The potential of plant protease enzymes as rennet alternatives for developing halal cheese product: A review

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ABSTRACT

Cheese, a derivative of dairy products made using the enzyme rennet, has received full attention because of the critical point for halalness from the milk coagulation process, which uses the rennet enzyme. Rennet enzymes can be obtained from the stomachs of animals such as cows, pigs, and goats, and they can also be produced from microbes. This very high risk of haram sources or unclean contamination has led to the development of cheese products using plant protease enzymes as a substitute for rennet enzymes. This study aims to highlight plant protease enzymes, characterize the enzymes produced, characterize cheese produced, and the potential of plant protease enzymes in replacing Rennet. Plants that have protease enzymes, such as noni, papaya, pineapple, red ginger, strawberries, pears, biduri, moringa, kiwi, tamarillo, and many other plants, have the potential to replace the rennet enzyme in making cheese. Thus, the doubts (mashbooh) arising from making cheese can be avoided by developing products from raw materials with guaranteed halal quality. Plant ingredients that can replace the rennet enzyme in making cheese are many and varied, for example, noni, papaya, moringa, bidi, pineapple, red ginger, kiwi, tamarillo, pears, *Balanites aegyptiaca*, strawberries, and many more. Doubtless, the problem of making cheese with Rennet can be avoided by developing cheese products from raw materials guaranteed to be halal. Limitations to plant protease enzymes on cheese production only apply to soft cheese, while it is difficult to produce hard cheese from plant enzymes.

Keywords: Halal, Cheese, Protease enzymes, Rennet

INTRODUCTION

Milk has high protein nutritional content, which is good for humans. Processing milk into cheese makes cheese high in fat (Syamsu & Elsahida, 2018). Cheese is coagulated milk from the coagulation process of milk proteins. It is usually made from the milk of mammals such as cows, buffalo, and goats. Generally, cheese in circulation is usually made from cow's milk (Estikomah, 2017). Cheese products derived from milk are a fermentation process of bacteria and casein coagulation with the help of a coagulation agent. The coagulating agent is the enzyme rennet (Faridah & Sari, 2019; Budiman et al., 2021; Nurgrahadi et al., 2020).

Rennet is an enzyme obtained from the stomach of mammals to digest milk (Patahanny et al., 2019). The source used to obtain Rennet can raise doubts about its halal status because Rennet can be obtained from non-halal animals or halal animals. However, the slaughter process is not carried out following Islamic law (Amen et al., 2020). The source of Rennet also comes from the stomach of a calf still breastfeeding its mother (Hutagalung et al., 2017). The limited availability of calves makes this enzyme difficult to obtain, making the selling price of Rennet expensive (Ardiana & Hernawati, 2019).

Based on this, using Rennet must be minimized by replacing other ingredients with the same function as Rennet in milk coagulation in cheese making. One enzyme with the same characteristics as Rennet is the protease enzyme, which can convert milk into cheese (Wulandari et al., 2021). Protease enzymes can be found in various microorganisms, such as plants, fungi, animals, and bacteria. This enzyme functions to hydrolyze peptide bonds in proteins into oligopeptides and amino acids. The use

of protease enzymes from plants is relevant as a source of commercial enzymes because of their abundant availability, which aligns with market demand for protease enzymes (Ishartani et al., 2011). Plant or microbial enzymes can reduce dependence on animal rennet (Fox et al., 2017). The proteolytic enzyme can coagulate milk protein (Anggraini et al., 2013).

The urgency to replace the rennet enzyme is starting to be researched a lot, especially from plant sources whose halal status is clear; this is because Rennet is one of the ingredients that has a critical point in the very high-risk category because it comes from animal ingredients whose halalness is difficult to trace (Purwanto, 2018). The protease enzyme belongs to the hydrolase group and functions to break down proteins. Peptide and protein bonds are hydrolyzed into simpler compounds, oligopeptides and amino acids (Sumardi et al., 2020).

Many plants have protease enzymes, such as papaya, pineapple, noni, red ginger, and moringa leaves (Table 1). This study aims to identify sources of vegetable ingredients that can replace the rennet enzyme in cheese making so that doubts about cheese making can be avoided by developing cheese products with raw materials that are guaranteed to be halal.

