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Enhancing cheese yield and milk clotting efficiency with pineapple bromelain enzyme through response surface methodology

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Abstract

The high demand for cheese products has impacted rennet enzyme production as the availability of ruminant stomachs becomes less and less limited. Therefore, the plant-based enzyme was the potential solution as an alternative to an animal-based enzyme. Bromelain enzyme is chosen due to its ability to coagulate milk as a proteolytic enzyme. This study aimed to obtain the optimum milk clotting activity (MCA) and yield of soft cheese by bromelain using response surface methodology (RSM) and to evaluate the proteolysis properties of soft cheese. Experimental design was generated using RSM by MINITAB software version 15 with three selected variables including bromelain concentration (0.2-1%), incubation time (1-5 hrs) and incubation temperature (45-55°C). The optimum condition for MCA and yield of soft cheese was achieved at a bromelain concentration of 0.53%, incubation time of 2.33 hrs and incubation temperature of 54.2°C, with predicted MCA and yield of 60.81 SU/mL and 29.88%, respectively. The optimum condition was verified with the MCA and the yield obtained was 60.16 SU/mL and 32.06%, respectively. Since there was no significant difference ($p>0.05$) between the predicted and verified MCA and yield values, thus it indicates that the predicted optimum condition by RSM can be accepted. The proteolysis of soft cheese produced using bromelain enzyme at optimum conditions was also evaluated and compared with commercial soft cheese. The result of proteolysis showed that proteolysis products such as alpha (α), beta (β) and kappa (κ) casein were visible at molecular bands of 30 kDa for all cheese samples. In conclusion, the bromelain enzyme from Josapine pineapple peel has a potential for rennet alternative with a significant value of MCA and yield and expected product of proteolysis.

1. Introduction

Cheese is the fresh or ripened solid or semi-solid product obtained by coagulating milk through the action of rennet or other suitable coagulating agents, acidification, or a combination of rennet and acidification methods. The coagulation of milk is the basic step in the manufacture of all types of cheeses. The addition of rennet or coagulating agents has been greatly used in the coagulation of milk to produce cheese. Calves' rennet contains a very high concentration of chymosin which is also known as rennin, which could account for up to 95% of the total proteases found in young calves' abomasum extract. Due to its ability to separate milk into curds and whey, rennet is very important for cheese production. In general, milk coagulation using calf rennet is the most used procedure (Siti Roha *et al*., 2022).

The growing demand for Halal-certified cheese among Muslim consumers worldwide must be fulfilled. This warrants comprehensive and rigorous studies, to find suitable plant-based enzymes that can be useful for cheese production as coagulants to cater for the increasing need worldwide. Bromelain is reported to be an alternative source for the production of cheese and is foreseen as a suitable rennet enzyme replacement in cheese production in Malaysia. Furthermore, the use of plant proteases in cheese manufacturing promotes greater acceptability by vegetarians and may improve their nutritional intake.

Pineapple or its name *Ananas comosus* is a perennial plant where from the family of Bromeliaceae. The pineapple fruit is edible and contains vitamins, enzymes, and antioxidants such as vitamin C, bromelain enzyme

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and manganese, respectively. In addition, research on the application of other plant-based enzymes in milk clotting has been conducted, for example, coagulant from *Solanum elaeagnifolium* by Gutiérrez-Méndez *et al*. (2019), flower extract from *Citrus reticulata* Blanco by Khan *et al*. (2019), kiwi and ginger extract by Fguiri *et al*. (2021) and coagulant from *Pergularia tomentosa* by Benyahia *et al*. (2021). The pineapple fruit is believed to be originated from tropical and subtropical America, and the fruit has been spread elsewhere, where the fruit now can be found to grow throughout the tropical and subtropical regions of the world (Sun *et al*., 2015; Hoque *et al*., 2019). In Malaysia, the pineapple fruits are ranked number nine in terms of production which places it as one of the popular types of fruit in this country. The pineapple variety Josapine is one of the popular pineapple varieties in Malaysia. This pineapple variety is a hybrid of the Johor variety ('Singapore Spanish' × 'Smooth Cayenne') with the 'Sarawak' variety ('Smooth Cayenne'). The Josapine fruit weight is between 1.1 to 1.3 kg and it has strong resistance to black heart disorder or internal browning caused by low temperature (Lasekan and Hussein, 2018).

