Simultaneous Hydrolysis of Proteins and Fats fro Waste Chicken Feathers.

Rousková, Milena
2018

SIMULTANEOUS HYDROLYSIS OF PROTEINS AND FATS FROM WASTE CHICKEN FEATHERS

Rouskova M.[1], Solcova O.[1], Sabata S.[1], Jiru M.[2], Hurkova K.[2], Hanika J.[1]

[1]*Institute of Chemical Process Fundamentals of the CAS, v.v.i., Rozvojova 135, 165 02 Prague 6, Czech Republic*
[2]*University of Chemistry and Technology Prague, Technicka 5, 166 28 Prague 6, Czech Republic*
rouskova@icpf.cas.cz

Abstract

The kinetics of the simultaneous hydrolysis of macromolecular proteins (keratin) and lipids (fat) from waste chicken feathers was investigated in this study. The perspective option pressure hydrolysis at elevated temperature was designed as one of the primary tasks within the research program in frame of the Competence Centre BIORAF, supported by the Technology Agency of the CR. The process development of simultaneous hydrolysis of proteins and lipids contained in the waste is focused on this potential alternative raw material for the preparation of valuable amino acids[1] and proteins of low molecular weight. In this study, the acidic hydrolysis kinetics of the waste biomass was performed using a batch stirred bench scale autoclave at elevated temperature and pressure of inert atmosphere from 0.9 to 2.3 MPa. Produced hydrolysate was filtrated and subsequently analyzed using HPLC/MS method at the UCT Prague.

Introduction

Poultry feathers represent a difficult waste from the food processing industry[2]. There are no rational usage and great troubles in case of both composting and incineration. There is a need to recover biogenic elements in accordance with the natural circulation. Hydrolysis of waste feathers can provide valuable amino acids and proteins in the mixture with acylglycerols and higher fatty acids.

The possibility of hydrolysate promises recovery in agricultural trade in a form of protective or stimulating plant sprays. Another possible use of products is, i.e., in the field of preparation[3] of biomaterials for regenerative medicine, cosmetics, biodegradable food packaging, functional ingredients in food etc.

The commonly applied hydrolysis using a strong mineral acid[4] or base[5] leads to the necessary subsequent recycling of the process solutions, including neutralization and the resulting formation of undesirable salts. It is therefore required to search for greener bio-waste treatment.

Materials and methods

Samples of waste chopped frozen chicken feathers (genotype Ross 308, age 39 days) were supplied by Rabbit Trhový Štěpánov, Corp. Dry matter was determined to 35 wt.%.

Hydrolysis was executed in batch stirred bench scale autoclave (volume 2 litres) under CO_2 atmosphere for 4 hours at elevated temperatures from 85 to 126 °C, working pressure was sustained in the range of 0.9 to 2.3 MPa. Produced hydrolysates were filtered.

Profiles of amino acids, low molecular weight proteins and lipid components were determined by HPLC/MS method at the Department of Food Analysis and Nutrition FFBT UCT Prague.

Results

During the study of waste feather hydrolysis, the influence of pressure and temperature on the creation of the monitored substances was pursued. The concentrations and distribution of individual amino acids (AA), the water-soluble low molecular proteins and the lipid portion components – triacylglycerols (TAG) and free fatty acids (FFA) were analyzed.

Profiles of selected amino acids

Totally 23 amino acids were determined in hydrolysate samples. To compare the amino acids concentrations, particularly those, which are dominantly present in the reaction products were selected in Figure 1 and 2. Their sum exceeded 70 % of the total identified quantity.

