapour pressure) was investigated. Starting the kinetic tests an inert gas (preferentially carbon dioxide) was introduced to the reactor in order to minimize undesired transformation of batch components by oxidation. Reaction time between 3 to 7 hours was applied (including heating up and cooling down of the reactor, i.e. 2 hours). Reaction temperature was maintained at 130 °C which corresponded to pressure 16.9 bars. The starting temperature for keratin and feathers fat hydrolysis was supposed to be 110 °C.

At the end of the test after a reactor cooling down the reaction product was separated by filtration to liquid hydrolysate (pH of the hydrolysate was 6.4 in all cases) and solid waste which was dried to investigate a mass balance equilibrium of hydrolysis. The mass balance of the individual tests was nearly identical. The mass deficit was undoubtedly caused by handling losses as emptying the autoclave, separation of reaction products, filtration and drying of the solid waste. Liquid filtrate was analysed using HPLC/MS and GC/MS methods. The total content of soluble peptides of low molecular weight and amino acids distribution were determined in the hydrolysate for all samples. Several samples were used for screening of feather fat hydrolysis products like free

fatty acids, mono-, di- and tri-glycerides of different fatty acids as well. The effect of reaction time on hydrolysate colour was tested using IR-FT method. In some experiments the dried waste was used for the 2nd step hydrolysis.

Kinetics of feathers hydrolysis

Time function of proteins concentration in hydrolysate of frozen dry feathers is illustrated in Figure 1. The first experiment corresponding to reaction time zero was aimed to find a reaction conversion during the first step of operation. In this starting test the reaction mixture was only heated up to temperature 110 °C and then cooled down to ambient temperature. The subsequent tests were done at the same temperature for different reaction time. The results showed that the reaction time of 3 h was sufficient to achieve the equilibrium protein's concentration in the hydrolysate. The equilibrium is probably limited by their solubility in reaction mixture, containing many other components – amino-acids, free fat acids and fatty acid glycerol's.

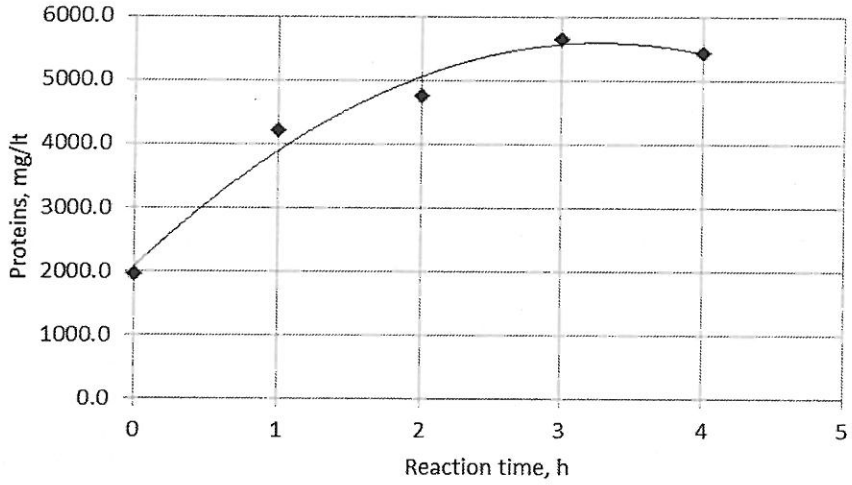

Figure 1. Proteins concentration versus reaction time of hydrolysis at 130 °C and 1.69 MPa

Formation of the soluble proteins and amino acids in the hydrolysate proceeds just in begin of the keratin hydrolysis, as well. The time dependence of the total content of amino acids is shown in Figure 2. It is interesting that during hydrolysis of dry feathers occurs a moderate decrease in summa of amino acids content. This fact can be associated with a parallel thermal decomposition some of them.

