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A REVIEW: APPLICATION OF BROMELAIN ENZYMES IN ANIMAL FOOD PRODUCTS

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ABSTRACT

This review focuses on the use of bromelain in various applications in animal products with the latest literature so that it can provide information on what parts of this enzyme can be applied. Bromelain is a protease enzyme found in stems (EC 3.4.22.32), leaves, crowns, fruit skins, fruit flesh (EC 3.4.22.33) and fruit tubers in pineapple. Bromelain has been exploited commercially in many applications in the food industry (baking industry), drinks industry (stabilizers in beer), tenders (meat), and pharmaceuticals (anti-tumorigenic agents). However, not all types of proteins can be hydrolyzed by bromelain, such as keratin which can only be hydrolyzed by keratinase, this is because the enzymes work specifically. In animal food products, bromelain is applied as a meat tenderizer, making of protein hydrolyzate products, cheese and also fish sauce product. The application of bromelain to animal food products has proven that hydrolyzed products using this enzyme can increase umami taste, which means that bromelain has great potential when applied to animal food products. Bromelain is useful in the processing of some animal food products because bromelain works specifically and is very active in animal protein such as milk, meat and collagen.

Keywords: Bromelain, Bromelain application, Animal food products.

INTRODUCTION

Protease enzymes already dominate the market, which is almost 60% of the total marketing of other enzymes (Wu at al., 2017). The main producers that produce proteases are Miles Laboratories, Gist-Brocades, Novo Industries and Genencor International (Feijoo-siota at al., 2011). Bromelain is one of the protease enzymes found in pineapple that has great potential in its application in the food industry. Research and isolation of bromelain has been investigated since 1894 (Neta at al., 2012). Bromelain is the main protease enzyme known for pineapple chemically since 1876 (Tochi at al., 2008) and was identified

for the first time by Marcano in 1891 (Upadhyay et al., 2010). Bromelain is found in stems (EC 3.4.22.32), leaves, crowns, fruit (EC 3.4.22.33), tubers and bark (Mohan et al., 2016 and Pavan et al., 2012). Pineapple stems have different biochemical properties and bromelain composition when compared to pineapple flesh (Pavan et al., 2012) and contain different mixtures of thiol-endopeptidase. The main component of bromelain is the proteolytic sulfhydryl enzyme which is the same as papain and fission (Tochi et al., 2008 and Gautam et al., 2010). However, bromelain is a glycoprotein whereas papain and fission are proteins (Chobotova et al., 2009).

Bromelain enzyme activity can be influenced by several factors, namely the source of the enzyme, pH, temperature, substrate and inhibitors (Chobotova et al., 2009). According to Liang et al., (2012) Fe³⁺ and Cu²⁺ metal ions are inhibitors that can significantly reduce bromelain activity. Optimum condition of bromelain enzyme is at pH 6.5 and temperature 50°C (Sree et al., 2012). Meanwhile, according to Mohapatra et al., (2013) the bromelain enzyme was stable at pH 3.0 - 7.0 and temperature of 40°C - 60°C. Based on the research of Manzoor et al., (2016), bromelain is stable at pH 5.5 - 8.0 and temperature 35.5°C - 71°C.

In the food industry, bromelain can be applied to non-animal products such as baking industry (Chalamaiah et al., 2012), stabilizers in beer and reduce the formation of foam (Benucci et al., 2011), anti-browning agents to inhibit browning in fruits (Chaisakdanugull et al. 2007) and enzymatic production of Virgin Coconut Oil (VCO) (Raghavendra et al., 2010). Based on the fact that the wide application of bromelain enzymes in the food industry, a review that focused on the application of bromelain in animal food products.

BROMELAIN ENZYME APPLICATIONS IN ANIMAL FOOD PRODUCTS

Bromelain enzymes are known to have very wide applications, such as those used in the food and beverage industry (Neta et al., 2012), in the pharmaceutical or medical industry (Dhandayuthapani et al., 2012) and cosmetics (Orsini, 2016). In this review, we will discuss the application of the bromelain enzyme in animal food products, which can be briefly seen in Table 1.

According to Taqwadasbriliani et al., (2013) bromelain works more active on animal protein, while papain works on vegetable protein. However, not all types of protein in animals that can be hydrolyzed by bromelain (such as keratin which is very well hydrolyzed by keratinase) (Mirdayanti, 2013), because of its specific working properties. Bromelain enzymes in pineapple can hydrolyze proteins in milk, meat and collagen (Dalimartha and Adrian, 2011).

