The Review Of
DIABETIC
STUDIES OPEN ACCESS

Comparative Analysis Of Proteolytic Activity Of Bromelain And Papain Enzymes On Soy Protein Isolate

Kumari Monica

Assistant Professor, Department of Botany, B.D. College, Patliputra University, Patna, India.
Email ID: kmonica1980@gmail.com, ORCID ID: 0009-0008-3329-3256

Abstract
The food and biotechnological industry use a myriad of proteolytic enzymes for protein modification, including papain and bromelain. This present study compares the performance of two plant derived cysteine proteases (Bromelain and Papain) as a function of their proteolytic efficiency on soy protein isolate (SPI). The enzymatic hydrolysis was performed in controlled conditions and the degree of hydrolysis (DH) was determined by O-phthaldialdehyde (OPA) assay method. The study of the enzymatic activity of papain, extracted from papaya, and bromelain, derived from pineapple, is significant because these plant-based cysteine proteases possess diverse applications in medicine, industrial biotechnology, and sustainable bioprocessing.

Keywords: Bromelain; Papain; Soy protein isolate; Degree of hydrolysis; OPA Assay.

1. Introduction

Proteolytic enzymes are biocatalysts which break peptide bonds in proteins and are widely used in food, biotechnology and pharmaceutical industries. Enzymatic hydrolysis is a valuable tool for food protein modification to enhance, for example, digestibility, solubility, emulsification and to produce peptides that may have bioactive properties (Adler-Nissen, 2000).

Soy protein isolate (SPI) is a major plant derived protein ingredient due to its high protein content, desirable amino acid profile and a wide range of functional properties. However, both the structure of soy proteins and presence of some antinutrient components can affect digestibility and technological properties. Enzymatic hydrolysis is a controlled process which could be used to make changes in the structural and functional properties of SPI and would allow production of protein hydrolysates with enhanced nutritional and functional properties (Radha & Kurup, 2002).

Bromelain, extracted from Ananas comosus, and Papain derived from Carica papaya are the most researched cysteine proteases of plant origin. Enzymes both have relatively broad proteolytic activity, and have been used for the hydrolysis and modification of food proteins. Their hydrolysis capabilities, however, may differ depending on their substrate specificity, catalytic properties and interaction with individual protein substrates. Thus a comparative evaluation of bromelain and papain on soy protein isolate as a common substrate can be helpful in providing information about their relative hydrolytic behavior under controlled conditions. Based on this, the present study was conducted to compare the proteolytic activity of bromelain and papain against SPI by observing the hydrolysis degree as a function of time, the respective hydrolysis kinetics were analyzed graphically, and the effect of the type of enzyme and the hydrolysis time was statistically evaluated.

2. Literature Review

2.1 Soy Protein Isolate as a Substrate for Enzymatic Hydrolysis

Soybean plant (Glycine max) is a valuable source of plant protein for human consumption and also has a wide range of uses in the food industry. Soy protein isolate (SPI) is a highly purified preparation of soy protein which consists mainly of the storage proteins glycinin (11S) and β-conglycinin (7S). These proteins are a significant component of the nutritional and functional attributes of SPI. The globular shape and intermolecular forces may, however, affect their susceptibility to the action of proteolytic enzymes.

Enzymatic hydrolysis of SPI has been extensively studied as a way to alter functional and nutritional properties. Proteolysis leads to the formation of smaller peptides and free amino groups that can alter characteristics like digestibility, emulsifying capacity, foaming ability, solubility etc. These changes can be more or less, depending on the nature of the enzyme, properties of the substrate, and conditions of hydrolysis.

2.2 Proteases and Enzymatic Protein Hydrolysis

Proteases are enzymes that catalyse the hydrolysis of peptide bonds in proteins and peptides. They are classified according to their catalytic mechanisms into major groups such as serine, cysteine, aspartic and metalloproteases. The proteases derived from plants have been secured due to their wide substrate particularity and the thought of using them in food and biotechnology.

Several factors including enzyme concentration, substrate concentration, pH, temperature and reaction time and enzyme–substrate interaction influence the efficiency of the enzymatic hydrolysis. The degree of hydrolysis (DH) is one of the most commonly used indicators of the amount of protein degradation and is the percentage of peptide bonds that are broken by the enzyme. Determination of DH is thus a useful parameter for comparing the proteolytic efficiency of different enzymes.