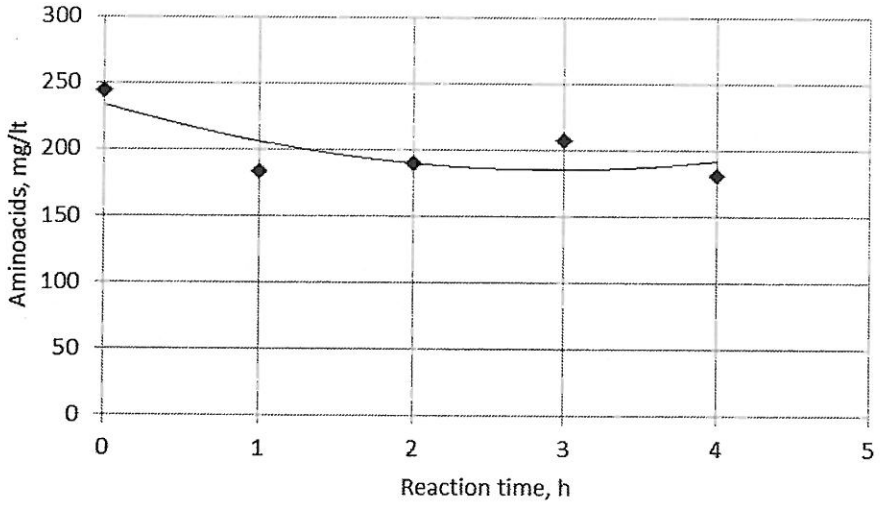

Figure 2. Summa of amino-acids concentration versus time of hydrolysis at 130 °C and 1.69 MPa

On the other hand, concentration of aspartic acids in the hydrolysate slightly increases to its limited value, see Figure 3.

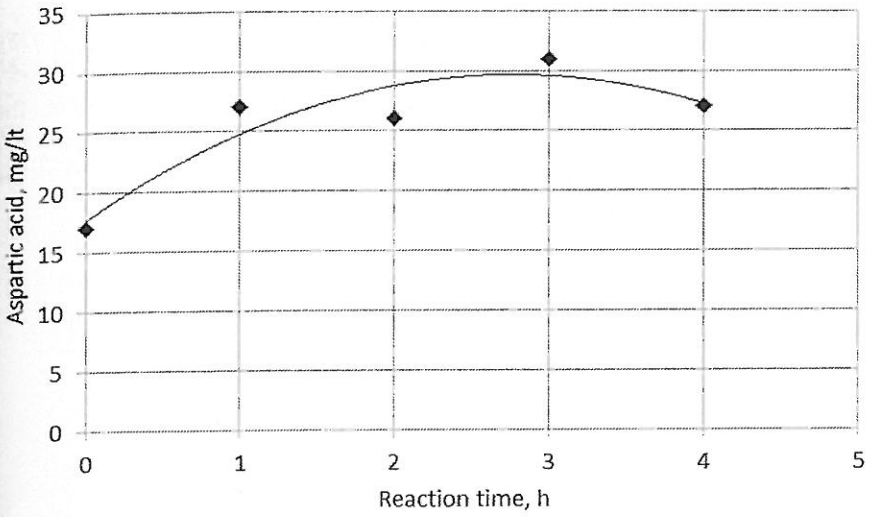

Figure 3. Aspartic acid concentration in hydrolysate versus reaction time at 130 °C and 1.69 MPa

Distribution of the individual amino acids in hydrolysates prepared during various reaction times is shown in Figure 4. In contrast to the increase in content of the aspartic acid hydrolysate it was possible to identify more dominant folder in the first phase hydrolysis – bioconversion glutamic acid and arginine. It's unclear why a large number of these two amino acids is created in autoclave by simply heating at temperature 110 °C only with subsequent cooling (i.e. short time of hydrolysis at low temperature).