The addition of the bromelain enzyme is not expected to reduce the quality of taste in the product, because taste is the main factor for consumers in choosing a product to consume. The enzymatic and non-enzymatic hydrolysis process is often associated with bitter effects. Based on research by Cheung and Li-Chan (2014), it was found that after 8 hours the hydrolysis process by bromelain showed a lower bitter taste and a better umami taste compared to alkalase and protamek hydrolase. The taste of umami in the hydrolysis products by the bromelain enzyme can suppress the bitter taste in the product (Rhyu and Kim,

2011). Likewise, the enzyme papain in making buffalo milk dangke by using the papain enzyme, will cause a bitter taste in the dangke produced, so that many consumers are dislike it, the more concentration of the papain enzyme added the bitter taste will increase (Sulmiyati and Said, 2018).

Table 1. Application of Bromelain Enzyme in Animal Food

Application	Reasons	References
Tenderization	– hydrolysis of myofibril protein in meat – hydrolysis for rejected meat in chicken and duck, hydrolysis in beef, goat and rabbit	Ketnawa & Rawdkuen (2011), Chaurasiya et al., (2015) , Nadzirah et al., (2016) , Doneva et al., (2018), and Novita et al., (2019)
Protein hydrolyzate	– hydrolyzes protein in fish meat or milk into protein hydrolyzate	Himonides et al., (2011), Wijayanti et al., (2016), and Prastari et al., (2017)
Cheese processing	– Assist the caking process in making cheese	Jaya & Didik (2009), and Komansilan et al., (2020)
Fish sauce processing	– Shorten the hydrolysis time of fish meat in fish sauce processing	Prasetyo et al., (2012), Kristianawati (2014), Bala et al., (2012), and Rabie et al., (2018)

Tenderization

Meat tenderness is one of the most common characteristics of meat quality. In the US, approved Angus beef has been introduced since the late 1970s, with the approval of consumer interest by its flavorful, juiceness, and tenderness (Koochmaraie and Geesink, 2010). Hard meat texture can be caused by various factors including the amount of intramuscular connective tissue, intramuscular fat and sarcomere length (Kemp at al., 2010). Meat softening during the postmortem ripening process or with additional combinations is a process characterized by variations in the complex of actomyosin and connective tissue which are defined as tenderisation (Bekhit at al., 2010; Rawdkuen et.a.; 2012; Kemp and Parr, 2012).

Several attempts have been made to increase meat tenderness at postmortem, such as technology to increase moisture in meat (Streiter at al., 2012), and enzymatic treatment (Pietrasik and Shand, 2011). Traditionally tender for meat is done by controlled meat remaining cool for 10 days to allow postmortem proteolysis by proteolytic enzymes such as cathepsin and calpain (Nowak, 2011).

The potential of proteolytic enzymes such as bromelain, papain, and ficin are known, for their use in tender meat (Arshad et al., 2014). Bromelain has been used to tenderize beef, chicken and squid (Ketnawa and Rawdkuen, 2011), goat meat (Bilee and Taapopi, 2008), chicken meat and rejected rejects (Koide at al., 2010) and pork (Leowsakulrat at al., 2011). Bromelain efficiently hydrolyzes several meat myofibril proteins, such as actomycin, titin, and nebulin, as a result of sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis (Hage at al., 2012). Sullivan & Calkins (2010), have also proven that bromelain can increase meat tenderness compare to other exogenous enzymes. The addition of bromelain can also produce a softening effect on myosin and other myofibrillar proteins from coarse dried sausages, as well as when applied to buffalo meat. According to the research of Jahidin and Monica (2018), soaking

buffalo meat using pineapple juice can increase tenderness in buffalo meat caused by the amount of connective tissue in hydrolyzed meat.