RESEARCH METHOD
Methods

This review was conducted based on information obtained on December 6th, 2023, in 2 databases, namely Science Direct and Google Scholar. Search articles using keywords related to plant protease enzymes to replace the rennet enzyme in cheese making, using the connecting words "and" and "or ."Search strategy in Science Direct: "plant or fruit and protease or cheese or milk" and in Google Scholar: "enzim protease untuk keju ."Articles are filtered within the last 10 years.

Based on population, intervention, comparison, and outcome (PICO), the research questions focus on:

1. What plants have the potential to be a source of protease enzymes that can replace the rennet enzyme?
2. What are the characteristics of cheese produced using plant protease enzymes?
3. Identification-Search articles in databases using keywords
4. Screening-Sorting articles based on title, keywords, and abstract
5. Eligibility-Sorting articles by doing full-text reading
6. Include-Determining the articles used in the review

This review aimsThisdetermine if plants withplants with protease enzymes can be an alternative to rennet enzymes when making cheese. When searching for articles, parameters for enzyme proteolytic activity, physicochemical properties of the cheese produced, and organoleptic test results of the cheese produced are used. In some articles, some parameters do not exist but can still be compared according to the topics discussed in each journal that discusses the same topic. After the article selection process is carried out manually, articles that enter the eligibility stage are identified to eliminate doubts that arose during previous processes.

The review was carried out using the PRISMA flow chart method depicted in Figure 1. At the identification stage, 1298 articles were identified from Science Direct and Google Scholar. Next, the title, keywords, and abstract are read at the screening stage. A total of 1248 articles were excluded, leaving 50 articles. Then, 22 articles entered the eligibility stage, and full-text reading article analysis was carried out, leaving 12 articles that would be reviewed further.

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Figure 1. PRISMA flowchart-The results of the systematic search between 2013-2023.

RESULT AND DISCUSSION

This review was carried out using the PRISMA flow chart method depicted in Figure 1. At the identification stage, 1298 articles were identified from Science Direct and Google Scholar. Next, the title, keywords, and abstract are read at the screening stage. A total of 1248 articles were excluded, leaving 50 articles. Then, 22 articles entered the eligibility stage, and full-text reading article analysis was carried out, leaving 12 articles that would be reviewed further.
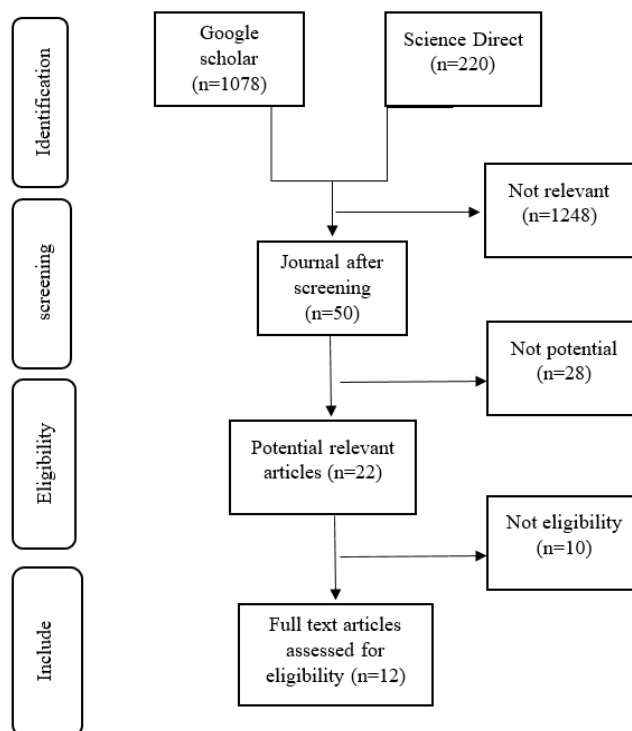
Protease Enzymes Extraction

Before being used in cheese making, protease enzymes from plant sources must be extracted first.

Table 1. Summary of plant sources with protease enzymes.

