Biotechnology and Bioprocess Engineering 25: 749-757 (2020)
DOI 10.1007/s12257-019-0351-8

pISSN 1226-8372
eISSN 1976-3816

RESEARCH PAPER

Reaction Stages of Feather Hydrolysis: Factors That Influence Availability for Enzymatic Hydrolysis and Cystine Conservation during Thermal Pressure Hydrolysis

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Received: 6 September 2019 / Revised: 23 March 2020 / Accepted: 23 March 2020

Abstract The vast amount of feathers generated (> 1 Mtons/a in Europe) in the poultry industry is an opportunity of upcycling by-product materials and improving sustainable practices. Feathers are potentially interesting materials as feed protein ingredients due to their high protein (> 85 wt%) and cystine content (> 7 wt%). However, due to their challenging recalcitrant nature, they have to be processed to make feather protein suitably digestible. The objective was to investigate the effects of temperature (120ºC-160ºC) and time (10, 30, and 60 min) in thermal pressure hydrolysis of feathers on availability for enzymatic hydrolysis (AEH) and cystine conservation. AEH is defined as degree of degradation of processed feather protein by two digestive enzymes pepsin and pancreatin (Boisen). The present study identified and assessed four temperature stages that take place during feather processing. The four temperature stages are 120ºC-135ºC, 140ºC-155ºC, > 160ºC, and the cooling-down phase. The second stage has the greatest influence on AEH. As well as temperature, hydrolysis time is also an essential parameter that had a major impact in the second stage (140ºC-155ºC). Both temperature and time influence negatively cystine content and stability. The present study demonstrates for the first time the importance of four reaction stages during feather hydrolysis and the impact of four stages on AEH of the obtained products.

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Keywords: feather, temperature, time, reaction stage, availability for enzymatic hydrolysis

1. Introduction

More than 1 million metric tons of feathers are produced annually as a by-product at European poultry slaughterhouses [1,2]. Feathers have excellent potential as protein feed ingredient [3], as they consist of mainly protein (> 85%) and have a high content of some essential amino acids, *e.g.* Ile, Leu, Phe, Thr, and Val [4]. Feathers are particularly high in cystine, a valuable sulphur-containing amino acid (> 7%) [5]. Cystine and cysteine are both building blocks of proteins. Within the cells of higher organisms, cystine is usually instantly reduced to cysteine, which plays an important role in metabolic pathways and in the biosynthesis of new protein structures [6,7]. Cysteine is not essential, as it can be synthesized in the liver, but only as the result of conversion from methionine [8]. Dietary cysteine and dietary cystine therefore reduce the need for dietary methionine [9]. Utilizing feathers in feed would therefore be a win-win, as this is a nutritious and sustainable use of an otherwise unused by-product [10].

The processing of feathers for feed use is challenging, however, in view of the molecular structures of feathers, which consist of ß-keratin with pleated sheets [11,12], also known as corneous beta-proteins. They are stabilized by various bounds, such as hydrogen bonds between sheets and peptide bonds [13]. In particular, the disulphide bonds of cystine result in the robust structure of keratin [14,15], which inhibits enzymatic hydrolysis during digestion [16]. To be able to use feather protein as feed, it is essential to unlock the keratin structure to allow enzymatic hydrolysis

[17]. This requires simultaneous modification of both primary and secondary structures of keratin, which results in smaller peptides and better availability for enzymatic hydrolysis (AEH).

To modify feather keratin, several different processing techniques are available. Most aim for a partial protein hydrolysis; this includes thermal pressure hydrolysis (TPH), enzymatic degradation, acidic or basic hydrolysis (change of pH), or combinations of these techniques [18-20]. As alternatives, microwave treatment [21] or co-extrusion of feathers with soybean meal have been reported [22]. Currently, TPH is the most used method for commercial feather processing. Temperature, time, and pressure are critical parameters that determine the AEH of feather proteins. The addition of enzymes and chemicals can be beneficial (Table 1).

The studies listed in Table 1 looked at several parameters and their influence on enzymatic hydrolysis. Most of these studies did not produce any insights into the reaction stages, but focused on data derived from *in-vivo* experiments, and were limited to the effects of time at a specific temperature. However, the effects of temperature and time may not be the same in all reaction stages. Furthermore, in most studies of protein hydrolysis, the reaction is terminated as soon as possible, without regard for the cooling phase. However, the cooling stage might affect the results as reactions may continue in the cooling stage. Evaluating the role of these cooling-stage reactions might help gain a better picture of feather hydrolysis and might also tie different research data resulting from different process designs together.