The enzyme used in cheese production comes from the extract of ruminant stomach. The enzyme, which is rennet can be obtained from the calf's stomach as its primary source. The utilization of a ruminant's stomach for extracting rennet is expensive and limited due to the availability of ruminant stomachs. Thus, the cost of cheese production is very high. Therefore, this problem led to the search for rennet enzyme alternatives. The plant-based enzyme has the potential to be the rennet alternative. This is because the plant-based enzyme is available and easy to get. Moreover, the use of plant-based enzymes in cheese production can be accepted by the vegetarian consumer. Also, the utilization of calf rennet was banned in several countries such as France, Germany, and the Netherlands. Moreover, the religious concern which is the Halal status of plant-based enzymes can be verified (Abd El-Salam *et al*., 2017).

During the canning and juicing process of pineapple, pineapple peels are usually discarded. During these productions, waste products such as pineapple peels and cores will waste about 35% of the whole fruit and lead to serious environmental pollution (Heryandi *et al*., 2017). The pineapple wastes are either used as animal feed or disposed of in the soil as waste. Pineapple peel principally consists of bromelain which is a plant-based enzyme. From the point of view of multipurpose utilization and environmental protection, the modification and utilization of pineapple peel' bromelain is important. It was foreseen as an effective substitution of rennet enzymes in cheese production, also providing added value such as increasing the cheese product's nutritional content. According to Komansilan *et al*. (2021), the physicochemical properties and profile of cottage cheese made from a crude extract of bromelain enzyme from pineapple were studied. The results showed that different bromelain concentrations had significant differences in the overall physicochemical properties of the cottage cheese and protein profile of cottage cheese. Therefore, the present research was focused on the optimization of the bromelain enzyme in the preparation of soft cheese and the quality characteristics of soft cheese.

2. Materials and methods

2.1 Preparation of samples

Pineapple variety Josapine with different maturity indices of 2, 5 and 7 used in this study were purchased from Pasar Moden Seksyen 6, Shah Alam, Selangor, Malaysia. The pineapple peel with maturity index 2, 5 and 7 were cut into small pieces and crushed in a food processor (Model PB-3203L, Pensonic, Malaysia) with the addition of water at a ratio of 1:1 to produce pineapple peel extract. Extraction was done at a chilled temperature (4°C). Next, the extract was filtered through a muslin cloth to remove the solid parts. The filtrate was collected and kept in a freezer (-20°C) to be used in further experiments (Gautam *et al*., 2010).

2.2 Ammonium sulphate precipitation

Firstly, the purification of the crude bromelain extract was carried out using the ammonium sulphate precipitation method. About 10 mL of crude bromelain extract was transferred into a centrifuge tube and placed in an ice bath. Then, about 6 g of ammonium sulphate was added pinch by pinch. Then, the sample was left for incubation for 1 hr until the appearance of precipitation was observed. Then, the incubated sample was centrifuged (Model 5420, Kubota, Japan) at 3500 rpm for 30 mins. Then, the pellet was collected by dissolving in 10 mL Tris buffer, while the supernatant was removed. Then, the purified bromelain underwent a desalting process. The salt in the purified bromelain was removed by diafiltration using a diafiltration machine (Gautam *et al*., 2010).