Plant Sources	Plant Parts	Name of Enzyme	Cheese Making	References
Noni	Fruit	Protease enzyme	V	(Adrian et al., 2015)
Moringa	Leaf	Protease enzyme	-	(Fathimah & Wardani, 2014)
Papaya	Sap	Papain Enzyme	V	(Pardede et al., 2013; Patahanny et al., 2019)
Red ginger	Rhizome	Zingibain Enzyme	V	(Nindyasari et al., 2022)
Biduri (*Calotropis gigantea*)	Extract	Protease enzyme	V	(Welin et al., 2023)
Pineapple	Fruit	Enzim bromelin	V	(Komansilan et al., 2019)
Pear (*Pyrus pyrifolia*)	Fruit	Protease enzyme	-	(Nam et al., 2015)
Balanites aegyptiaca	Fruit	Protease enzyme	-	(Beka et al., 2013)

Plant Sources	Plant Parts	Name of Enzyme	Cheese Making	References
Kiwi	Fruit	Actinidin Enzyme	V	(Puglisi et al., 2013)
Tamarillo	Fruit	Bromelain enzyme	-	(Li et al., 2018)
Strawberry	Fruit	Protease enzyme	V	(Wulandari et al., 2021)

Based on research by Adrian et al. (2015), extraction of the noni protease enzyme begins by separating the noni from the stalk and then washing it thoroughly. One hundred grams of noni were blended and added with 200 ml of 100 mM potassium phosphate buffer pH 7 containing stabilizers (ascorbic acid and EDTA) until a homogeneous mixture was obtained. After that, filtration is carried out using a thin filter cloth, and the filtrate is obtained. The filtrate was centrifuged at 10,000 rpm for 30 minutes at a temperature of 4°C. The supernatant was taken, and its proteolytic activity was measured. The noni protease enzyme was characterized after the enzyme was partially purified by adding 60% ammonium sulfate/500 ml crude extract of the protease enzyme when the enzyme had been separated from other impurity components.

Then, based on research by Fathimah & Wardani (2014), the protease enzyme from Moringa leaves was extracted using the stabilizer ascorbic acid and EDTA. It was found that the stability of the enzyme was at a temperature of 40-60°C with a pH of 4-7. The HgCl2 inhibitor inhibits the protease enzyme from Moringa leaves so that this enzyme can be categorized as a cysteine protease.

Furthermore, based on research by Pardede et al. (2013), the papain enzyme can be obtained from papaya. Papaya sap was added with an activating solution (14 grams of $NaHSO_3$ and 3 grams of NaCl in 1 liter of distilled water) in a 1:1 ratio. Stir the mixture, filter it and collect it in a petri dish. The results from the filter are dried in the sun until dry. The dried sap is made into fine papain flour. The activating solution reduces the disulfide bonds in the pro-papain compound, resulting in active papain.

Moreover, finally, based on research by Nindyasari et al. (2022), red ginger is washed to obtain the zingibain enzyme, and the skin is peeled. After that, grind it using a juicer until the juice is obtained. Phosphate buffer solution (pH 8.0) was added in a 1:1 ratio of filtrate to buffer. The resulting enzyme mixture is stored in a refrigerator at 0–5°C. The protease enzyme extraction process involves chemical compounds and is guaranteed to be halal.

Protease Enzyme Activity

Enzyme activity in cheese making is important because it is an indicator that influences milk coagulation, which produces curd quality. Total curd is the amount of milk casein that coagulates after undergoing coagulation and is separated from whey (Budiman et al., 2017).

Several types of protease enzymes used in cheese-making have different enzyme activities. The differences in protease activity values for each plant depend on the compounds that make up the plant, which influence protease levels, the ability (kinetic properties) of each protease as a catalyst, physical properties (optimum conditions), and the type of protease contained in the plant (Ratnayani et al., 2015).

Enzyme activity using noni fruit showed a total specific enzyme activity of 1.67 U/ml from the crude enzyme supernatant. However, after purification with ammonium sulfate, the specific activity of the enzyme after being precipitated increased by 2.11 U/ml (Adrian et al., 2015).