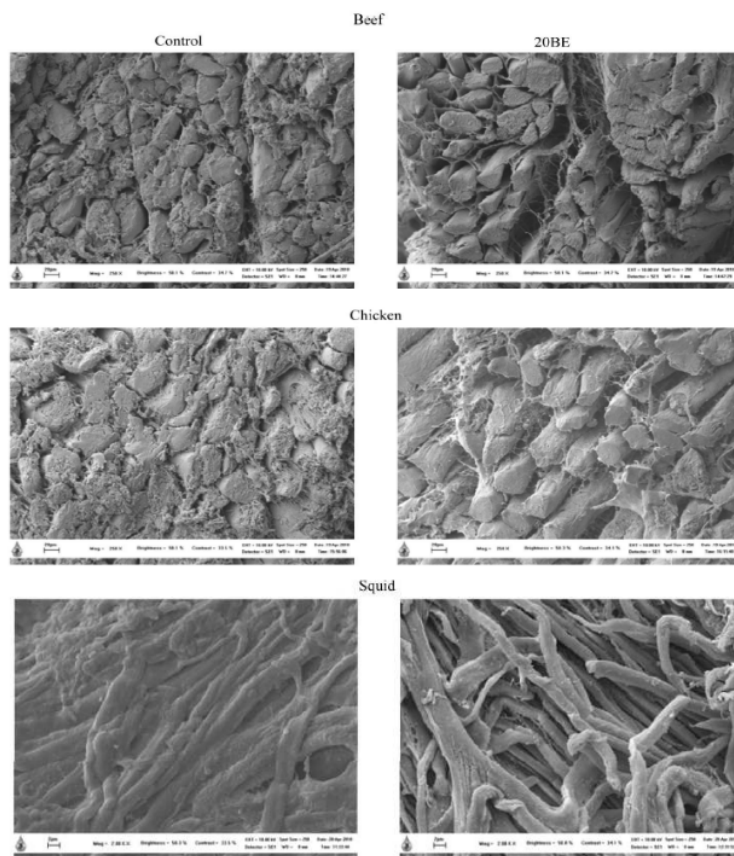


Fig. 1. Micro structure of the sample with no treatment (control) and with the addition of 20% (w / w) bromelain extract powder. 250x magnification for cattle and chicken samples, 2000x magnification for squid samples, at a voltage of 10kV (Ketnawa and Rawdkuen, 2011)

Note: The Fig has been through the permission of the researcher.

Ketnawa and Rawdkuen (2011) use 20% bromelain powder (w / w) produced from pineapple skin to tenderize beef, chicken meat and squid meat. The results can be seen in Fig.1. which shows the changes in the structure of each meat sample observed by using scanning electron micrographs. Rawdkuen and Benjakul (2012) also reported that enzymes can increase collagen solubility and structural changes through action on collagen cross-links.

Based on the results of Wada et al., (2002), the bromelain enzyme greatly influences the structure of the myosin heavy chain (MHC) and actin filament (AC) of the myofibrillar protein which is the main protein in muscle meat. Furthermore, this enzyme is active in breaking down major muscle proteins

(connective tissue or collagen and myofibrillar protein), resulting in over-tenderization and soft-textured meat (Rawdkuen at al., 2010).

Protein hydrolyzate

The results of hydrolysis in meat such as fish meat has the potential to produce protein hydrolyzates containing peptides which are lower molecular weight and contain free amino acids (Nurhayati at al., 2014). Hydrolyzate is usually used as a nutritional and functional additive with emulsification, aeration, bioactive and anti-oxidative properties (Nalinanon at al., 2011), to animal feed and microbial growth media (Aspmo at al., 2005).

The process of protein hydrolyzate production enzymatically by protease treatment such as the bromelain enzyme. Based on research by Wijayanti et al., (2016) the addition of 6% bromelain can produce up to 85.00% protein content in protein hydrolyzate which is produced by hydrolysis time for 6 hours. This is in accordance with the research of Harahap et al., (2018) which showed an increase of 15% protease can produce protein in the protein hydrolyzate of rebon shrimp reaching 84.81%. The higher the concentration of proteolytic enzymes used, the protein hydrolysis process will increase which causes an increase in the content of dissolved nitrogen in fish hydrolyzate (Hasnaliza, 2010). Ang and Ismail-Fitry (2020) state that protein hydrolyzates made with the help of the enzyme bromelain not only increase protein content, but also create a umami taste in chicken meat hydrolyzate and contain high free glutamic acid.

Cheese processing

Bromelain enzymes can also help coagulation of milk, which is one of the important steps in cheese production. Cheese production will be affected by pH, temperature, incubation time and enzyme addition (Kapoor and Metzger, 2008). Previous research by Jaya & Didik (2009) showed that the use of the bromelain enzyme can be used to produce cottage cheese which has 10.34% - 12.77% protein, 70.5% - 85.78% humidity, and pH at 4.36 - 4.75.

The addition of the bromelain enzyme also contributes to the casein coagulation process (Prasetyo at al., 2012). Geantaresa and Supriyanti (2010) showed that the protease enzyme would hydrolyze k-casein (kappa casein) in the surface area of the casein micelles to para-kappa-casein. In addition, when the pH approaches isoelectric (pH 4.6-4.7), casein micelles are coagulated into gels through hydrophobic interactions (Pardede at al., 2013).