Besides striving for optimal AEH, another important issue is to avoid lanthionine formation (from cystine) during feather processing. During processing, the disulfide bond of cystine can be cleaved, which can lead to lanthionine formation [23,24]. Fig. 1 shows several pathways that can

Table 1. Effects of temperature and time on feather hydrolysis for the availability for enzymatic hydrolysis (AEH), using two methods to assess AEH, namely chicken growth *in vivo* and pepsin digestibility *in vitro*

Temperature (°C)	Pressure (kPa)	Time (min)	Other factors	Measurement	Finding	Reference
121	-	30	-	Chicken growth	No growth support	[16]
121	-	18 h	-		Moderate growth support with amino acid supplementation	
142		30			With amino acid supplementation, equal to soybean protein	
-	-	-	Sodium sulphide		No effect	
146		30 - 70	Moisture 50 - 70%	Pepsin	No significant effect of time	[35]
-	200 - 340	20 - 60		Chicken growth	Similar effects of pressure and time change	[44,46]
-	340	30, 50, 70	Moisture 60%	Pepsin Chicken growth	Positive effect of time on both	[47]
-	340	30 - 70	NaOH 0.2 - 0.6%	Pepsin	Positive effect of prolonged conditions; Inverse relationship between amino acid content and AEH	[48]
-	207, 310, 414	24		Pepsin	Positive effect of pressure	[45]
-	207, 276, 345	30	pH 5, 7, 9	Pepsin	Positive effects of pressure and pH	[19]
	207 - 724	106 to 4.5 h		Pepsin	No indication of negative effect of high pressure if time was appropriate	[38]

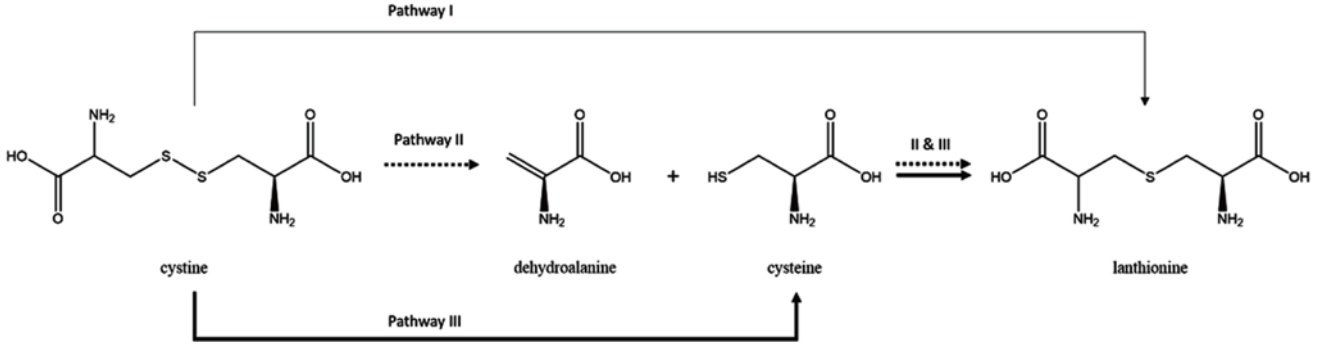

Fig. 1. Reaction pathways of cystine conversion to lanthionine. Pathway I: direct conversion of cystine to lanthionine. Pathway II: dehydroalanine as intermediate [41,49]. Pathway III: cysteine as intermediate [7,50].

result in lanthionine formation. As lanthionine plays no role in the biosynthesis of proteins, it has no nutritional value.

We have investigated the effect of temperature and time on hydrolysis in THP of feathers, with AEH and cystine conservation as outcomes. Based on our evaluation of the existing knowledge, we hypothesized as follows:

(1) Hydrolysis of keratin can be subdivided into different reaction stages related to different changes in AEH.

(2) Time may have different effects on AEH in different reaction stages.

(3) The final cooling stage after the reaction significantly affects AEH because the reaction continues during the cooling process.

(4) Temperature and time impact the hydrolysis reaction significantly but differently, and therefore also cystine integrity.

2. Materials and Methods

2.1. Raw materials treatment

Feathers were collected from a poultry slaughterhouse within less than 4 h after plucking. They were directly transported to the laboratory at 7°C and within 8 h. In the laboratory, the feathers were separated from non-feather material (heads and feet). Subsequently, the feathers were mixed into a homogenous matrix, washed with water, dried at 60°C in an oven (Memmert, Germany) until constant weight, and stored in closed a container at room temperature until use.