Purification of the cysteine protease enzyme in pears using 1 mM and 10 mM cysteine buffer showed enzyme activity of 142.3–165.8 U in the 10 mM sample and the 1 mM cysteine sample showed a lower total activity of around 46 U. So, it is known that the higher level of purification of a protein, the higher the protease enzyme activity will be (Nam et al., 2016).

Another research conducted by Fathimah & Wardani., (2014), who extracted the protease enzyme from Moringa leaves, showed that the activity of the protease enzyme when 0.3% ascorbic acid was added, had a proteolytic activity of 2.26 U/mg, then the addition of 15 mM EDTA had specific activity for the protease enzyme from Moringa leaves, namely of 2.01 U/mg. Meanwhile, adding a combination of 0.3% ascorbic acid and 15 mM EDTA further increased the specific protease activity of Moringa

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leaves, namely 2.45 U/mg. These data show that adding antioxidants to the protease enzyme will increase the enzyme activity value.

Kiwi fruit is also known to have protease enzyme activity, which can coagulate milk, as in research by Puglisi et al. (2014), who tested kiwi fruit extract as a cow's milk coagulant in making mozzarella cheese. Kiwi extract showed specific activity against a total casein of 299 U/mg. The protease enzyme activity of kiwi is lower when compared to strawberry extract, which produces an enzyme activity of 1600 SU/ml (Wulandari et al., (2021).

Ratnayani et al. (2015) showed that the crude protease activity of papaya, taro, and chayote plant sap was 0.9194 U/mL, respectively; 0.0123 U/mL; and 0.0264 U/mL. Another study also showed that the highest enzyme activity of bromelain extracted at different pH levels was extracted from pineapple tubers at pH 7.0, amounting to 1.081 units/g (Masri, 2014). Based on this, it is known that the type of material and pH used will influence the enzyme activity value produced.

Types of Protease Enzymes

In biological processes, enzymes are useful as biocatalysts. Enzymes consist of groups of proteins that can carry out chemical changes. Protease enzymes have two meanings, namely peptidase and proteinase. Protease enzymes can catalyze the hydrolysis of protein molecules into simpler ones called proteinases. Protease enzymes hydrolyze peptides into amino acids are called peptidases (Supriyatna et al., 2015). Protease enzymes are generally defined as proteolytic enzymes that can break peptide bonds in proteins (Yuniati et al., 2015).

Protease enzymes can be grouped into cysteine proteases, serine proteases, aspartic proteases, metalloproteases, and threonine proteases, which are based on the catalytic mechanism and amino acids in the active site (Mótyán et al., 2013; Sun et al., 2018). Cysteine proteases have the amino acid cysteine in their active site. Serine proteases have the amino acid serine in their active site. Aspartic proteases have aspartic amino acids in the enzyme's active site (Jisha et al., 2013). The work of each protease enzyme has a different pH (Nam et al., 2015).

Plants with cysteine proteases include papain from papaya, bromelain from pineapple, zingibain from ginger, actidin from kiwi and protease from pears. Protease from pears works optimally at pH 5.3-7.0 and 40-70°C (Nam et al., 2015). The protease enzymes papain and bromelain can break down proteins into amino acids because the papain and bromelain enzymes contain the amino acid cysteine (Rachmania et al., 2017).

Serine protease is an endopeptidase enzyme with serine in its active site, which works through protein hydrolysis (Patel, 2017). The tamarillin enzyme from Dutch eggplant works as a serine protease enzyme. In its working process, this serine protease does not use reducing and chelating agents (Li et al., 2018). The action of serine protease will be inhibited by metal ions, chelating agents, EDTA, and organic solvents (Patel, 2017). Serine protease can maintain its activity and is stable at relatively high temperatures, as well as its ability to work over a wide pH range. The presence of oxidizing agents does not affect the action of the serine protease enzyme (Li et al., 2018).

Aspartic protease has optimal activity at pH 3-5.5 and is stable in the acid-to-neutral pH range. The optimal activity of aspartic protease at acidic pH makes it also called acid protease (Wei et al., 2023). *Balanites aegyptiaca* fruit has protease enzyme activity in two forms, namely aspartic protease and serine protease (Beka et al., 2013). Meanwhile, strawberries contain the serine protease enzyme used to coagulate milk (Wulandari et al., 2021).

