

Profiles of enzymatic hydrolysis of different collagens and derivatives over time

ABSTRACT

The interest in collagen use in the food industry to replace synthetic agents is growing every day. Among the derivatives, has the hydrolysate, which dissolves easily in water and brine, and can be incorporated into foods or drinks. The objective of this study was to determine the degree of hydrolysis (DH) of crude collagen fiber, powdered collagen fiber, gelatin and two samples of hydrolyzed collagen. The substrates were subjected to individual action of three proteolytic enzymes: papain, bromelain and collagenase (microbial origin). The substrates were incubated in sodium phosphate buffer (0.1 M, pH 7.0) on an orbital shaker at 55 °C for different periods of time. In 120 minutes of hydrolysis, the crude collagen fiber showed 37.2% of DH using collagenase, the highest value compared to other substrates. The lowest values for DH were obtained for the hydrolysates collagen, with less than 1 % of DH. For hydrolysis in 60 minutes, the DH values were similar to those obtained in 120 minutes of hydrolysis, with minor variations over time. The hydrolytic ability of the collagenase and bromelain was similar and superior compared with the papain.

KEYWORDS: Collagens. Degree of hydrolysis. Proteolytic enzymes. Hydrolysis time.

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INTRODUCTION

In recent years, there is an increasing interest in the intentional modification of proteins to obtain the protein hydrolysates, these with many uses in foods for special purposes such as non-allergenic formulations of infant, geriatric food, sports drinks, and dietetic food (DAMODARAN et al., 2010).

These hydrolysates can be obtained by chemical or enzymatic hydrolysis (KOBBLITZ, 2008). The enzymatic method is most appropriate, because the addition of enzymes allows control of the process, improving the properties of the final product, different from what occurs in the chemical hydrolysis. Furthermore, the enzymatic hydrolysis process is simple, efficient and involves mild alkaline conditions which do not destroy the proteins (SCHMIDT; SALLAS-MELLADO, 2009). The reaction conditions used, such as: substrate concentration, ratio enzyme/substrate, incubation time and physicochemical conditions (such as pH and temperature) which determine of the degree of hydrolysis (BENITEZ et al., 2008).

The enzymes used for obtaining of protein hydrolysates are proteases (EC 3.4) also known as peptidases. The peptidases are enzymes that belong to the class of hydrolases and they act by breaking the peptide bonds of peptides and proteins (BON, 2008). The substrates susceptible to the action of proteases include many common proteins, such as: rice protein (QIANG et al., 2012), gluten (KONG et al., 2007), maize protein (ZHOU et al., 2012), casein, albumin and collagen (KOBBLITZ, 2008). Collagen is the most abundant protein in the human body, is organized into high strength fibers and has low solubility in water. A typical collagen molecule has as its main characteristic the triple helix conformation (formed from three polypeptide chains) and their content of amino acids, containing glycine (about 33 % of total), proline (about 10 % of the total) and 4-hydroxyproline (approximately 10 % of total) (MOTTA, 2005, NELSON; COX, 2006).

Using collagen as raw material, are commonly obtained the partially hydrolyzed collagen (gelatin) and the hydrolyzed collagen (DEMAN, 1999; DAMODARAN et al., 2010). For industrial production purposes, gelatin is obtained from collagen using acid or alkaline hydrolysis. It is a substance widely used as a

food additive to enhance the texture, water retention capacity and stability of food products. Collagen hydrolysate is obtained by chemical or enzymatic hydrolysis under controlled conditions (SCHRIEBER; GAREIS, 2007, WANG et al., 2009).

The collagen hydrolysate may be obtained through the use of various proteases. Plant proteases can be used in the hydrolysis reactions and this group is papain (EC 3.4.22.2) and bromelain (EC 3.4.22.4). Papain is a cysteine protease extracted from the latex of the fruit of papaya (*Carica papaya*), having proteolytic activity at pH values from 5.0 to 9.0 and its optimal activity observed at temperatures from 60 °C to 70 °C. Bromelain is also a cysteine protease and is extracted from peduncle of the pineapple fruit, with optimal activity between pH 6.0 and 8.0 and rapidly losing its activity at temperatures above 70 °C (KOBELITZ, 2008). Due to their high efficiency of hydrolysis, can also be employed microbial proteases such as collagenase, which may have endopeptidase action (VIEIRA, 2007).

Collagen hydrolysate has as characteristics and properties the low viscosity in aqueous solution, neutral odor, colorless, emulsifying property, film-forming capacity, wettability, dispersibility, high solubility, low compressibility in powder and low allergenicity. The collagen hydrolysate provides great potential for use in preparations for skin care and hair care, in solid dosage forms of drugs and in numerous foods since they can be easily mixed with other products without causing alteration of the sensory properties of the product (DENIS et al., 2008). There is a great interest in the enzymatic hydrolysis of collagen for production of biologically active peptides with antioxidant and antimicrobial capacity (GÓMEZ-GUILLÉN et al., 2011).