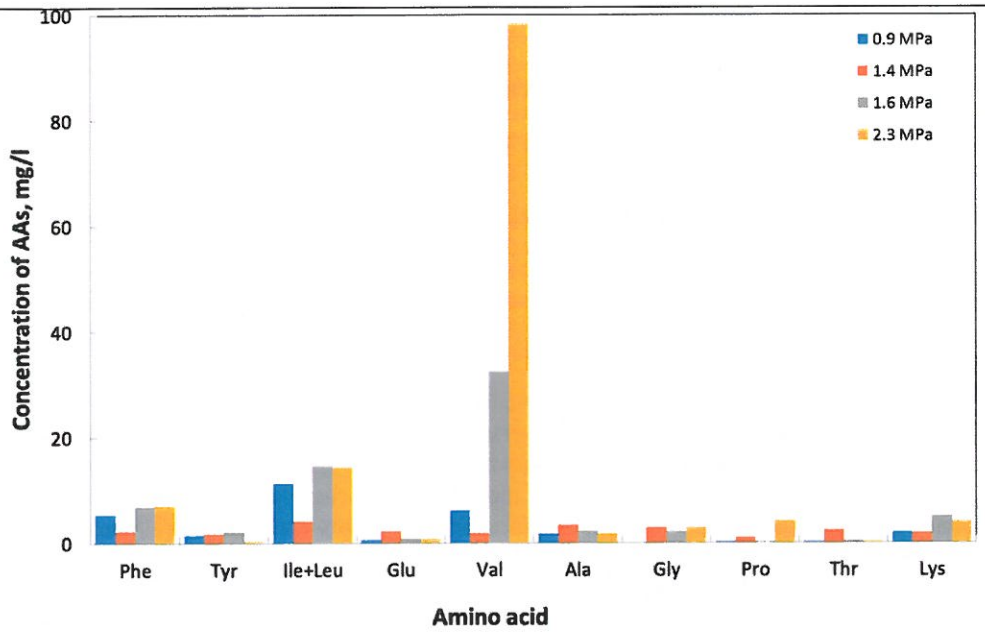

Figure 1. Pressure effect on amino acids composition (temperature 113-115 °C)

The results of experiments depicted in Figure 1 were performed in this case at temperatures of 113-115 °C, a reaction time of 4 hours and a pressure range of 0.9 to 2.3 MPa. The total amount of amino acids grew with an increasing pressure of 0.9 - 1.4 - 1.6 and 2.3 MPa in the order of 29.9 - 27.5 - 68.6 - 135.4 mg/l, which from the pressure of about 1.5 MPa shows the strong dependence of the concentration of the total amount of amino acids in the hydrolysate on the operating pressure of the hydrolysis process. This form of dependence is probably related also to the content of free fatty acids in the hydrolysate resulting from the hydrolysis of lipid components of the feathers.

The most significant influence of pressure can be seen in the increase of the valine content. It, due to the non-reactive aliphatic side chain, belongs to the family of hydrophobic amino acids. It is incorporated into the internal structure of proteins, and therefore very limitedly accessible. It is evident that the increased reaction pressure contributes to its release from the structure of the processed material.

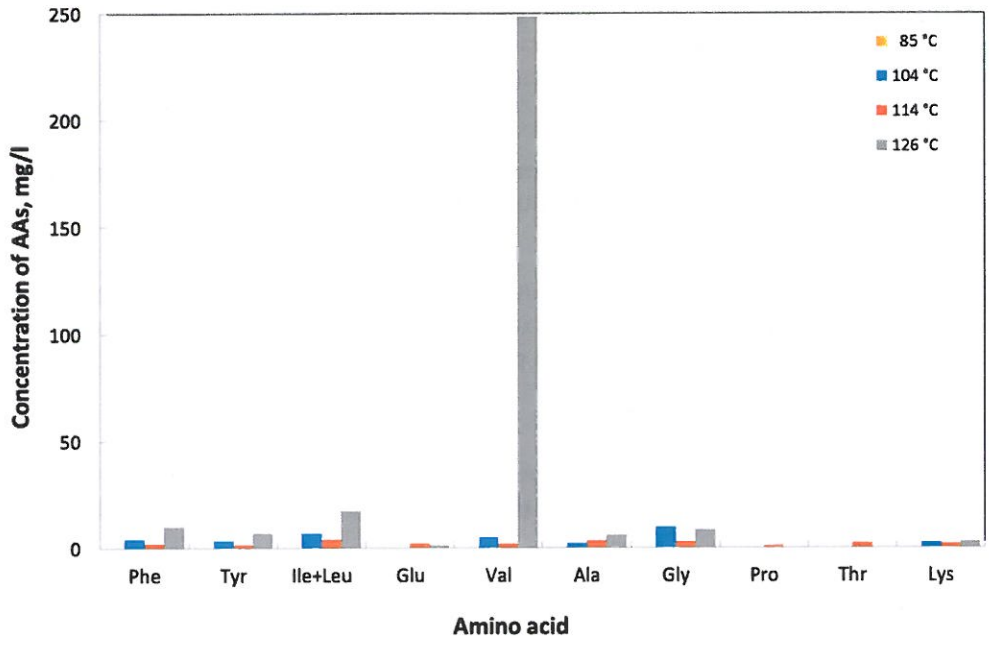

Figure 2. Temperature effect on amino acids composition (pressure 1.3-1.5 MPa)

Similarly, in the case of the temperature dependence of the total amino acid concentration (demonstrated by the results in Figure 2), it cannot be expected a monotonously growing trend in accordance with the Arrhenius

equation. However, it should be realised that the hydrolysis of keratin to amino acids represents a system of subsequent reactions. The total amount of amino acids grew with an increasing temperature of 85 - 104 - 114 and 126 °C in the order of 0.2 - 36.8 - 27.5 - 304.6 mg/l. The first stage represents hydrolysis of the keratin to water-soluble proteins with a lower molecular weight. Thus, the temperature dependence of amino acid formation in the second reaction stage has a more complex shape.