Types of Cheese

Cheese has different types depending on the processing and characteristics of the cheese produced (Hendrasty et al., 2022). According to Wulandari et al. (2021), cheese is grouped into 2 types based on its texture: hard cheese and soft cheese.

Meanwhile, according to BPOM RI (2019), based on density, there are four types of cheese, namely very hard cheese, hard cheese, semi-hard cheese, and soft cheese). The non-fat solids (PTL) content of very hard cheese is less than 51%, the PTL content of hard cheese is 49-56%, the PTL content of moderately hard cheese is 54-69%, and the water content of soft cheese is more than 67%.

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One type of soft cheese is mozzarella cheese, which is elastic, stringy, and soft. Mozzarella cheese is a typical Italian cheese made from buffalo milk (Sunarya et al., 2016). Mozzarella cheese is a soft cheese not cooked in the manufacturing process; it is also called fresh cheese. (Sari et al., 2014; Nur et al., 2015).

Apart from mozzarella, another type of soft cheese is cottage cheese, which, in its manufacture, can be consumed immediately after the curd is taken; this causes this cheese to have a high water content (Putri et al., 2013). Sukainah et al. (2021) reported that soft cheese, such as cottage cheese, contains more than 52% to 80% water with low-fat content. Apart from that, there is also a type of semi-solid cheese called Gouda cheese, a semi-hard cheese originating from the Netherlands. This Gouda cheese has a soft texture and a taste that is not sour, savory, and slightly salty (Chairunnisa, 2007).

In this study, the cheeses produced were cottage cheese, mozzarella cheese, and soft cheese. These three types of cheese are included as soft cheese. According to BPOM RI (2019), cottage cheese is a soft cheese made from fresh or fresh milk with no fat or recombined milk without ripening.

Organoleptic Characteristics of Cheese

Organoleptic determines the level of preference for the cheese produced, whether in terms of color, texture, aroma, or taste (Negara et al., 2016). A cheese product with a high organoleptic value indicates that the product produced is acceptable to the human senses. This shows that if the organoleptic value of a product is good, people will consider consuming it. Organoleptic are signs of identification and attractiveness of a product, which include color, taste, aroma, and texture (Nasution & Marya, 2021)

Based on journals, Adrian et al. (2015) show the organoleptic value of cottage cheese where the manufacture of this cheese uses protease enzymes from noni as much as possible 0.02% (M1), 0.04% (M2), dan 0.06% (M3) produce an assessment of taste, smell, color, texture, and preference for the final result of cottage cheese. The highest odor value is M3 cheese, which has a value of 5.85. The hedonic taste assessment was highest for M2 cheese, namely 5.80, where this value was also close to the value of the control beef rennet cheese, 5.95. The hedonic assessment of cow rennet cheese in terms of color is 5.95, and the cheese made from the noni protease enzyme is closest, namely M2 cheese, with a value of 5.70. The texture attribute of cheese using noni protease was the highest, M3 5.70. The M3 texture value is closest to the control value of 5.80. The result of adding up the preference values for this attribute is closest to the value of cow rennet cheese, and the highest is noni enzyme cheese 0.04% (M2).

Pardede et al. (2013) make cottage cheese from papaya sap using papain enzyme. The papain enzyme used in making cottage cheese is at a concentration of 300 ppm (K3), 500 ppm (K5), 700 ppm (K7), and 1000 ppm (K10). The results of organoleptic testing show that the K5 sample has the highest value, so the K5 sample is the most preferred of the other samples.

Welin et al. (2023) made soft cheese using protease enzymes from bidi leaf juice. Soft cheese is made using biduri leaf juice with concentrations of 1.5% (p1), 2% (P2), and 2.5% (P3). This research shows that the higher the addition of biduri leaf juice, the less it is liked because of the bitter taste and distinctive aroma of biduri leaves that appear in soft cheese.