The obtaining of bioactive peptides of collagen has attracted attention due to the demand for functional foods as well as the study of the bioavailability, functional properties and mechanisms of action of these compounds. Some studies demonstrate that collagen peptides have an antimicrobial activity, antioxidant activity and anti-hypertensive activity. The antioxidant activity of the bioactive peptides is due to the capacity of these compounds to chelate metals, such as copper. The antimicrobial activity is due to the capacity of the peptides to inhibit the growth of micro-organisms, by linking them with the bacterial cell membrane (PRESTES et al., 2014).

Advantages of hydrolyzed collagen has also extended to the food industry with functional properties, since studies have proven that it has the potential to contribute favorably to the treatment and prevention of osteoarthritis and osteoporosis besides having the auxiliary capacity weight loss, causing a feeling of satiety (SCHRIEBER, GAREIS, 2007).

The application of hydrolyzed collagen in meat products can be an alternative to increase of collagen ingestion by the modern consumer. The main functionality of hydrolyzed collagen in meat are: increased water retention, improved of texture, improved of fat retention, increased of yield after cooking, improving of slicing, no interference with the natural color of the product to which is added and decreased of the exudation of fluid on fresh produces (PRESTES, 2013).

In the current days in which the consumer seeks health and safety in the food it consumes, the collagen is an ingredient that offers numerous possibilities of application, with much to be explored and studied. Therefore, the objective of the present study was evaluate the efficiency of the use of papain, bromelain and collagenase in the hydrolysis of different samples of collagen and obtain the enzymatic hydrolysis profiles over time, to verify the possible differences between enzymes and profiles obtained for substrates of the same origin but with different physical and chemical characteristics.

MATERIAL AND METHODS

The experimental analyzes were performed at the Bioprocess Laboratory and Microbiology Laboratory in Department of Food Engineering - State University of Santa Catarina (Pinhalzinho, SC, Brazil).

Was evaluated four different substrates: crude collagen fiber (granulometry of 1.80 mm and 1.92 mm) and powdered collagen fiber (granulometry of 0.45 mm and 0.57 mm), provided by JBS Collagen (São Paulo, SP, Brazil), hydrolyzed collagen Peptiplus® (called in this study of collagen hydrolysate A) provided by Gelita South America (Mococa, SP, Brazil), hydrolyzed collagen Peptan B® (called this study of collagen hydrolysate B) and gelatin (derived from collagen) (Bloom \leq 260 g) provided by Rousselot (Amparo, SP, Brazil), all of bovine origin.

Two proteases of plant origin, papain (AB Enzymes GmbH, Darmstadt Germany) and bromelain (Pharmanostra- Rio de Janeiro, RJ, Brazil), and a collagenase of microbial origin (Corolase® 7089, AB Enzymes GmbH, Darmstadt, Germany) were used in the hydrolysis of substrates, all of which were submitted to individual action of three enzymes under the same operating conditions.

PROCEDURE FOR THE ENZYMATIC HYDROLYSIS

The dispersions were prepared in Erlenmeyer flasks of 250 mL in the proportion of 5 % substrate and 8 % of the enzyme (on the substrate mass) in sodium phosphate buffer (0.1 M, pH 7.5). The Erlenmeyer flasks (containing enzyme, substrate and buffer) were placed in an orbital shaker (Solab - Piracicaba, SP, Brazil) at temperature of 55 °C (WHITAKER et al., 2003) and 50 rpm of agitation for 120 minutes (AVRAMENKO et al., 2013; SEGURA-CAMPOS et al., 2013).

After the hydrolysis time, the samples were heated in a water bath at 75 °C for 10 minutes for denaturation and subsequent inactivation of the enzyme. The reaction medium was centrifuged in centrifuge (Quimis - Diadema, SP, Brazil) for 10 minutes and the supernatant obtained was used for determining the Degree of Hydrolysis (SCHMIDT; SALLAS-MELLADO, 2009). All experiments were performed in triplicate.

DETERMINATION OF THE DEGREE OF HYDROLYSIS (DH) OF THE SUBSTRATES

After centrifugation and removal of supernatant, the DH was expressed according to the ratio of amount of protein (mg/mL) using the Biuret method. The method consists in determining the protein concentration of the sample through the reaction there of with Biuret reagent and subsequent absorbance at 540 nm. The amount of protein is calculated by correlation with the linear equation obtained from a calibration curve using bovine serum albumin as standard. For obtaining the values of protein after centrifugation of the hydrolysate, 2 mL of supernatant was transferred to a test-tube to which was added 8 mL of Biuret reagent. The test tubes remained at rest for 30 minutes at room temperature and after this time, samples were read in spectrophotometer (Biospectro – Curitiba, PR, Brazil) at 540 nm (MACEDO et al., 2005).