2.2. Experiment setup

Four experiment series of hydrolysis were performed (Fig. 2). The experiments were executed in randomised order

2.2.1. Experiment series I - effects of temperature and time on hydrolysis reaction

The experiments were executed in randomised order. The feather material was prepared by using a cutting mill (Retsch SM 300, 1- mm sieve, Germany). 30 g of prepared feathers and 90 g of Milli-Q water were stirred until a homogenous feather mass formed. The mass was added to the reactors (20 ± 2 g each, room temperature) (PARR 5000 Multiple Reactor System, USA), together with a small magnetic rod. The temperature profile was set by using a process controller (PARR 4870, USA). Temperature was directly measured inside the feather mass. Time measurement was started directly after the target temperature was reached (which was within 10 to 12 min). Stirring began at 100°C. The experiments were stopped after the desired time. The reactors were then immediately put into freshly prepared ice containers until the vessel was opened (after, on average, 5 min). The hydrolyzed feather mass was collected, weighed, and dried overnight by 60°C until constant weight was reached (weight difference < 0.1%), manually milled and sieved (0.8-mm sieve) and then stored in a glass container at room temperature until further analysis. The weights before and after hydrolysis reaction are given in Table S1 as supplementary data.

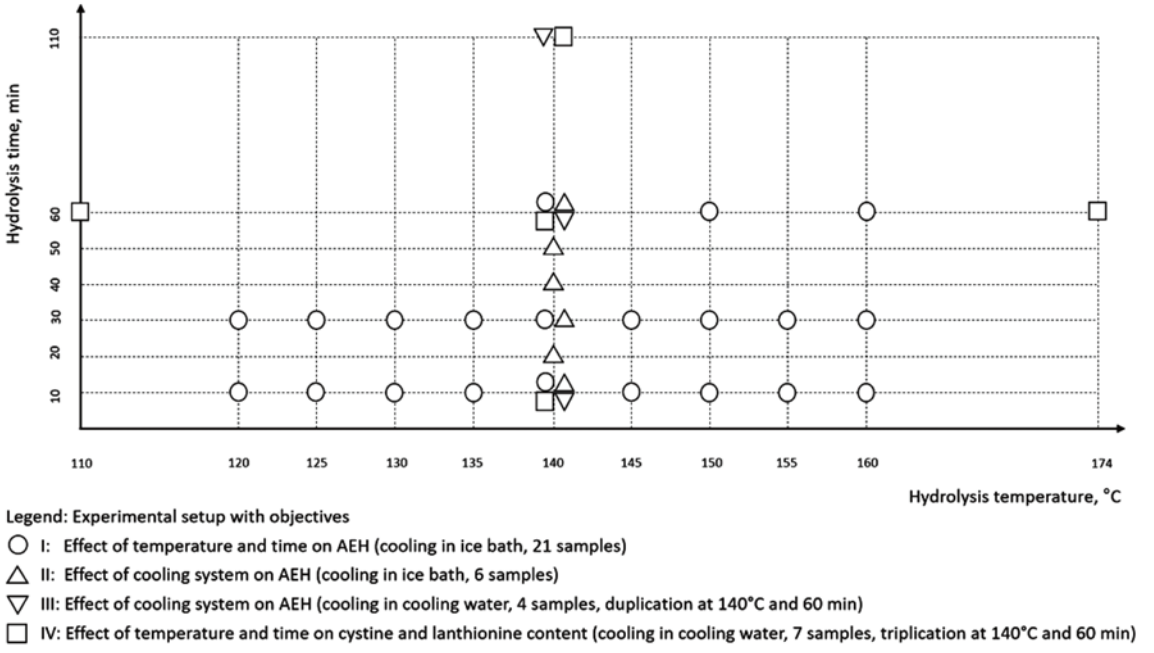

Fig. 2. Experimental setup with three specific objectives. Experiment series I and II were designed according to application relevance. Experiment series III and IV were designed by Statgraphics Centurion XVII (Statpoint Technologies, USA). The point at 140°C and 60 min was defined as center point and triplicated.

2.2.2. Experiment series II and III - effects of cooling-stage reactions on AEH

To evaluate the effects of possible cooling-stage reactions on AEH, experiments II and III with different cooling systems *i.e.*, with significantly different durations for the cooling-stage reaction were performed. Effects of set process time and actual hydrolysis time on AEH were evaluated separately. The actual hydrolysis time was calculated according to the following formula:

$$actual\ hydrolysis\ reaction\ time = set\ process\ time + cooling\ time \quad (1)$$

The reactors of experiment II were cooled down in an ice container. The cooling time was 5 min on average. The experimental procedure was the same as for Experiment I (PARR 5000 Multiple Reactor System, USA).