The experiments were in this case carried out under reaction times of 4 hours, temperatures of 104 to 126 °C and pressure of 1.3-1.5 MPa.

The results show not only an increase in the total amount of determined amino acids. Especially notable is the dominant increase in the concentration of phenylalanine (Phe), isoleucine/leucine (Ile + Leu) mixture and, in particular, valine (Val) amino acids. The vigorous change in valine content in hydrolysates at temperatures above 115 °C represents release from the internal structure of feathers from the order of units mg/l to 248 mg/l. A branched-chain amino acid mixture, i.e. leucine/isoleucine/valine, most commonly in a 2/1/1 ratio, is one of the popular dietary supplements in bodybuilding. However, there are no detail studies to determine long-term safe and effective doses[6].

Proteins

Other studied substances belonged to low molecular proteins resulting from the cleavage of disulphide bridges in the keratin molecule.

Figure 3 shows the analogous trends in the influence of reaction pressure and temperature on the content of soluble low molecular weight proteins in chicken feather hydrolysates. For example, from the trends of dependence on these figures, it can be assumed that the limit concentrations of the proteins in the reaction mixture are thermodynamically limited by their limited solubility in the actual reaction mixture. Although the change in pressure to the total amount of obtained proteins had virtually no effect, the increase in temperature significantly increased the cleavage of keratin and releasing of proteins into the reaction solution.

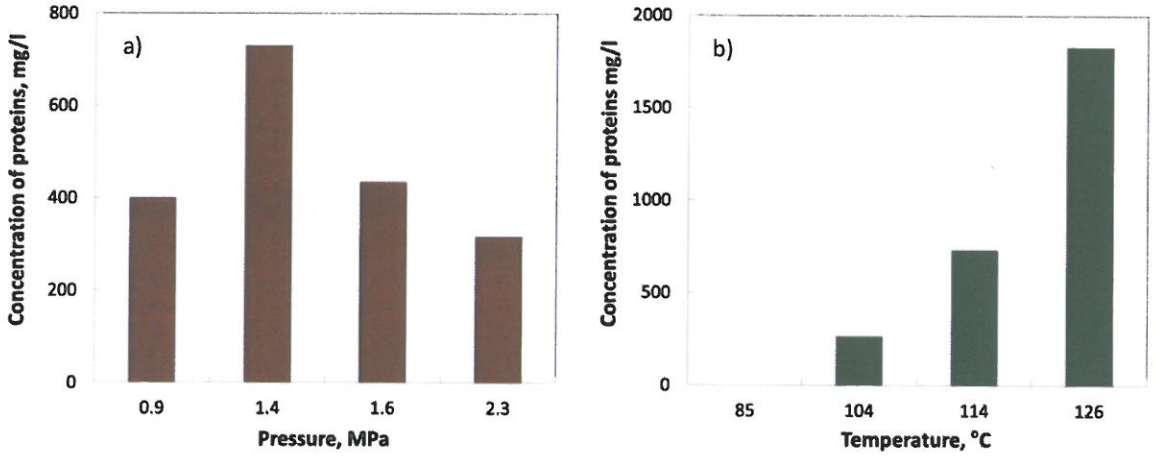

Figure 3. Pressure and temperature effects on protein content in hydrolysates;
a) temperature 113-115 °C, b) pressure 1.3-1.5 MPa

Lipid components - triacylglycerols and free fatty acids

In addition to the representation of proteins and amino acids, the presence of lipid components and their derivatives in chicken feather hydrolysates were studied in detail in this work. Chromatographic analysis determined the distribution of triacylglycerols (TAG) and free fatty acids (FFA) as the main components of animal fat. Twenty TAGs ranging in length of chain C44 to C54 and main FFA were analysed. Only the most represented TAGs contained in all samples were selected for simplification. Relative representation of TAGs ratio was available. Summary of the results are presented in Figures 4-6.