For the calculation of DH, was using a relation between protein concentration in the supernatant obtained without addition of the enzyme (without enzyme treatment), and the protein concentration in the supernatant with the addition of the enzyme (with enzyme treatment).

PROFILES OF HYDROLYSIS OF COLLAGEN AND DERIVATIVES OVER TIME

After obtaining the DH values for all substrates at 120 minutes of process, was studied the hydrolysis over of the 300 minutes to obtain hydrolysis profiles of DH substrates had better values in 120 minutes in order to verify a possible increase in DH over time. The hydrolysis profile was monitored at 60 minutes intervals for the three enzymes tested, individually. The DH was determined as described previously.

After obtaining the hydrolysis over 300 minutes profile, was studied the process of the hydrolysis over a 60 minutes using the enzyme showed best potential to hydrolysis of the substrates. The sample collection was carried out about every 15 minutes. All experiments were performed under the same operating conditions and determination of the DH. The experiments were performed in duplicate.

STATISTICAL ANALYSIS

The statistical analysis of experimental results was performed using the Statistica® 10.0 (STATSOFT, Inc.) and Tukey's test at the 95% level of confidence.

RESULTS AND DISCUSSION

DEGREE OF HYDROLYSIS (DH) OF THE SUBSTRATES

The most widely used parameter to describe the result of a hydrolysis process is the Degree of Hydrolysis (DH) (BENITEZ et al., 2008), defined as the fraction of cleaved peptide bonds, which is usually expressed as a percentage (DAMODARAN et al., 2010). The average of percentage of hydrolysis obtained in 120 minutes of process for each substrate, the standard deviation and the Tukey test (at the 95 % level of confidence) are shown in Table 1.

Table 1- Values of the Degree of Hydrolysis (DH) obtained in 120 minutes of process using bromelain, papain and collagenase to all substrates

Substrate	DH (%) ± Standard Deviation		
	Bromelain	Papain	Collagenase
Crude collagen fiber	35.99 ^{dB} ± 0.27	33.95 ^{eA} ± 0.37	37.25 ^{cC} ± 0.44
Powdered collagen fiber	8.33 ^{aA} ± 0.21	6.27 ^{dB} ± 0.08	7.46 ^{bA} ± 0.78
Gelatin	7.58 ^{aA} ± 0.08	3.33 ^{cB} ± 0.30	7.43 ^{bA} ± 0.22
Collagen hydrolysate A	3.83 ^{cA} ± 0.53	1.94 ^{bB} ± 0.23	2.09 ^{aB} ± 0.32
Collagen hydrolysate B	0.94 ^{bAB} ± 0.27	0.47 ^{aA} ± 0.16	1.41 ^{aB} ± 0.16

Lower case letters same, in column, are averages of DH same for the same enzyme in 95 % level of confidence, using the Tukey Test. Uppercase letters same, in line, are averages of DH equal for the same substrate in 95 % level of confidence, using the Tukey Test.

Fonte: Elaborado pelo autor (2015).

Comparing the same substrate in relation the enzyme (in line), it can be observed that the major DH obtained was 37.25 % for the crude collagen fiber using collagenase, followed by 35.99 % using bromelain and 33.95 % using papain, and these values are statistically different ($p < 0.05$) by Tukey test.

Verifying in relation to the same enzyme for different substrates, it is noticed that for bromelain, the highest average of DH was obtained for the crude collagen fiber, whose value is statistically different ($p < 0.05$) from the other substrates. For powdered collagen fiber and gelatin, the average values of DH are same statistically ($p > 0.05$). The hydrolyzed collagens A and B were the substrates whose the DH value was inferior, statistically different from each other. By using papain, all the DH values are statistically different ($p < 0.05$). For collagenase, the DH of crude collagen fibers differs significantly from the others substrates, and the powdered collagen fiber and gelatin and collagen hydrolysates A and B showed no significant difference ($p < 0.05$), respectively.

The crude collagen fiber (coarse fraction) and the powdered collagen fiber (fine fraction) are from by-products of tanneries, such as dermis and subcutaneous tissue, that were previously subjected to chemical and heat treatment, but the powdered collagen fiber is subjected to higher temperatures and subsequent grinding (PEREIRA et al., 2011).