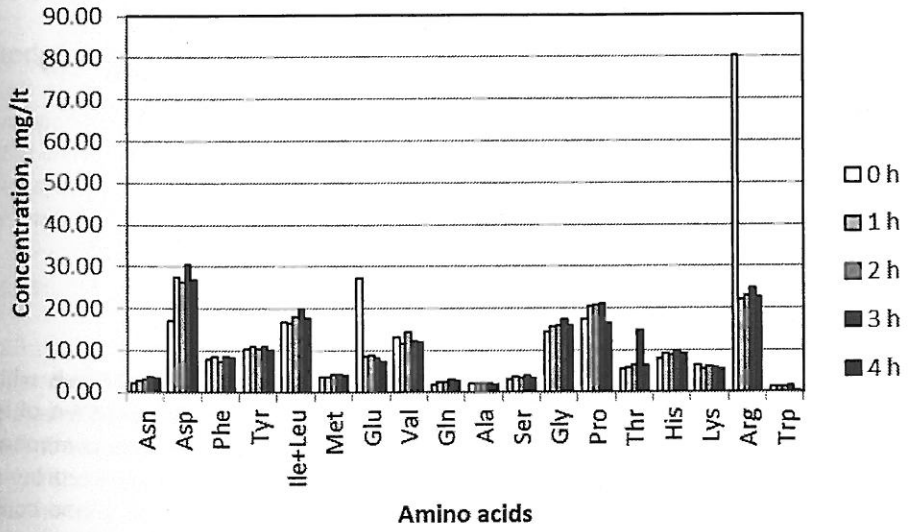

Figure 4. Representation of amino acids in the hydrolysate, depending on reaction time (0 to 4 h)

According to the representation of the low molecular weight proteins in the hydrolysate the optimum reaction time is equal approx. 3 h, while the content of amino acid reaches the equilibrium values already in the first stage of the hydrolysis. After 1 h hydrolysis their summa concentration in the hydrolysate in the course of decomposition reactions gradually decreases.

It cannot be excluded that the achievement of a steady-state representation of the amino acids in the hydrolysate can be related to the limited solubility of the individual amino acids. The reason consists in a complex hydrolysate composition.

The higher reaction conversion of keratin is reached with increasing reaction time. The intensity of a yellow discoloration of the hydrolysate was observed in this case. Figure 5 brings measured UV-VIS-FT spectra of hydrolysates (reaction time from 7 h -top to 3 h -bottom) after their filtration. This simple analytical method can be recommended as a reaction product quality test using absorbance value at light wave 350 nm as an example. Further, it should be stressed that very good absorbance of hydrolysates predetermine their application as anti-stress agent for plants controlling sun light intensity.