The reactors of Experiment III were cooled down in cooling water at 10°C. The cooling time was calculated as the time from which the experiment was stopped till the time the vessel was opened. The setting range for hydrolysis temperature and time was broader than for Experiments I and II in order to record more details about the hydrolysis reaction. The combinations of settings were designed by using the Statgraphics Centurion XVII program (Statpoint Technologies, USA). The feather mass for Experiment III was prepared by using a mincer (Tafel, Le Hachoir 1700, Germany, 7.5 mm perforated disc). Five hundred gram of prepared material (feathers and water, 70 wt% moisture) were filled into the reactor (Kiloclave, Büchiglasuster, Switzerland). The reactor was equipped with a central stirring unit, running during the entire experiment. The hydrolysis temperature and time were set by using a process controller btc PID temperature controller, Büchiglasuster, Switzerland). The temperature was directly measured inside the feather mass. The system heated in 46 min to about 140°C. The experiments were automatically stopped according to the time setting. The reactors were immediately put into cooling water at 10°C until the vessel was opened. The hydrolysed feather mass was collected, weighted, dried in a freeze-dryer (Dieter Piatkowski, -45°C, 24 h) until constant weight. The material was then manually milled, sieved (0.8-mm sieve), and stored in a container at room temperature for further analysis.

2.2.3. Experiment series IV - effects of temperature and time on cystine and lanthionine content

The samples from experiment III were analysed for amino acid composition.

2.3. Analysis and statistics

The moisture content was analysed according to EC regulation [25] (oven: Memmert, Germany), in triplicate. The ash content analysis was adapted from EC regulation [25] by using thermo-gravimetric analysis (Mettler Toledo) and carried out in duplicate. The nitrogen content was measured by the Dumas method (FlashEA® 1112 Organic Elemental Analyzer, Thermo Scientific), in duplicate. The samples were pre-dried in an oven overnight at 60°C (Memmert, Germany). The pre-dried samples of 10 to 15 mg each were wrapped in an aluminium tin (nitrogen-free) and annealed at 900°C.

Availability for enzyme hydrolysis (AEH) is defined as degree of degradation of processed feather protein by two digestive enzymes: pepsin and pancreatin. This method for enzymatic hydrolysis with pepsin and pancreatin represents the well-established Boisen protocol for *in-vitro* digestibility [26]. Here it is used as a method to indicate trends for *in vivo* digestibility without the need for animal studies [27]. This so-called Boisen method was used with two adaptations [27]. The first adaptation was to use a shaker to create a reaction atmosphere (39°C and 60 rpm, Kuhner Climo-Shaker ISF1-X, Germany). The second adaptation occurred in the pepsin stage. The mixture with protein, buffer, and HCl was shaken for 30 min in the same shaker (Kuhner, 39°C, 60 rpm) to achieve better homogenisation for the pH adjustment (VWR pHenomenal® 1100 H, Germany). The AEH was calculated from the following equation:

$$AEH\ (wt\%) = (N_{Sample} - N_{Residue}) / N_{Sample} * 100\% \quad (2)$$

Where N_{Sample} and $N_{Residue}$ is the nitrogen content in grams of respectively the crude sample and the undigested residue after enzymatic hydrolysis.

The samples from Experiment III were analysed for cystine and lanthionine content in µmol / 100 g crude protein (CP). Cystine content was analysed by Eurofins, Germany according to ISO 13903: 2005 [28]. Lanthionine content was analysed by InVivo, France according to EU regulation 152/2009 27-01-2009.

Experimental data was statistically analysed with the Statgraphics Centurion XVII program (Statpoint Technologies, USA) and Excel 2016. Significance was set to three levels ($P < 0.001$, $P < 0.01$, and $P < 0.05$). The three levels of significance were used due to the sample size in order to show the detailed difference between the results.

3. Results and Discussion

3.1. Effects of hydrolysis time on availability for enzymatic hydrolysis

3.1.1. Effects of using durations of 10 or 30 min

The influence of hydrolysis times, using short hydrolysis times of 10 and 30 min on AEH was investigated first. It

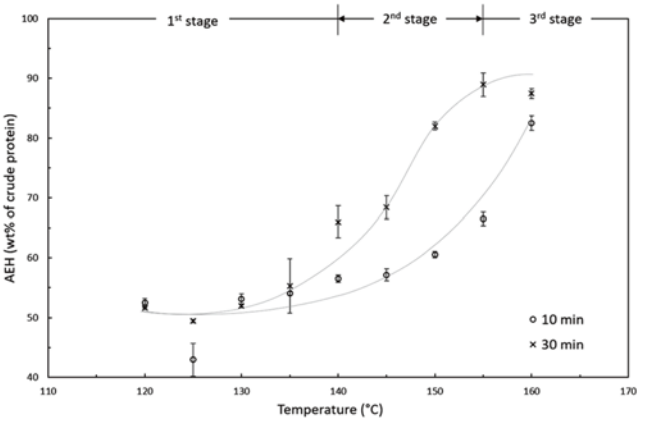

Fig. 3. Effects of hydrolysis times on availability for enzymatic hydrolysis (AEH) at different temperatures (lines to guide the eye). The average value of two analyses was taken as the data points on the diagram. Correlation between temperature and AEH: R2 = 0.78 and P < 0.05 for 10 min whereas R2 = 0.94 and P < 0.001 for 30 min.