In a study by Wolf et al. (2009), about the characteristics of collagen fibers and powdered collagen fiber, the authors conclude that the collagen fibers are more susceptible to structural changes compared to the powdered collagen fiber, due

to a higher hydration capacity, thus explaining the highest value of DH obtained for crude collagen fiber obtained in this study. Another factor that may explain the higher value DH crude collagen fiber is that in the treatment to obtain the powdered collagen fiber may have occurred a break of peptide bonds or disorganization in the protein structure, thereby, the enzymes do not have as many links peptide available for the cleavage in crude collagen fiber (SCHRIEBER; GAREIS, 2007).

Similar results to those found for the crude collagen fiber of this study were found by Apinunjarupong et al. (2009) who obtained values of DH ranging from 19.00 % at 36.50 % of protein contained in the external part of the rice grain hydrolyzed with 6 % of bromelain.

The DH values of the powdered collagen fiber and gelatin were similar. These values can be explained because gelatin is already a product obtained by partial hydrolysis of collagen (GÓMEZ-GUILLÉN et al., 2011). Thus, as the powdered collagen fiber, the availability of peptide bonds would be available in a smaller quantity.

The smaller DH values were obtained for the samples of hydrolyzed collagen A and B, and the sample B presented the lowest value of DH of all the substrates used. The low DH these substrates is due to the fact that they both already presented almost completely hydrolyzed, thus limiting the action of enzymes due to low availability of peptide bonds for cleavage.

The collagen fiber is a product obtained from collagen in raw form while the gelatin is produced from the hydrolysis of collagen. Collagen hydrolysate is obtained by chemical and enzymatic hydrolysis under controlled conditions. The difference between the collagen hydrolysate and gelatin is that the collagen hydrolysate is dissolved in water or brine, the vast majority does not present gelling capacity. When collagen is in the form of fiber or powder, presents differences in properties compared with gelatin. Studies have shown the gelation properties of the collagen fibers and their use as raw material for the production of biocomposites. However, the emulsifying properties have not been widely exploited, despite its traditional use as a binder of water and fat in meat products (PRESTES, 2013; PRESTES et al., 2014).

According with Table 1, is observed also that the bromelain and collagenase presented the better proteolytic capacity for all substrates. The lowest DH values were obtained using the papain, which was less efficient, especially for sample gelatin, with value of DH of 3.33 %, whereas the bromelain and the collagenase presented, respectively, DH values of 7.58 % and 7.43 %.

Low values of DH were also obtained in the study of Kong et al. (2008), in the process of hydrolysis of soybean protein, for 180 minutes, the papain reached a DH near of 6 %, whereas when used Alcalase, the DH was 3 times higher, reaching 18.30 %.

The bromelain and papain are cysteine proteases of plant origin, whose action is the activation of cysteine radical by a histidine, doing the function of nucleophile that attacks the peptide bond (COELHO et al., 2008). The results demonstrate that, although the two enzymes exhibit the same mode of action under the conditions used, the bromelain presented a higher proteolytic potential than the papain.

All of the enzymes have an optimum temperature to achieve maximum activity, wherein the enzyme has a constant activity for a period of time (BOBBIO and BOBBIO, 2003). Ketnawa et al. (2010) in a study conducted for hydrolysis of bovine collagen, found a higher activity for bromelain at 55 °C. Thus, the temperature used in this study may have influenced so that bromelain has reached its maximum activity, unlike of the papain.

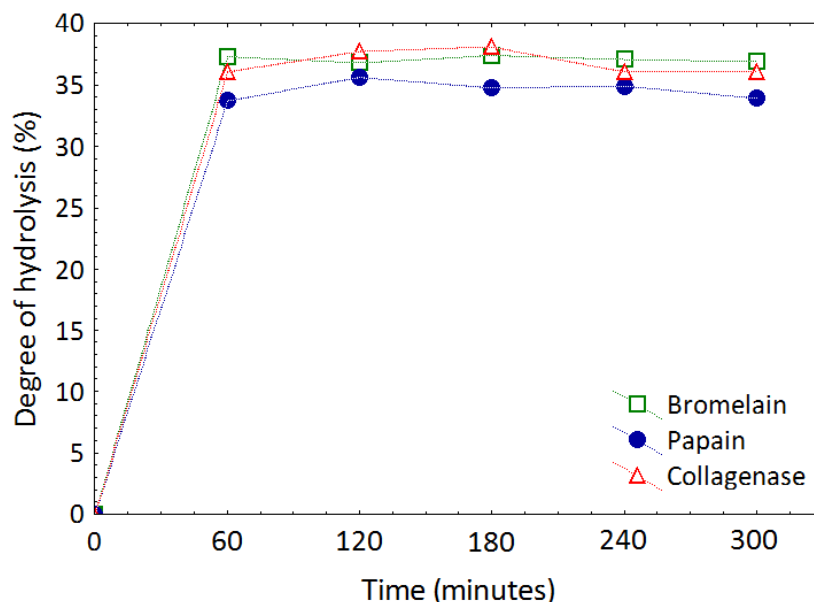
PROFILES OF DEGREE OF HYDROLYSIS OVER DIFFERENT TIMES

The substrates with larger average of DH in 120 minutes of hydrolysis, was studied over 300 minutes of hydrolysis in order to verify a possible increase in the value of DH. In Figure 1 are shown the profiles of the DH obtained over of 300 minutes of process to the crude collagen fiber.