2.3 Bromelain and Its Proteolytic Activity

Bromelain is a mixture of proteolytic enzymes that is obtained mostly from pineapple (Ananas comosus). Commercial preparations of bromelain can include several enzymatically active proteins, but the main proteolytic enzymes in bromelain are the cysteine proteases. Bromelain shows a wide substrate specificity and has been studied extensively for its protein hydrolyzing ability in both plant and animal proteins.

Bromelain catalytic activity is affected by the pH, temperature, enzyme concentration and the incubation time. It has been found to be useful in the modification of food proteins and the preparation of peptide fractions with different physicochemical and functional properties, because of its ability to cleave peptide bonds. But, the efficiency of the hydrolysis by bromelain depends on the type and structural properties of the protein substrate and the reaction conditions used.

2.4 Papain and Its Proteolytic Activity

Cysteine proteases are also called papain, which is the major enzyme found in the latex of the plant, Carica papaya. It is one of the most studied plant proteases and has a relatively broad substrate specificity. Papain is capable of breaking down a variety of proteins to yield peptides of lower molecular weight and leaving more free amino groups.

The activity of papain is highly sensitive to environmental factors such as pH, temperature, enzyme to substrate ratio, and incubation period. It is known to have proteolytic properties, and is used in food processing, protein modification and biotechnology. However, the degree of hydrolysis obtained with papain is dependent on the nature of the protein to be hydrolysed and the conditions under which hydrolysis occurs.

2.5 Enzymatic Hydrolysis of Soy Proteins

The enzymatic hydrolysis process has proved to be an effective process for the modification of soy protein molecular and functional properties. Soybean protein has been subjected to hydrolysis by various proteases such as papain, microbial proteases, and trypsin and pepsin. Proteolytic cleavage

would decrease the molecular size of proteins, thereby increase the number of terminal amino groups and potentially improve the solubility, digestibility and other functional properties.
Soy proteins are prone to enzymatic hydrolysis and the accessibility of the peptide bonds plays a role in determining the susceptibility of soy proteins. The structure of glycinin and β-conglycinin is distinct, and may hence show different sensitivity to proteolytic attack. Thus, the type of protease can significantly affect both the amount and the type of hydrolysis.

2.6 Factors Affecting Proteolytic Hydrolysis

A number of interacting variables influence the extent of enzymatic hydrolysis. The conformation, catalytic activity and the accessibility of the substrate are affected by pH and temperature, and the ratio of enzyme to substrate will determine the number of available catalytic sites in relation to the amount of substrate. Another factor is reaction time, since hydrolysis may be fast in the first few steps, and slower as the bonds that are susceptible to hydrolysis are increasingly removed.
When comparing two different proteases it is especially important to standardize these variables. A uniform set of experimental conditions ensures that variations in the hydrolysis are more clearly related to the intrinsic catalytic properties and substrate specificity of the enzymes than to variations in experimental conditions.

2.7 OPA Method for Assessment of Protein Hydrolysis

The o-phthaldialdehyde (OPA) assay is one of the most common analytical methods used for the monitoring of enzymatic hydrolyses of proteins. It is based on the reaction of OPA with primary amino groups formed upon peptide-bond cleavage. A rise in number of OPA-reactive amino groups indicates the degree of proteolysis.
The assay can be repeated and is quick so it is ideal for the monitoring of changes in protein hydrolysis over time. The OPA reactive group of bromelain and papain can therefore be determined, and the hydrolytic behaviour of these two enzymes compared under the controlled experimental conditions.

2.8 Comparative Assessment of Bromelain and Papain

Bromelain and papain are both proteins derived from plants and are cysteine proteases, but present some differences in their catalytic property and substrate specificity, which could lead to different rates and extents of protein hydrolysis. It is not possible to compare their proteolytic performance solely from the results of independent studies, due to the fact that the conditions of the experiments differ significantly.
It is important to compare them directly with the same protein substrate under the same reaction conditions to get a more reliable estimate of their relative hydrolytic activity. This is especially applicable to SPI as the structure of glycinin and β-conglycinin can impact on enzyme access and enzyme cleavage.