was found that a hydrolysis time of 30 min led to a higher AEH than 10 min at the same temperature (Fig. 3). During hydrolysis three stages were identified, namely the first stage from 120°C to 135°C, the second stage from 140°C to 155°C, and the third stage at 160°C. During the first stage from 120°C to 135°C, hydrolysis times of 10 and 30 min had similar effects on the AEH increase (2% to 5% AEH difference). During the second phase from 140°C to 155°C, using a hydrolysis time of 30 min resulted in a significant increase of AEH relative to 10 min (17% to 36% AEH difference). In the third stage at 160°C, increasing the hydrolysis time from 10 to 30 min had a smaller effect on AEH (6% difference).

These observations match those of Milczarek *et al*. [29] by using Differential Scanning Calorimetry (DSC), who suggested looking at different stages to understand the thermal transition of human hair keratin. They reported that keratin processing involves a thermally activated process of releasing water and observed two temperature points for structural changes, 140°C and 155°C. Up to 140°C (mostly around 70°C), keratin first releases loosely bound water. Above 140°C, hair keratin begins its essential structural change by losing strongly bound water and at 155°C to 160°C, and the amorphous phase changes into a crystalline microfiber phase. In the third reaction stage, with the well-ordered crystalline phase, the reaction gradually reaches its maximum. This is also supported by the findings of Takahashi *et al*. [30], who found two endothermic transition temperature peaks (using DSC) for rachis of fowl feathers in wet conditions (130°C and 150°C). In their study, the first peak was weak and broad whereas the second one appeared strong and sharp. The authors reported that the endothermic transition stopped at 160°C due to the high thermal stability of the structure built by intra- and inter-disulfide bonds. Furthermore, Brebu and Spiridon [31] reported that feathers begin to decompose at 155°C, with a gradual release of ammonia and CO_2. This indicates the occurrence of decarboxylation and deamination of released amino acids.

Although in the experiments only one temperature point was observed in the third reaction stage, we still hypothesize that hydrolysis of feather keratin takes place in three different reaction stages (followed by the cooling stage as the fourth stage).

3.1.2. Effects of using a duration of 10, 30, or 60 min in the second stage

The effects of hydrolysis time (10, 30, or 60 min) on AEH in the second stage were investigated next (Fig. 4).

Fig. 4 shows that at higher temperature a shorter time to reach a similar AEH is needed. At 140°C, the hydrolysis time contributed linearly to the AEH increase, from 56 wt% CP at 10 min to 75 wt% CP at 60 min. At 150°C, however, a hydrolysis time of 30 min instead of 10 min had a greater influence on AEH (21% increase from 61 wt% CP to 82 wt% CP) than using 60 min instead of 30 min (5% increase from 82 wt% CP to 87 wt% CP). At 160°C, hydrolysis times of 10, 30, and 60 min had a similar influence on AEH (a 5% increase of 82 wt% CP to 87 wt% CP for 30 min instead of 10 min and a further 3% increase of 87 wt% CP to 90 wt% CP for 60 min).

Thus, less time was required at higher temperatures to obtain similar AEH results. These finding are supported by a study on the thermal conversion of sulfur-containing amino acids by Sohn and Ho [32], who reported that the increase in ammonia generation from cysteine is much greater between 110°C and 150°C than between 150°C and 180°C. Yablokov *et al*. [33] studied the thermal decomposition of cysteine and cystine and found that cysteine and cystine

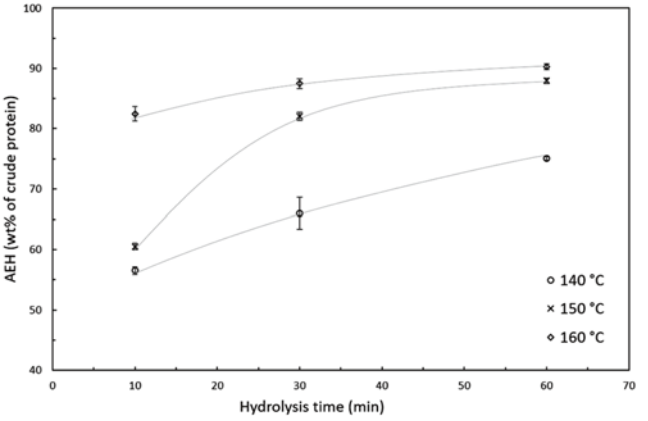

Fig. 4. Influence of hydrolysis time on availability for enzymatic hydrolysis (AEH) at 140, 150, and 160°C (lines to guide the eyes). The average value of two analyses was taken as the data points on the diagram.

have similar thermal decomposition behaviors as well as their mutual transformation. The mutual transformation of cysteine and cystine during the reaction is complex because interchange of S-S and H-S bonds can lead to the creation of new S-S bonds [34].