It is observed that in the first 60 minutes of the process, the reaction occurs at a high rate, in which a large number of peptide bonds are hydrolyzed. Subsequently, there is a decrease in the enzymatic hydrolysis rate, reaching an apparent steady stage where small variations of DH occur until 300 minutes. Values exceeding the 30 % of hydrolysis have been observed in the first 60 minutes of the

process for the three enzymes tested and the bromelain show a better performance, with a DH near of 37 %.

Figure 1 - Hydrolysis profiles over of 300 minutes of process for the crude collagen fiber using bromelain, papain and collagenase

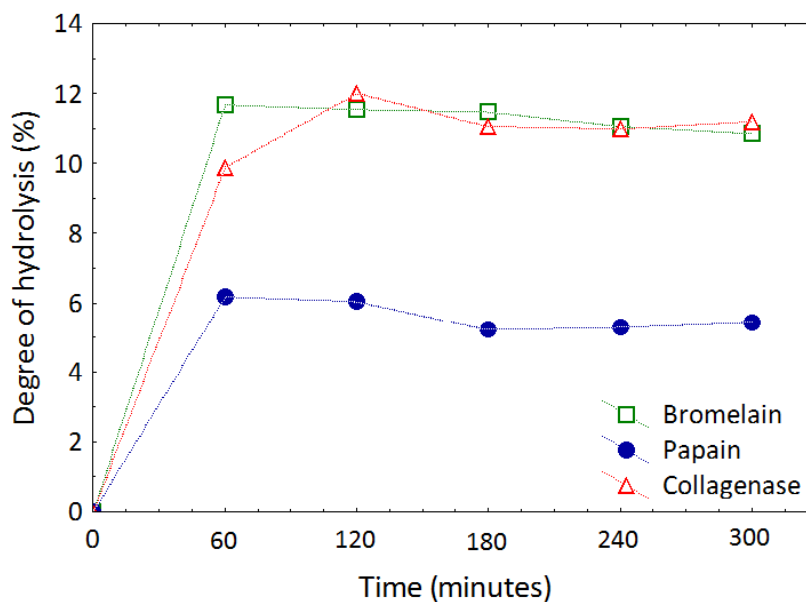


Fonte: Elaborado pelo autor (2015).

A similar profile was also obtained by Kong et al. (2007) for hydrolyzing wheat gluten during 300 minutes using Alcalase, trypsin, pancreatin and a commercial protease. The reaction occurred at a high rate during the first 30 minutes and, posteriorly, was characterized by an equilibrium phase.

The Figure 2 show the profiles of DH obtained over of 300 minutes to the powdered collagen fiber. As in the crude collagen fiber, the powdered collagen fiber had a high hydrolysis rate in the first hour of reaction, followed of a reduction of rate up to achieve a constant phase (Figure 2), evidenced by the profiles obtained with the use of bromelain and collagenase. For the first 60 minutes of reaction, the bromelain showed a value of DH next to 12% followed by collagenase (approximately 10 %) and papain (approximately 6 %). While the papain and bromelain remained at a constant level of hydrolysis (between 60 and 120 minutes of process), the collagenase led to an increase of approximately 9 % to 12 % of DH. Thus, there was a higher affinity of the collagenase for the substrate. This can be justified by collagenase present high specificity for collagen molecules having efficient mechanisms of action for the degradation of this substrate (LIMA et al., 2009).

Figure 2 - Hydrolysis profiles over of 300 minutes of process for the powdered collagen fiber using bromelain, papain and collagenase