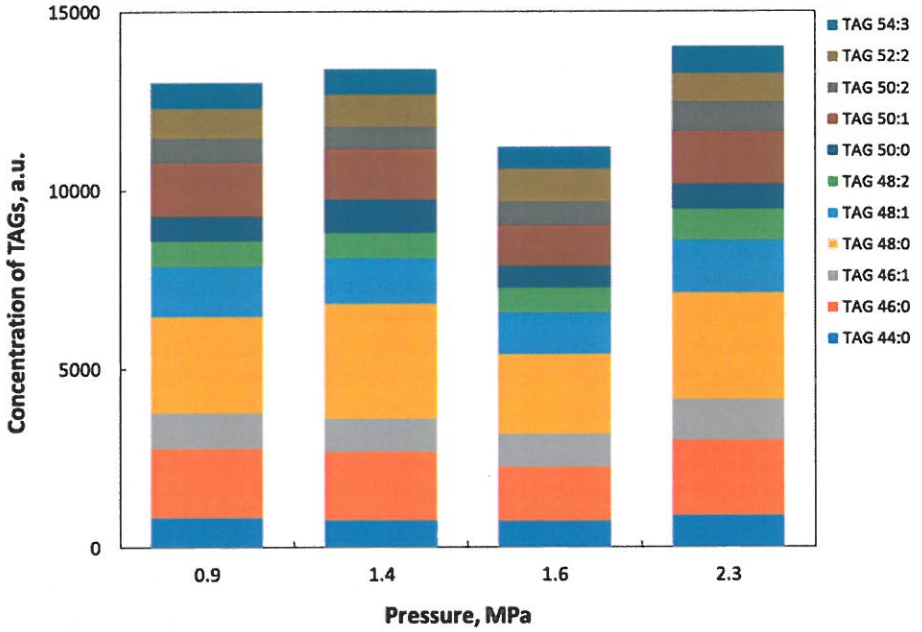

Figure 4. Pressure effect on TAGs composition (temperature 113-115 °C)

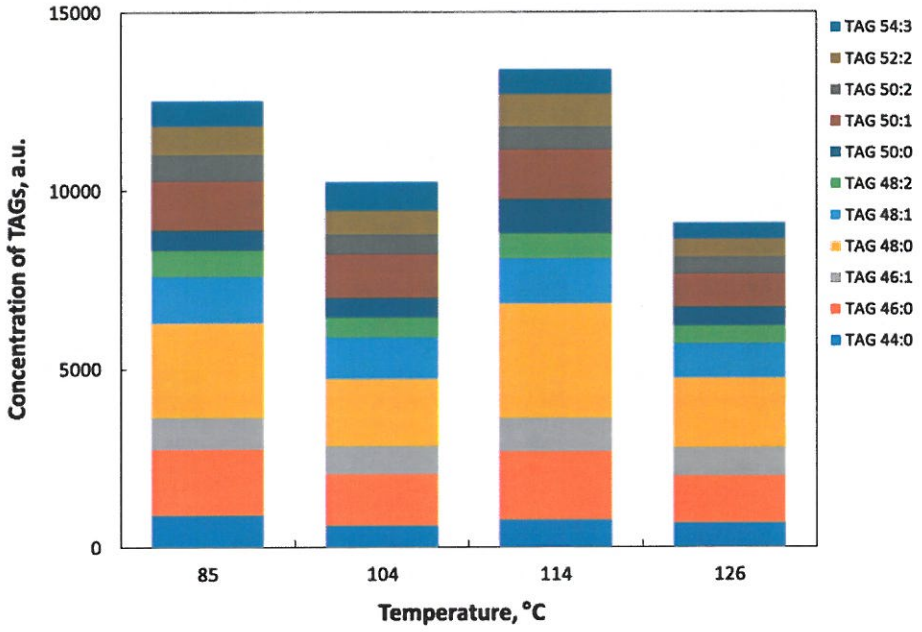

Figure 5. Temperature effect on TAGs composition (pressure 1.3-1.5 MPa)

No obvious trends are evident from the course of relative concentration dependencies of TAGs and major free fatty acids on temperature and pressure. Very complex interaction of individual organic components of the reaction system does not allow for a clearer interpretation of the obtained data. In this respect, more intense future research of the monitored parameters will be necessary.

An insignificant effect contributing to the complexity of this reaction system is the limited solubility of the individual reaction products in the liquid phase of the reaction mixture as well as the decomposition reactions of solid phase containing some less stable substances.