Due to the chosen temperature limit of 160°C the investigated hydrolysis time range is only part of the entire reaction curve. Nevertheless, the results show clearly that hydrolysis time influences AEH differently at different temperatures (Fig. 4).

In summary, the feather hydrolysis reaction can be seen as subdivided into three main reaction stages, followed by the cooling stage as the fourth reaction stage. In terms of impact of AEH, the second stage is the most influential and the most effective temperature range for affecting AEH is 140°C to 150°C.

3.2. Effects of the cooling-stage reactions

As mentioned, the cooling-stage reactions during cooling and its effect on AEH were also studied. Cooling could be referred to as the fourth stage and its duration may significantly affect AEH because smaller proteins, peptides, and amino acids are produced further in this stage.

Two different cooling systems for this investigation were used, one with ice (6 samples, Experiment II) and one with cooling water of 10°C (4 samples, Experiment III.1). The time for ice cooling was calculated as 5 min on average, whereas the time for water cooling was read from the control system as 43 to 59 min (Fig. 5).

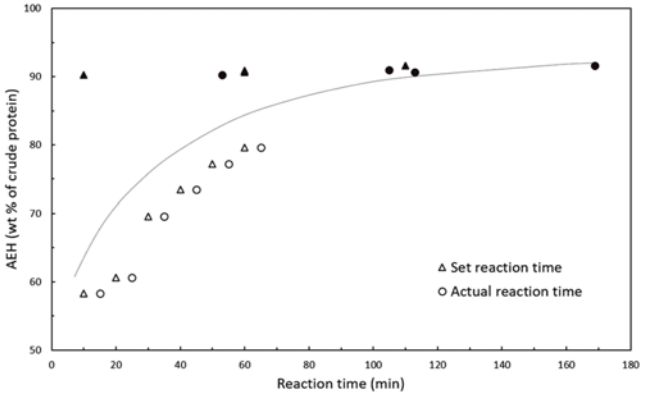

Fig. 5. Effects of set process time and actual hydrolysis time on availability for enzymatic hydrolysis (AEH) at 140°C (line to guide the eye). Four samples indicated by black dots and triangles processed in Kiloclave in Experiment III (R2 = 0.94 and P < 0.05 between set hydrolysis time and AEH whereas R2 = 0.92 and P < 0.05 between actual hydrolysis time and AEH). Six 50-mL samples indicated by open symbols processed in PARR 5000 multiple reaction system in Experiment II (R2 = 0.97 and P < 0.01 between set hydrolysis time and AEH whereas R2 = 0.97 and P < 0.001 between actual hydrolysis time and AEH). Total evaluation of 10 samples: R2 = 0.39, P > 0.05 between the set hydrolysis time and AEH whereas R2 = 0.85 and P < 0.001 between actual hydrolysis time and AEH.

The correlation of 10 samples between the set hydrolysis time and AEH was not significant (R2 = 0.39, P > 0.05) whereas the correlation between actual hydrolysis time and AEH was significant (R2 = 0.85, P < 0.001) (Fig. 5). The difference was caused by 4 samples that had longer cooling times (43-59 min, experiment III) and showed a weak correlation between hydrolysis time and AEH (R2 = 0.94 and P < 0.05 between set hydrolysis time and AEH, R2 = 0.92 and P < 0.05 between actual hydrolysis time and AEH). Without these 4 samples, the correlation was strong (R2 = 0.97, P < 0.01 between set hydrolysis time and AEH, R2 = 0.97 and P < 0.001 between actual hydrolysis time and AEH). In a study by Papadopoulos *et al*. [35], hydrolysis time was found to have no significant effect on AEH (pepsin) at 146°C (94.02% at 30 min, 91.8% at 50 min, and 93.94% at 70 min). The positive effect of hydrolysis time on AEH for the same temperature could be an indication of the occurrence of cooling-stage reactions that occurred during cooling. Therefore, care has to be taken when comparing data since the cooling phase, which is not always reported, can have a significant influence on the reported results. Since most studies about protein hydrolysis are designed with stopping of the reaction as soon as possible at the desired temperature and time settings, we were not able to find any literature about possible cooling-stage reactions. Volkin and Klibanov [36] found that cystine degrades its sulfide bonds above 100°C in a first-order reaction. When heating is stopped, the stored material holds the temperature in the heat-isolated reactor for a certain time and therefore, the reaction continues. In addition, energy is generated through the exothermic formation of new bonds between freed amino acid residues as well as in other inter- and intra-chain interactions. As a result, more disulfide bonds are cleaved and the unlocked molecular structure provides more access for continued enzymatic degradation.