Komansilan et al. (2019) used the enzyme bromelain from pineapple to make cottage cheese. Cottage cheese was added 3% of the bromelain enzyme to several pH treatments (K1 pH 4.4, K2 pH 4.50, K3 pH 5.40, and K4 pH 5.60). The results of making mozzarella cheese with the bromelain enzyme were subjected to an organoleptic test on the effect of pH differences by adding 3 % bromelain. These results show that the pH variations do not affect the taste of bromelain enzyme mozzarella cheese. The color attribute of mozzarella cheese produced by the bromelain enzyme with several pHs shows a color difference. The aftertaste of mozzarella cheese from the bromelain enzyme with pH variations shows no bitter aftertaste.

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Proximate Characteristics of Cheese

In general, proximate cheese analysis is carried out, namely the analysis of water, ash, fat, protein, and carbohydrate content (Rohmatussolihat et al., 2015). Negara et al. (2016) reported that cheese contains 19.4% protein, 21.6% fat, and 2.20% carbohydrates. Meanwhile, mozzarella cheese has a fat content in dry matter of 35-45%, water 52-56%, salt around 1%, and a pH ranging from 5.1-5.4 (Yulia et al., 2015).

Research conducted by Adrian et al. (2014) obtained the results of nutritional component analysis of cottage cheese using noni, which showed the best value at a concentration of 0.06% with yield parameters of 10.86%, protein content of 26.30% and fat content of 17.98%, while for water content the control showed the highest value of 51.86%. %. This research shows that the best value is shown at the highest concentration of noni, and the more enzyme concentration is used, the more the nutritional value of the cheese will increase.

Making mozzarella cheese using the zingibain enzyme as a coagulant showed that the protein content in mozzarella cheese is around 18-21% with the addition of 1.75% zingibain enzyme (35 ml of ginger extract). Meanwhile, the fat content obtained was 10.68%, and the water content obtained was 51% (Nindyasari et al., 2022).

Using the papain enzyme as a coagulant in making cottage cheese also has a different nutritional value for each concentration added. The concentrations used in making cottage cheese are 300 ppm, 500 ppm, 700 ppm, and 1000 ppm with the highest water content of 58.75% with the addition of 1000 ppm enzyme, then the highest ash content produced is 6.09% with a concentration of 500 ppm, the highest fat content was 9.23% with an enzyme concentration of 1000 ppm, the protein content was 16.84% with a concentration of 700 ppm, and the highest amount of carbohydrate was obtained at a concentration of 300 ppm at 25.98% (Pardede et al., 2013).

In the research of Wulandari et al. (2021), who made fresh cheese by carrying out one of the tests, the water content obtained a value ranging from 50.29 to 58.33%, which is included in the soft cheese category. The water content produced depends on the type of coagulant used. A good coagulant will help the particle shrinkage process in whey separation so that more water will be separated, and the resulting cheese will have a low water content value.

Different enzyme extraction processes also cause the purity of the enzyme to be different. The research of Sajuthi et al. (2010) found that the purer an enzyme, the higher its activity. This, of course, will affect the yield of the resulting cheese.

Cheese Yield

Yield is a percentage determined by comparing the weight of the cheese produced with the weight of fresh cow's milk as raw material (Karlina et al., 2021). This also shows how effective the enzyme is used in making cheese, so the yield value is an important parameter to determine the use of enzymes.

The yield of cheese produced using plant protease enzymes is quite varied; this is due to the influence of milk composition, pH and processing temperature, enzyme purity, the concentration of protease enzyme extract, the addition of acid, and the addition of other chemical compounds, such as stabilizers (Adrian et al., 2015; Razig & Babiker, 2009). Sumarmono & Suhartati (2012) also reported that cheese produced using fruit extract generally produces a 14-18% yield range. Differences in yield in cheese making are influenced by milk composition, type of acidulant, milk pasteurization method, and whey pressing method.

Adding a bacterial starter to the cheese-making process risks it being non-halal. The critical point of halalness is the medium used to grow the bacteria. If the media contains unclean or haram ingredients, this will determine the haraam of the bacteria. Also, if bacteria are produced from the genetic engineering of humans or pigs, then the bacteria are haram (Amen *et al*., 2020). Therefore, the bacterial aspect will also be removed to ensure the halalness of cheese products, apart from the rennet enzyme replacement aspect.