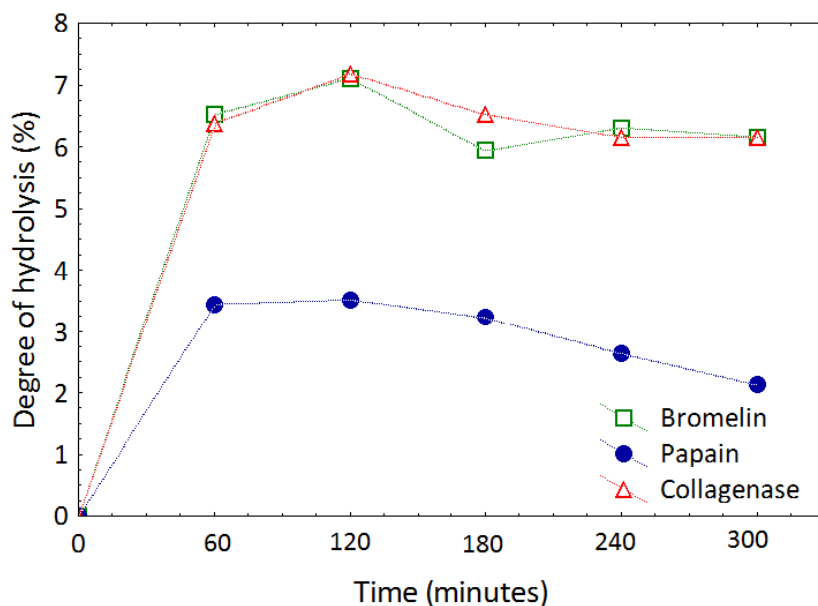


Fonte: Elaborado pelo autor (2015).

The hydrolysis profiles for the gelatin, over of 300 minutes, are shown in Figure 3. The collagenase and bromelain showed an increased rate of hydrolysis up to 120 minutes of reaction. Subsequently, there is a decline (180 minutes - 240 minutes), justified, possibly, by triggering a reverse reaction to the hydrolysis, with formation of new peptide bonds. For the papain, the DH has already been achieved for the first 60 minutes and after that there was a decline that followed linearly to 300 minutes of reaction.

The continuous profile can be attributed to several factors such as: the reduction of the concentration of peptide bonds available for cleavage, the competition between the original substrate and the peptides formed during the reaction or decrease in enzymatic activity/enzyme denaturation (KUROZAWA et al., 2009).

Figure 3 - Hydrolysis profiles over of 300 minutes of process for the gelatin using bromelain, papain and collagenase



Fonte: Elaborado pelo autor (2015).

The results of DH (%) for the crude collagen fiber in 60 minutes and 300 minutes, standard deviation and Tukey's test at the 95 % confidence level are shown in Table 2.

Analyzing the DH values for the crude collagen fiber, shown in Table 2, it is observed that for all three enzymes, individually analyzed with respect to time, there was no significant difference ($p > 0.05$) between the DH in 60 and in 300 minutes of reaction.

Table 2- Values of the Degree of Hydrolysis (DH) obtained in 60 minutes and 300 minutes for crude collagen fiber

Enzyme	DH (%) \pm Standard Deviation	
	60 minutes	300 minutes
Bromelain	37.30 ^{aA} \pm 0.35	36.88 ^{aA} \pm 0.29
Papain	33.73 ^{bA} \pm 0.24	33.91 ^{bA} \pm 0.37
Collagenase	36.08 ^{aA} \pm 0.36	36.13 ^{aA} \pm 0.29

Lower case letters same, in column, are averages of DH same for the same time, in 95 % level of confidence, using the Tukey Test. Uppercase letters same, in line, are averages of DH same to for the same enzyme in 95 % level of confidence, using the Tukey Test.

Fonte: Elaborado pelo autor (2015).

Regarding the same time, we can see that the value of DH obtained using papain, in 60 minutes of reaction, differ statistically ($p < 0.05$) of the collagenase

and bromelain, with the value of DH lower for papain. The same occurred to the time of 300 minutes with the papain, lower and different statistically ($p < 0.05$) of the DH obtained of the other enzymes.

The DH results of powdered collagen fiber for 60 minutes and 300 minutes of hydrolysis, the standard deviation and Tukey's test at the 95 % confidence level are shown in Table 3. Between the times, it is found that in 60 minutes of reaction using bromelain, the DH value obtained showed a different statistically ($p < 0.05$) of the value of DH obtained for 300 minutes. Also, it was observed that there was a reduction in the DH value of 60 initial minutes until the 300 minutes of hydrolysis. Some enzymes are inhibited by high concentrations of product formed or lack of reagent in the reaction media, causing the displacement in direction of the reaction, which may have occurred when used papain and bromelain, characterized by decay profile (WHITAKER et al., 2003; BIAZUS et al., 2006).

Unlike other enzymes, the collagenase showed an increased DH of the 60 minutes to 300 minutes of the process, with the average of DH statistically different ($p < 0.05$). Comparing to the same time, it is verified that the DH values obtained for powdered collagen fiber, in 60 minutes of process, differ statistically ($p < 0.05$) and the largest DH was obtained for the bromelain, followed by collagenase and, finally, papain. In 300 minutes, the behavior of bromelain and collagenase is reversed, with higher value of DH for collagenase, with all the averages of DH statistically different.