It appears that the hydrolysis reaction proceeds during the cooling phase. Therefore, the cooling phase has to be regarded as an extension of the reaction time, which directly affects AEH.

To quantify the effect of these cooling-stage reactions on AEH, it is also necessary to take the dimension of the reactor into consideration. The larger the reactor, the greater this effect will be as it takes longer to heat a larger amount of material to the desired temperature and longer to cool it down again. In line with this, the cooling-stage reactions will also continue longer if a similar-size reactor contains more material. As more energy is stored in the system, cooling will take longer; also, more energy will be generated through the formation of new bonds. These influences were not further studied here.

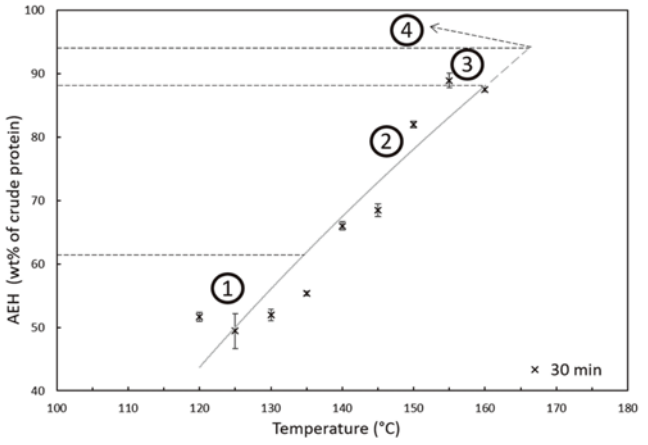

Fig. 6. The four stages of feather hydrolysis (line to guide the eye). First stage: 120°C-135°C. Second stage: 140°C-155°C. Third stage: 160°C. Fourth stage: cooling. The average value of two analyses was taken as the data points on the diagram. Correlation between temperature and availability for enzymatic hydrolysis (AEH) at 30 min: R2 = 0.94, P < 0.001.

Thus feather hydrolysis takes place in four different stages including the cooling stage, (Fig. 6).

3.3. Effects of temperature and time on cystine and lanthionine content

Next, the effect of temperature (106°C to 174°C, 60 min) and time (10, 60, or 110 min, 140°C) on cystine and lanthionine content was explored (Fig. 7 and Fig. 8).

Both temperature and time had a significant negative effect on cystine content. As hydrolysis temperature increased, cystine content decreased by 46% between 106°C and 140°C (from 31.94 μmol / 100 g CP to 17.39 μmol / 100 g CP) (Fig. 7). The cystine loss was 94% between 106°C and 174°C (from 31.94 μmol / 100 g CP to 1.97 μmol / 100 g CP) (Fig. 7). With increased hydrolysis time at 140°C from 10 to 110 min, the cystine content decreased by 38% (from 21.98 μmol / 100 g CP to 13.67 μmol / 100 g CP); see Fig. 8. Compared with the clear effect on cystine content, the effect of temperature and time on lanthionine content was not straightforward. As temperature increased, lanthionine content first increased between 106°C and 140°C (from 2.24 μmol / 100 g CP to 10.07 μmol / 100 g CP) and then decreased between 140°C and 174°C (from 10.07 μmol / 100 g CP to 2.74 μmol / 100 g CP) (Fig. 7). With increased time at 140°C from 10 to 110 min, lanthionine increased constantly from 8.47 μmol / 100 g CP to 11.44 μmol / 100 g CP (Fig. 8).

These observations of cystine conversion are consistent with findings by Lagrain *et al*. [34]. They reported that cystine conversion follows first-order kinetics; at the same hydrolysis time, they observed less cystine at higher temperatures. Furthermore, Latshaw [19] and Papadopoulos

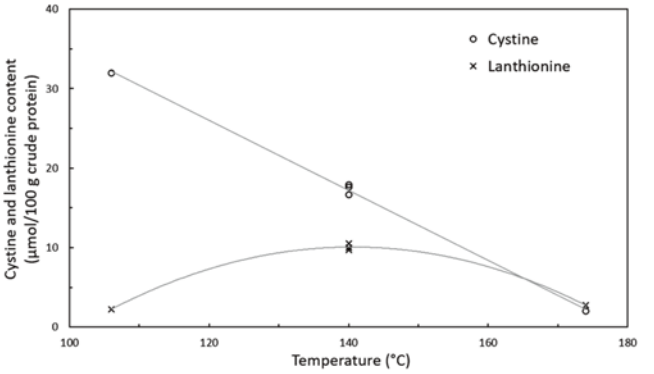

Fig. 7. Effect of temperature on cystine content and on lanthionine production at 60 min (lines to guide the eye).