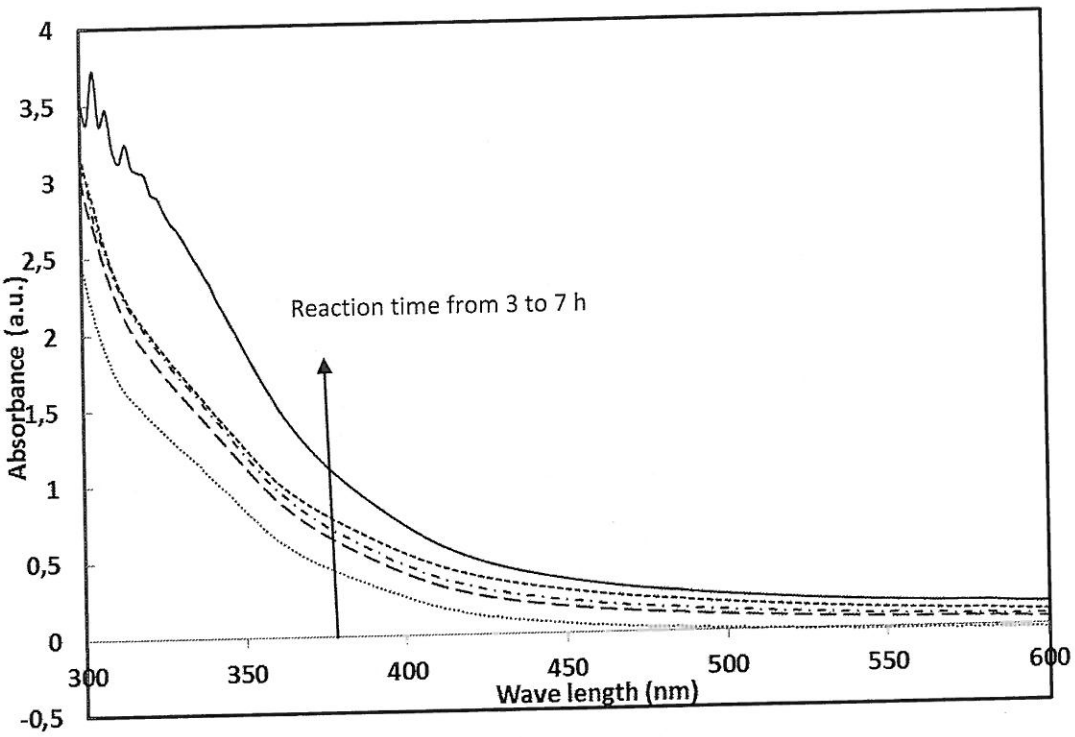

Figure 5. Effect of reaction time on absorbance spectrum of hydrolyzates (UV-VIS/FT)

Products of feather fat hydrolysis

As was mentioned above, parallel reaction to proteins transformation is feather fat hydrolysis which takes place in the reaction system as well. The following Figure 6 shows the relative representation of free fatty acids, monoacylglycerols, triglyceroles of fatty acids and the total content of the following components. Two different autoclaves volume 2.0 and 2.5 litres were used for these experiments. A very good reproducibility of the experiments was stated. It's worth noting a fact that the reaction product did not contain any diacylglycerols and only a tiny amount of monoacylglycerols. The presence of free fatty acids (FFA) as a good surfactant has a positive effect on hydrolysate application in agriculture as spraying agent of plants. A surface active agent supports a very good wettability of plant's sheets. This effect, together with the sun light intensity prevention (see above), is appreciate very much as well.

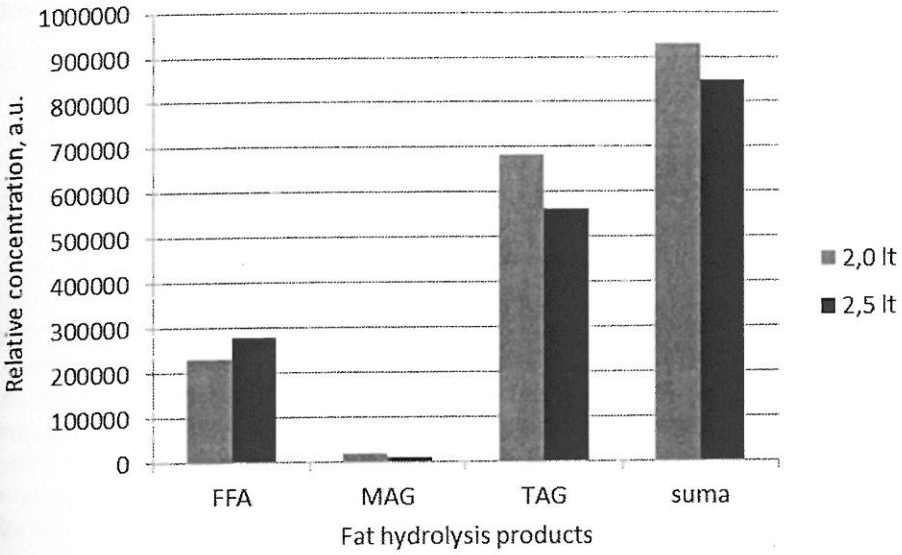

Figure 6. comparison of the concentrations of lipids in hydrolysates of hydrolysates from autoclaves and 2.0 2.5 L capacity

Conclusion

Recycling economy is a strong imperative of the current mankind epoch and a focus on a bio-element's recycling should stay in the first line. It was shown that the waste chicken feathers from agriculture and food industry represent very good raw material not only for intensification of this segment of economy but also for synthesis of chemical specialties, representing by amino-acids and low molecular proteins. The next steps in research and development of the chicken feather hydrolysis should be focussed on the process scale up and final products formulations as well.

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Financial support from the Technology Agency of the Czech Republic under the Competence Centre BIORAF (project No. TE01020080) and Strategy AV21, Foods for the future is very much acknowledged.
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