Table 3 - Values of the Degree of Hydrolysis (DH) obtained in 60 minutes and 300 minutes for powdered collagen fiber

Enzyme	DH (%) \pm Standard Deviation	
	60 minutes	300 minutes
Bromelain	11.68 ^{aA} \pm 0.01	10.85 ^{aB} \pm 0.01
Papain	6.18 ^{bA} \pm 0.19	5.43 ^{bB} \pm 0.09
Collagenase	9.89 ^{cA} \pm 0.00	11.20 ^{cB} \pm 0.09

Lower case letters same, in column, are averages of DH same for the same time, in 95% level of confidence, using the Tukey Test. Uppercase letters same, in line, are averages of DH same to for the same enzyme in 95% level of confidence, using the Tukey Test.

Fonte: Elaborado pelo autor (2015).

Spellman et al. (2009) using temperature 50 °C and pH 7.0 for protein whey hydrolyzed with collagenase (Corolase® - 7089), obtained after 240 minutes of reaction, DH value of 9.6 %, approaching the values found in this study.

By comparing between the crude collagen fiber, powdered collagen fiber and gelatin, the lower DH values were obtained for the gelatin, as shown in Table 4.

Table 4 - Values of the Degree of Hydrolysis (DH) obtained in 60 minutes and 300 minutes for gelatin

Enzyme	DH (%) ± Standard Deviation	
	60 minutes	300 minutes
Bromelain	6.53 ^{aA} ± 0.10	6.16 ^{aA} ± 0.00
Papain	3.45 ^{bA} ± 0.10	2.13 ^{bB} ± 0.31
Collagenase	6.38 ^{aA} ± 0.10	6.16 ^{aA} ± 0.21

Lower case letters same, in column, are averages of DH same for the same time, in 95 % level of confidence, using the Tukey Test. Uppercase letters same, in line, are averages of DH same to for the same enzyme in 95 % level of confidence, using the Tukey Test.

Fonte: Elaborado pelo autor (2015).

For the conversion of collagen (molecular weight of the 345,000 and 360,000 Da) in gelatin (molecular weight of the 10,000-65,000 Da - only in some cases reaching 250,000 Da), the controlled hydrolysis is required (OCKERMAN and HANSEN, 1994), demonstrating a difference between the collagen samples (crude and powdered) and the gelatin, characterized as a partially hydrolyzed molecule due to the manufacturing process.

For all enzymes, it was verified that the values of DH in 300 minutes was lower compared to the DH values obtained in 60 minutes, but only for papain a significant difference was observed ($p < 0.05$) between the two times. As is the class of hydrolases, proteases are able to perform the reverse reaction, with forming the peptide bonds (KOBBLITZ, 2008). This reaction is also known as the plastein reaction, which occurs, primarily, from a series of reactions involving the initial proteolysis followed by peptide bonds of resynthesis (DAMODARAN et al., 2010). When the hydrolysate containing the enzyme is concentrated to 30-35 % of solids, and incubated, the enzyme recombines the random peptides, generating new peptide bonds.

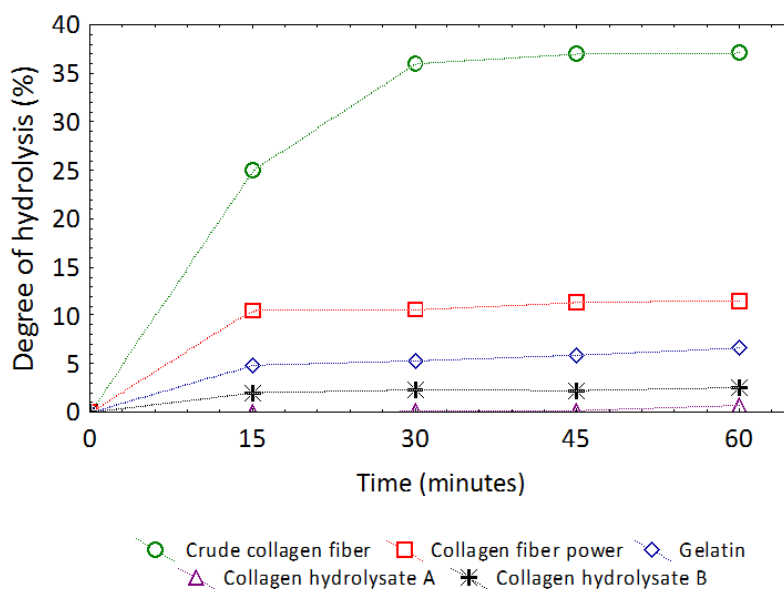
Based on the results, it can be observed that the bromelain and the collagenase have a proteolytic capacity similar for all samples, being efficient in the

hydrolysis of substrates. Based on the obtained profiles, a new study of the hydrolysis regarding the time, since for these substrates, already in the first 60 minutes of reaction, there was obtained the highest DH values, with some small increments in until 300 minutes of process.