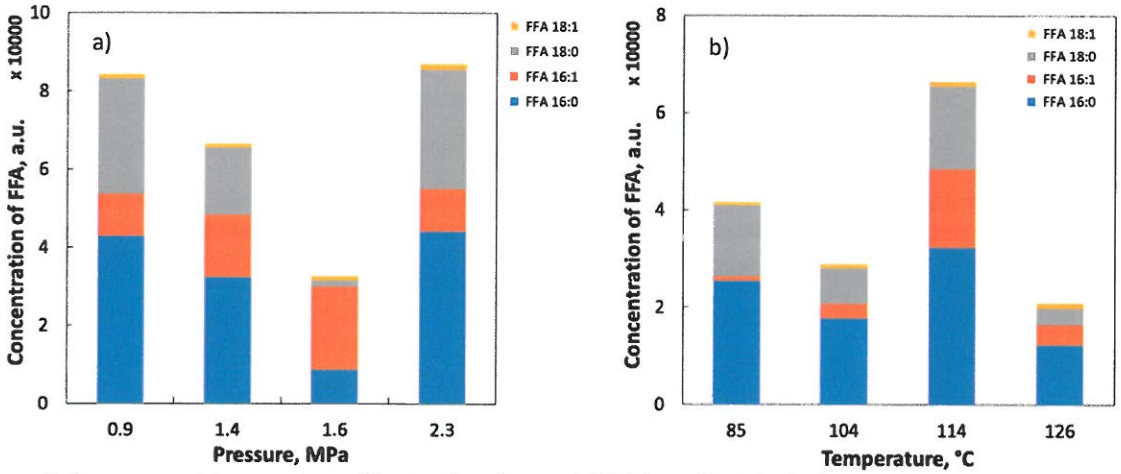

Figure 6. Pressure and temperature effect on free fatty acid (FFA) profile in hydrolysates; a) temperature 113-115 °C, b) pressure 1.3-1.5 MPa

Conclusions

The study was focused on comparing the influence of hydrolytic treatment conditions of waste feathers on obtaining nutritionally valuable components - amino acids, low molecular peptides and lipid part (triacylglycerols and/or free fatty acids). The experiments were carried out in a temperature range of 85 to 126 °C, pressure of 0.9 to 2.3 MPa uniformly for reaction time of 4 hours under an oxygen free atmosphere. The increase in pressure had a positive effect on the yield of essential amino acids, the content of peptides and triacylglycerols did not show a significant change. Effect of temperature increase was apparent above 125 °C, where the concentration of valine markedly increased in the hydrolysates. The content of proteins in hydrolysates increased significantly, the concentration of TAGs and free fatty acids did not manifest a visible trend.

It is evident that acid treatment of feathers involves a complex system of many parallel hydrolytic destruction of ester and peptide bonds of many components in three-phase reaction mixtures. The obtained complex image of the studied reaction system, however, appropriately contributes to the understanding of the individual reactions in the studied system. For the scale-up of the process a future research and development are necessary.

Abbreviations

AA	amino acid
Ala	alanine
FFA	free fatty acid
Glu	glutamic acid
Gly	glycine
Ile+Leu	isoleucine+leucine
Lys	lysine
Phe	phenylalanine
Pro	proline
TAG	triacylglycerol
Thr	threonine
Tyr	tyrosine
Val	valine

Acknowledgement

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 acknowledged.

References

1. Hanika J., Solcova O., Hajslova J., Zachariasova M., Jiru M., Kastanek P., Bizkova Z., Hrstka Z., Fulin T.: A method of preparing a mixture of proteins and amino acids with a predominant content of aspartic acid, CZ Pat. 306431 (2016).
2. Hanika J., Solcova O., Kastanek P.: Proc. 3rd. Int. Conf. Chem. Technol., Mikulov, April 13-15, 2015, p 435.
3. Sinkiewicz I., Staroszczyk H., Sliwinska A: J. Food Biochem. *42*(2), n/a (2018).
4. Bouhamed S. B. H., Kechaou N.: Bioproc.Biosyst. Eng. *40*(5), 715 (2017).
5. Lee Y. S., Phang L.-Y., Ahmad S. A., Ooi P. T.: Waste Biomass Valori. *7*(5), 1147 (2016).
6. Helms E. R., Aragon A. A., Fitschen P. J.: J. Int. Soc. Sports Nutr. *11*, 20 (2014)