2.9 Research Gap and Rationale

The hydrolytic activity of both the bromelain and papain, on a variety of protein substrates, has already been demonstrated. But, the results of published studies differ in experimental conditions, and as a result, it is hard to determine which is more efficient compared to the other towards SPI. Moreover, there are few studies that have assessed the two enzymes, bromelain and papain, together and under the same experimental conditions and the same analysis.
Hence, a comparative study of these two plant enzymes under controlled conditions is needed to characterize their proteolytic performance towards SPI. Bromelain and papain were tested under standard conditions and the hydrolysis of standard proteins was followed during a defined incubation time, with the OPA assay. This method allows the comparison of the temporal hydrolysis profile and degree of hydrolysis generated by the two enzymes and their suitability for enzymatic modification of soy protein.

3. Materials and Methods

3.1 Materials

Soy protein isolate (food grade) was used as the substrate. Analytical grade bromelain and papain were purchased from the commercial market. All the reagents used in buffer preparation and in OPA assay were of analytical grade.

3.2 Preparation of Substrate

SPI was dissolved in distilled water to make a 5% (w/v) solution and stirred continuously for 2 h to ensure complete hydration. The pH was optimized for both enzymes at 7.0 by using a phosphate buffer as follows: Optimal Activity Conditions for both enzymes are:

The optimal pH is 7.0 (phosphate buffer) and the optimal temperature is 50 °C.
Papain: pH 7.0 (phosphate buffer), 50 °C
From literature reports of optimal conditions for each enzyme)

3.3 Enzymatic Hydrolysis

Hydrolysis reactions were conducted individually using 1:100 enzyme–substrate ratio (w/w) of bromelain and papain. Reaction mixtures were incubated at 50 °C and samples were taken after 0, 15, 30, 60, 120 and 240 min incubation, and enzyme activity was stopped by heating at 95 °C for 5 min.

3.4 Determination of Degree of Hydrolysis

The degree of hydrolysis (DH) was determined using the method of O-phthaldialdehyde (OPA) described by Cupp-Enyard (2008).
DH was measured using O-phthaldialdehyde (OPA) assay, a method that measures the amount of free amino groups that are released during hydrolysis.

$$\mathrm{DH} = \frac{\text{Amt of free } \mathrm{NH_2} \text{ released}}{\text{Total peptide bonds in SPI}} \times 100$$

Absorbance was measured at 340 nm and DH was calculated as a percentage of the peptide bond that was broken to total peptide bonds in the SPI.

3.5 SDS -PAGE Analysis

SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis), which is commonly used as a method to separate proteins with molecular masses between 5 and 250 kDa was used for the analysis of enzymatic activity of both the enzymes. It was found that

- Bromelain Hydrolysis: Partial digestion of higher molecular mass polypeptides (β-conglycinin and glycinin) which are present.
- Papain Hydrolysis: Breakdown is more extensive, high molecular weight bands disappear and low mass peptides appear.

3.6 Statistical Analysis

All measurements were repeated three times and the results given as means. The statistical significance of the result between bromelain and papain treatments was analyzed.

4. RESULTS

Both enzymes showed a time dependent increase in DH (Table 1). Papain always exhibited greater DH values than bromelain at all the time points. Papain's DH was 57.2% after 240 min of incubation, while bromelain's DH was 43.2% after 240 min of incubation.

Table 1: Degree of Hydrolysis (%) of SPI

Time (minutes)	Bromelain (%)	Papain (%)
15	12.5	18.5
30	20.4	28.7
60	32.8	45.1
120	38.2	52.0
240	43.2	57.2

Table 1: Comparative proteolytic activity of bromelain and papain on soy protein isolate as measured by degree of hydrolysis over time.

Papain had significantly higher rate of hydrolysis than the bromelain in all the incubation periods. The graphical representation (Figure 1&2) shows that the papain is more kinetic and has higher proteolytic efficiency which are in agreement with previous reports (Radha & Kurup, 2002).