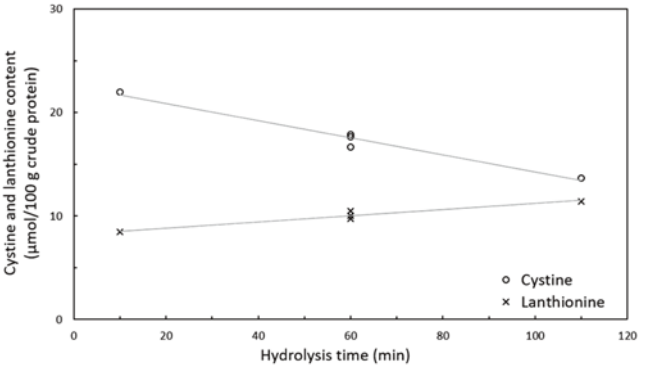

Fig. 8. Opposite effects of hydrolysis time on cystine and lanthionine content at 140°C (lines to guide the eye).

[17] observed that increasing the time or pressure has a significant negative effect on cystine content in the feather meal.

It is generally accepted that lanthionine formation is related to cystine conversion and that prolonged conditions or higher temperatures or pressures promote lanthionine formation. By contrast, our observations for lanthionine formation are not consistent with some earlier findings. Papadopoulos *et al*. [37] reported a positive effect of hydrolysis time on lanthionine formation. Moritz and Latshaw [38] found that lanthionine content increases with pressure (207-517 kPa) for a hydrolysis time of 36 min. However, Asquith and Otterburn [39] observed a similar phenomenon as we did and reported lower lanthionine content in wool keratin at 180°C than at temperatures below 160°C (dry heating).

The divergent results indicate the complexity of cystine conversion to lanthionine. There are three generally accepted reaction pathways of lanthionine formation from cystine conversion: I: direct conversion, II: cysteine as intermediate, and III: dehydroalanine as intermediate. Our results suggest that pathway III was the main reaction pathway (Fig. 1) [34]. It is also possible that pathway I and

II were involved in the conversion because of the interchange of H-S and S-S bonds as well as the catalytic effect of present cysteine on cystine conversion [34,39,40].

Heating at alkaline pH or heating above 200ºC promotes pathway III [34,41]. Furthermore, different types of reactions under thermal treatment are involved [35,42], as the reactants are not only the amino acids in the keratin chain, but also carbohydrates and fats in the absence of reducing substances.

Some researchers have reported that lanthionine content is related to AEH [43,44] and suggested that lanthionine is a reasonable indicator for AEH to evaluate processed feather meals. The results reported here do not support that and indicate that lanthionine may not be the final product of cystine conversion. Damodaran [41] suggested the cross-linking of lanthionine and lysinoalanine as one of possible reasons for further reaction of lanthionine. However, the consequence of protein cross-linking is a decrease of the AEH, which is in contrast with the positive correlation between AEH and temperature found in our study.

It is obviously essential to balance the process to obtain a high AEH and maintain a high cystine content [45]. It is an unavoidable consequence to lose cystine through cleavage of the disulfide bonds when aiming on higher AEH. However, the results reported here show that lower process temperatures and shorter times facilitate cystine conservation. This is in agreement with the work of Papadopoulos [23]. In addition, Moritz and Latshaw [38] have indicated that the nutritional value does not decrease if process time is chosen optimally in combination with high pressure. Our results suggest that keeping the temperature at 140°C at the beginning of the second phase helps conserve cystine.

4. Conclusions

Feather hydrolysis occurred in four stages, namely between 120ºC and 135ºC, between 140ºC and 155ºC, the third stage beginning at 160ºC, and an often overlooked cooling stage serving as the fourth stage. The second stage had the greatest influence on AEH and the optimal hydrolysis temperature was between 140°C and 150ºC. Cooling-stage reactions impacted AEH positively. Higher temperatures and longer reaction time had negative effects on cystine conservation. At 140ºC cystine and lanthionine are inverse linearly correlated to each other indicating that cystine is converted in to lanthionine at that temperature.

To quantify the effect of cooling-stage reactions on AEH, it is necessary to take the dimension of the reactor into consideration. For industrial application, further research will be needed.

Acknowledgments

This work was financially supported by Saria International GmbH.

The authors declare no conflict of interest.

Neither ethical approval nor informed consent was required for this study.

Nomenclature

AEH, availability for enzymatic hydrolysis; TPH, thermal pressure hydrolysis; wt% CP, wt% of crude protein; DSC, Differential Scanning Calorimetry; μmol / 100 g CP, μmol / 100 g crude protein

Electronic Supplementary Material (ESM)

The online version of this article (doi: 10.1007/s12257-019-0351-8) contains supplementary material, which is available to authorized users.

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