The Figure 4 shown the profiles for the hydrolysis of all substrates studied (crude collagen fiber, powdered collagen fiber, gelatin, collagen hydrolysate A and collagen hydrolysate B) over of 60 minutes using the bromelain as biocatalyst (enzyme with the higher results of DH).

For the crude collagen fiber, the rate of reaction increased until 30 min of the hydrolysis, with a DH value of 35.99 % and in 60 minutes, the value of DH was 37.10 %. For the collagen fiber powder, gelatin and the hydrolyzed collagen A, in the first 15 minutes, the reaction progressed rapidly, with average values of DH of 10.58 %, 4.84 % and 1.99 %, respectively. After this time, the reaction was slower and in 60 minutes, the DH values were 11.61 %, 6.59 % and 2.53 %, respectively.

Figure 4 - Hydrolysis profiles for all substrates over 60 minutes of the process using bromelain



Fonte: Elaborado pelo autor (2015).

Unlike other substrates, to collagen hydrolysate B, in the first 15 minutes of reaction, there was no hydrolysis. An average value of DH of 0.07 % was observed after 30 minutes of reaction, and in 60 minutes of process, was obtained 0.71 % of DH. Similar results were obtained by Lamsal et al. (2007) for soy protein and

bromelain, which showed that the reaction progresses rapidly for the first 15 minutes, then immediately, of the a constant profile.

CONCLUSION

Using the crude collagen fiber as substrate for hydrolysis, was obtained the highest average values of Degree of Hydrolysis (DH) for the 3 enzymes under study, bromelain, papain and collagenase, with values above of 33 %. For be hydrolyzed collagen, smaller DH values were obtained for the collagen hydrolysate B, with DH average values of less than 1 %, suggesting that this substrate has been almost completely hydrolyzed in the manufacturing process, different from the hydrolyzed collagen A. During 300 minutes of reaction, it was verified that the highest DH values are obtained during the first 60 minutes of hydrolysis, with minor variations over time. For the crude collagen fiber, the first 30 minutes, the reaction rate was high, reaching to the DH value close to that obtained in 60 minutes. For other substrates, the DH profile was similar, but only in the first 15 minutes of hydrolysis. The proteolytic ability of collagenase and bromelain was similar, leading to higher values of DH, thereby demonstrating that both have the potential for hydrolysis of the substrates studied. There are large numbers of studies on the applications of collagen hydrolysate in the most diverse areas; however, there is little literature on the study of the DH. The protein hydrolysates obtained could be used in food products in order to obtain functional properties, technological properties such as increased water retention and decreased fluid exudation.

Perfis de hidrólise enzimática de diferentes colágenos e derivados ao longo do tempo

RESUMO

O interesse no uso de colágeno na indústria de alimentos em substituição aos agentes sintéticos cresce a cada dia. Dentre os derivados, temos os hidrolisados, que se dissolvem e água e salmoura com facilidade, podendo ser incorporados em alimentos ou bebidas. O objetivo do presente estudo foi verificar o comportamento do Grau de Hidrólise (GH) da fibra de colágeno bruta, fibra de colágeno em pó, gelatina e duas amostras de colágeno hidrolisado. Os substratos foram submetidos a ação individual de três enzimas proteolíticas, sendo duas de origem vegetal, a papaína e a bromelina, e uma de origem microbiana, uma colagenase. Os substratos foram incubados em tampão fosfato de sódio 0,1 M pH 7,0 em agitador orbital a 55 °C por diferentes períodos de tempo. Em 120 minutos de hidrólise, a fibra de colágeno bruta apresentou 37,2 % de GH utilizando a colagenase, o maior valor obtido em relação aos demais substratos. Os menores valores de GH foram para os colágenos hidrolisados, com valores de GH inferiores 1 %. Para a hidrólise em 60 minutos, os valores de GH obtidos foram próximos aos obtidos em 120 minutos de hidrólise, apresentando pequenas variações no decorrer do tempo. A capacidade hidrolítica da colagenase e da bromelina foram semelhantes e superiores a papaína.

PALAVRAS-CHAVE: Colágeno. Grau de hidrólise. Enzimas proteolítica. Tempo de hidrólise.

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Recebido: 28 out. 2015.

Aprovado: 18 abr. 2017.

Publicado: 27 mai. 2017.

DOI: 10.3895/rbta.v11n1.2755

Como citar:

ILTCHENCO, S.; KEMPKA, A. P.; PRESTES, R. C. Profiles of enzymatic hydrolysis of different collagens and derivatives over time. **R. bras. Technol. Agroindustr.**, Ponta Grossa, v. 11, n. 1, p. 2165-2185, jan./jun. 2017. Disponível em: <<https://periodicos.utfpr.edu.br/rbta>>. Acesso em: XXX.

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