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Table 2. Summary of cheese yield from plant protease enzymes

Added Enzyme	Milk	Concentration of enzymes/extracts containing enzymes	Yield	References
Rennet	0.3 liter	0.02 %	9.46 %	Adrian et al., 2015
Noni protease enzyme	0.3 liter	0.02 %	8.99 %	Adrian et al., 2015
		0.04 %	9.93 %	
		0.06 %	10.86 %	
Ginger red Zingibain Enzyme	2 liter	1.00 % (1:1)*	6.05 %	Nindyasari et al., 2022
		1.25 % (1:1)*	6.30 %	
		1.50 % (1:1)*	6.86 %	
		1.75 % (1:1)*	7.02 %	
		2.00 % (1:1)*	6.96 %	
		1.00 % (0:1)*	6.75 %	
		1.25 % (0:1)*	6.88 %	
		1.50 % (0:1)*	7.10 %	
		1.75 % (0:1)*	7.13 %	
		2.00 % (0:1)*	7.69 %	
Pineapple bromelain enzyme	28 liter	3 %	-	Komansilan et al., 2019
Papaya papain Enzyme	1 liter	300 ppm (0.03 %)	6.43 %	Pardede et al., 2013
		500 ppm (0.05 %)	9.05 %	
		700 ppm (0.07 %)	8.21 %	
		1000 ppm (0.07 %)	7.48 %	
Biduri leaf protease enzyme		1.5 %	19.50 ± 1.53 %	Welin et al., 2023
		2.0 %	24.03 ± 1.47 %	
		2.5 %	29.56 ± 4.16 %	
Kiwi actinidin Enzyme	10 liter	-	10.6 %	Puglisi et al., 2013
Strawberry protease enzyme	1.5 liter	20 %	21.53 ± 3.46 %	Wulandari et al., 2021
		30 %	23.597 ± 1.83 %	
		40 %	23.81 ± 2.42 %	

Benefits and Limitations of Rennet Substitution

Halal food has become one of the main requirements in food labels, especially in regions where the population is predominantly Muslim, such as Indonesia. Atma et al. (2017) said that cheese products found daily and widely consumed are also a concern regarding their halalness. Cheese products are critical biotechnology products, especially milk coagulation, due to the addition of rennet enzymes, which can come from animals or be produced using microbes. To minimize the risk of a tipping point in halal cheese, many vegetable sources have been researched that have the potential to replace the rennet enzyme.

If you look at the extraction process, the protease enzyme from the plant does not use a microbial growing medium. However, it uses a simple process that does not involve haram or non-halal ingredients. Farkye (2004) said the yield of cheese produced using the rennet enzyme ranges from 9-15%. The varied yields from these plant sources can still be an alternative for making cheese, and it does not rule out the possibility that other plant sources can also be used to make cheese. There are still many plant sources that have the potential to have protease enzymes that can replace the rennet enzyme.

The availability of plant materials is very high, and they are abundant in Indonesia, such as noni, papaya, red ginger, moringa, biduri, pineapple, pear, kiwi, tamarillo, strawberry, and other plants which also produce protease enzymes, which can be used to produce cheese. It uses plant protease enzymes, so its sustainability will be longer than that of the rennet enzyme, which must be taken from animals or

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produced by microbes. All the considerations that have been made show the potential of plant protease enzymes to replace the rennet enzyme in making cheese so that its halal quality is guaranteed.

Apart from its relatively high potential, there are also limitations to plant protease enzymes, namely that the characteristics of the cheese produced are mostly soft cheese, and it is difficult to produce hard cheese. Further research is needed to combine treatments so that plant protease enzymes can also be used to produce hard cheese.

CONCLUSION

The sources of plant ingredients that can replace the rennet enzyme in making cheese are many and varied, for example, noni, papaya, moringa, biduri, pineapple, red ginger, kiwi, tamarillo, pears, *Balanites aegyptiaca*, strawberries and many more. Doubtless, the problem of making cheese with Rennet can be avoided by developing cheese products from raw materials guaranteed to be halal. Limitations to plant protease enzymes on cheese production are only soft cheese that is applicable while it is difficult to produce hard cheese from plant enzymes.

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