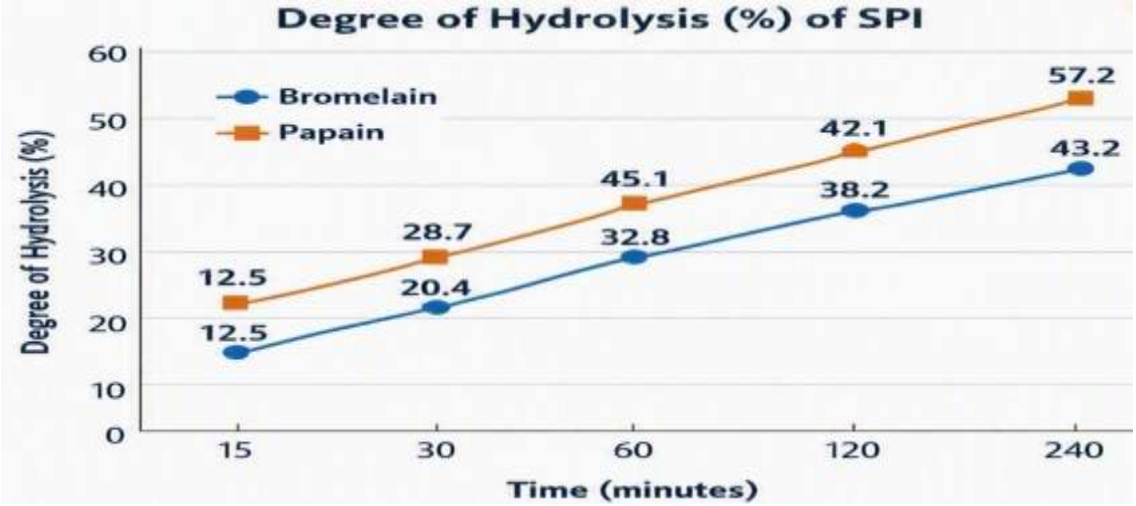

Figure 1. Graphical Representation of Comparative proteolytic activity of Bromelain and Papain on Soy Protein Isolate.

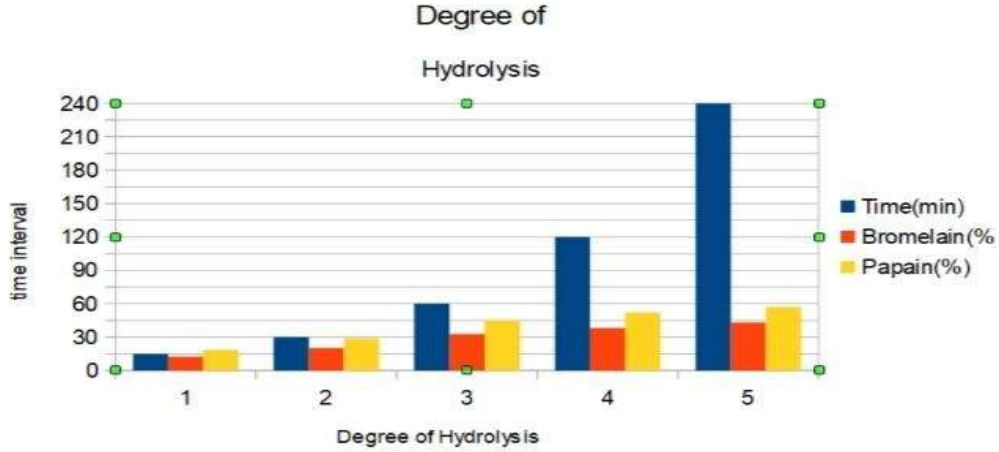

Figure 2: Bar graph showing difference in Bromelain and Papain with respect to time.

5. DISCUSSION

The papain had a slightly higher DH compared to the other enzymes, indicating that it has a higher hydrolytic activity towards the SII under the experimental conditions used. The difference could be due to the wide range of substrates that papain can break down and its catalytic properties which can allow it to break peptide bonds in complex plant proteins (Ha et al., 2012). Bromelain also showed significant proteolytic activities but its lower DH values were possibly due to differential recognition of the substrate, cleavage specificity or accessibility of susceptible peptide bonds within the soy protein matrix.

The observed differences between the two enzymes did reach statistical significance, and the consistent increase in DH associated with papain across the hydrolysis period represents an experimentally observable trend. Many factors are important in determining the extent of hydrolysis of a protein, such as enzyme concentration, nature of the protein, length of the reaction, pH, temperature and the interaction between enzymes and their substrates (Adler-Nissen, 2000). For the conditions explored in the present study, papain thus showed a higher level of soy protein hydrolysis, while bromelain showed a comparatively lower degree of hydrolysis. The different hydrolytic activities might be beneficial in choosing proteases for use in applications where either relatively extensive or more controlled modification of soy protein is desired.