Fish Sauce processing

Protease is widely used in fish and seafood processing (Suresh at al., 2015), covering a variety of applications such as fish sauce processing. the process of making fish sauce Traditionally is mixing fish with salt and fermented to produce fish sauce by taking up to a year to two years to complete the fermentation process (Visessanguan at al., 2004; Akolkar at al., 2010). Enzymes are solution to overcome this (Fraatz, 2014). The enzymes that are commonly used are protease enzymes for various applications

such as papai, fisin and bromelain (Suresh et al., 2015). Bromelain has been investigated as an enzyme supplement to accelerate the rate of fermentation of fish sauce (Koochmaraie and Geesink, 2006).

In the research of Dewi et al., (2016) showed that the addition of crude bromelain enzyme with a concentration of 9% can shorten the fermentation process to 3 days with good quality, both in terms of organoleptic (sensory value 5.89) and proximate value (protein 77.86%, fat content 6.81%). The addition of the bromelain enzyme also increases the yield of fish sauce produced. In the Nanda study (2018), which made fish sauce from tuna powder with the help of the enzyme bromelain 12% concentration obtained the highest average yield of 83.74%. Likewise with the study of Widyastuti et al., (2014), with the raw material of the stomach contents of manyung fish, the yield of fish sauce obtained was 78.84% with different enzyme concentration.

According to Prasetyo et al., (2012) bromelain enzyme can digest fish muscle tissue in a short time, then produce fish sauce with a distribution and component of nitrogenous concentration that is almost the same as hydrolyzate. However, bromelain activity during this process will decrease gradually during the fermentation of fish sauce due to high salt content (25% NaCl) and acidic (pH 5.5) [40].

ADVANTAGES OF THE BROMELAIN ENZYME

Bromelain enzyme is a protease enzyme that is easy to produce because of the large amount of pineapple available and almost all parts of the pineapple plant contain bromelain. In Indonesia in 2017 pineapple production increased by 399,833 tons or 28.64% (BPS, 2017). With the availability of this raw material, many suppliers produce bromelain such as Sigma, MP Biomedicals, Pfaltz & Bauer, Beta Pharma, and Enzyme Development Corporation (Feijoo-Siota and Villa, 2011).

Processing pineapple into bromelain certainly can help farmers, and pineapple industries both on a large scale and small scale in increasing the economic value of pineapple. According to Ketnawa et al., (2012) (Ketnawa et al., 2012) commercial bromelain prices can reach 2,400 USD / kg. In its application to synthesize proteins, bromelain is more widely used compared to other proteases. According to Sekizaki et al., (2008), the enzyme fisin, papain is still little used to be applied, while bromelain is widely used in amino acid cistensis and peptides (Lang et al., 2009).

BROMELAIN APPLICATIONS IN OTHER FIELDS

Because of the advantages of the bromelain, bromelain is widely applied to other industries such as the baking industry, the beer industry, the pharmaceutical and pharmaceutical industries, and in the manufacture of detergents.

Bromelain is used in the baking industry to hydrolyze gluten, making roasting masses easier. In this case, due to the faster reaction rate, wide optimal pH and temperature and the lack of amylase or pentosanase side activity (Polaina and MacCabe, 2007). Bromelain has also been studied to obtain hypoallergenic flour because of its ability to break down the glutenin glnen-gln-Gln-Pro-Pro epithelial glutenin (Tanabe et al., 1996).

In beer processing, bromelain is used to obtain good colloidal properties at low temperatures, thereby eliminating the formation of foam on beer (Jones, 2005). When compared to papain, bromelain is more widely used in brewing. This is because additive-free beer is valid in several European countries (Feijoo-Siota and Villa, 2011).

The health field bromelain also has important benefits where bromelain is a pharmacologically active compound that acts as a strong anti-tumorigenic agent (Neta at al., 2012). In breast carcinoma cells, bromelain affects MCF-7 cells by slowing down the growth inhibitory response and activating the autophagy process (Bhui at al., 2010).

CONCLUSIONS

Bromelain is a protease enzyme that can be obtained from pineapples, both from the stem, leaves, crown, skin and flesh of the fruit. Bromelain has been studied extensively because it can be applied in various industries. In animal food processing has been widely used for tendering to meat, making protein hydrolyzate, processing cheese and making fish sauce. However, not all types of animal protein can be hydrolyzed optimally by this enzyme such as keratin can only be hydrolyzed optimally by the enzyme keratinase. Bromelain has the opportunity to be mass produced because pineapple is widely cultivated and almost all parts of the pineapple plant contain bromelain.

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