CONCLUSION

In the present study, the proteolytic activity of bromelain and papain enzymes was comparatively determined using soy protein isolate (SPI) as the common substrate in the hydrolysis condition. Both enzymes showed a time-dependent increase when measured using the degree of hydrolysis (DH) measured by the O-phthaldialdehyde (OPA) assay. But papain always gave higher DH value than bromelain during incubation period. The hydrolysis time of 240 minutes resulted in a DH of 57.2% for papain and 43.2% for bromelain, and it is this higher value of DH that shows that a higher degree of protein degradation was achieved by papain under the experimental conditions used. The SDS-PAGE observations confirmed this trend, where papain had the most significant degradation of higher molecular weight soy protein fractions and the appearance of lower molecular weight peptides. Bromelain also had significant hydrolytic activity and relatively lower level of extensive protein degradation. The results indicate that the observed hydrolytic variation may be due to substrate specificity, catalytic properties, and accessibility of the susceptible peptide bonds. The differences in the reported hydrolysis values were not statistically significant, but the general trend was in the increasing direction with papain, suggesting this may be useful for larger scale hydrolysis. Bromelain could prove beneficial in situations where a more limited degree of protein modification is desired. Overall, the study gives a comparative basis for choosing the plant proteins for hydrolysis of soy protein in food and biotechnological applications.

References

1. Mora, L., & Toldrá, F. (2023). Advanced enzymatic hydrolysis of food proteins for the production of bioactive peptides. Current Opinion in Food Science, 49, 100973.
2. Mamboya, E. A. F., & Amri, E. (2012). Papain, a plant enzyme of biological importance: A review. Am. J. Biochem. Biotechnol, 8(2), 99-104.
3. Ashaolu, T. J. (2020). Applications of soy protein hydrolysates in the emerging functional foods: A review. International Journal of Food Science and Technology, 55(2), 421-428.
4. Mamboya, E. A. F., & Amri, E. (2012). Papain, a plant enzyme of biological importance: A review. Am. J. Biochem. Biotechnol, 8(2), 99-104.
5. Coscueta, E. R., Campos, D. A., Osório, H., Nerli, B. B., & Pintado, M. (2019). Enzymatic soy protein hydrolysis: A tool for biofunctional food ingredient production. Food chemistry: X, 1, 100006.
6. Cupp-Enyard, C. (2008). Sigma's non-specific protease activity assay-casein as a substrate. JoVE (Journal of Visualized Experiments), (19), e899.

7. Rout, P., Chakraborty, C., & Hossain, S. (2024). Functional characterization of enzyme-hydrolysed soy and whey protein isolates: A comparative approach. Food Chemistry Advances, 5, 100745.
8. Nielsen, P. M., Petersen, D., & Dambmann, C. J. J. O. F. S. (2001). Improved method for determining food protein degree of hydrolysis. Journal of food science, 66(5), 642-646.
9. Tacias-Pascacio, V. G., Castañeda-Valbuena, D., Tavano, O., Murcia, A. B., Torrestina-Sánchez, B., & Fernandez-Lafuente, R. (2023). Peptides with biological and technofunctional properties produced by bromelain hydrolysis of proteins from different sources: A review. International Journal of Biological Macromolecules, 253, 127244.
10. Ha, M., Bekhit, A. E. D. A., Carne, A., & Hopkins, D. L. (2012). Characterisation of commercial papain, bromelain, actinidin and zingibain protease preparations and their activities toward meat proteins. Food Chemistry, 134(1), 95-105.
11. Ramli, A. N. M., Manas, N. H. A., Hamid, A. A. A., Hamid, H. A., & Illias, R. M. (2018). Comparative structural analysis of fruit and stem bromelain from Ananas comosus. Food chemistry, 266, 183-191.
12. Sui, X., Zhang, T., & Jiang, L. (2021). Soy protein: Molecular structure revisited and recent advances in processing technologies. Annual Review of Food Science and Technology, 12(1), 119-147.
13. Sun, X. D. (2011). Enzymatic hydrolysis of soy proteins and the hydrolysates utilisation. International Journal of Food Science and Technology, 46(12), 2447-2459.
14. Tsumura, K. (2009). Improvement of the physicochemical properties of soybean proteins by enzymatic hydrolysis. Food Science and Technology Research, 15(4), 381-388.
15. Zang, X., Yue, C., Liu, M., Zheng, H., Xia, X., & Yu, G. (2019). Improvement of freeze-thaw stability of oil-in-water emulsions prepared with modified soy protein isolates. Lwt, 102, 122-130.
16. Xu, Y., Yang, Y., Ma, C. M., Bian, X., Liu, X. F., Wang, Y., ... & Zhang, N. (2023). Characterization of the structure, antioxidant activity and hypoglycemic activity of soy (Glycine max L.) protein hydrolysates. Food research international, 173, 113473.