2.3 Preparation of soft cheese

Firstly, approximately 100 g of fresh milk was transferred into a plastic cup and heated to 32°C. Then, about 5 drops of calcium chloride solution were added and allowed to stand for 10 mins. After that, approximately 0.2 g of mesophilic starter culture was added and stirred, and the mixture was allowed to ripen for 1 hr. Next, the bromelain enzyme (range 0.2% to

1.0%) was weighted. The bromelain enzyme solution was then added, and the timer was set to measure the milk clotting activity (MCA). The timer was stopped and the time was recorded when the formation of coagulation was observed for MCA determination. The milk was incubated in an incubator oven (Model Innova 40, Brand New Brunswick Scientific, Country United States) at the predetermined temperature (range 45-55°C) for the predetermined time (range 1 to 5 hrs) without mixing or stirring. After incubation, the milk was poured into a muslin cloth to collect the curd, and the whey was left to drain for 1 hr. The cheese was then allowed to ripen for 7 days at a chilled temperature. Finally, after 7 days, the cheese was weighed to determine the yield. The optimum condition obtained from the RSM was used to produce bromelain standard cheese using the same step for the bromelain pineapple peel cheese.

2.4 Determination of milk clotting activity

The milk-clotting activity (MCA) was determined using the procedure described by Abd El-Salam *et al.* (2017). The time between the addition and the appearance of clots was recorded and the total MCA was calculated as follows.

$$MCA\left(\frac{SU}{mL}\right) = \frac{2400}{t} \times \left(\frac{V_s}{V_e}\right)$$

Where t = coagulation time (s), V_s= Substrate volume, milk (mL) and V_e= Extract volume, enzyme (mL)

2.5 Determination of yield

The cheese yield was calculated as follows.

$$Yield = \frac{(\text{weight of cheese})}{(\text{weight of milk} + \text{weight of bromelain})} \times 100$$

2.6 Experimental design

In this experiment, response surface methodology (RSM) was used in optimizing the enzymatic process parameter to achieve optimal milk clotting activity (MCA) and yield of soft cheese. The experimental design and analysis were performed using MINITAB statistical software version 15. The experimental design was conducted based on central composite design (CCD). Three independent variables were used including enzyme concentration (X_1), incubation time (X_2) and incubation temperature (X_3). The ranges for these three independent variables were enzyme concentration (0.2-1.0%), incubation temperature (45-55°C) and incubation time (1-5 hrs) as shown in Table 1.

Table 1. The coded and uncoded values are used in the optimisation of enzymatic conditions.

	(-α)	-1	0	1	(α)
Bromelain concentration (%)	0.2	0.4	0.6	0.8	1.0
Incubation time (hr)	1	2	3	4	5
Incubation temperature (°C)	45	47.5	50	52.5	55

2.7 Proteolysis of soft cheese

The proteolysis in soft cheeses was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). First, the running buffer (MES SDS) (1X) and sample buffer (Laemmli) (1X) were prepared. Next, the gel was prepared and the electrophoresis cell was assembled by removing the comb and tape from the gel. Then, the inner and outer buffer chambers of the cell were filled with the running buffer (1X). The samples used for the analysis were the protein ladder, bromelain pineapple peel cheese, bromelain standard cheese, and Laughing Cow cheese. The samples were heated at 80-85°C for 5 mins. Subsequently, the samples were loaded onto the gel. Once ready, the cell was connected to a power supply, and the gel was run in two stages: first stage (70 V, 110 mA, 15 mins) and second stage (120 V, 110 mA, 60 mins). After the electrophoresis was completed, the power supply was turned off, and the gel cassettes were opened. The gel was gently removed from the plate and floated in deionized water. Then, the gel was stained with Coomassie blue staining. The gel image was captured and stored in a chiller immersed in deionized water (Laboratories Bio-Rad, 2012).

2.8 Data analysis

All experiments were performed in triplicate. The data obtained were analyzed using a software, Statistical Package for the Social Sciences (SPSS). The analysis of variance (ANOVA) tests was performed on the optimization of Milk Clotting Activity and Yield.

3. Results

In this research work, bromelain protease was extracted from pineapple peel. The impact of enzyme concentration, incubation temperature and time on MCA and yield of soft cheese was optimized by response surface methodology. The prepared soft cheese was analysed for proteolysis using SDS PAGE.

3.1 Optimisation of soft cheese production

In this present study, response surface methodology was used to determine the optimum condition for the factors affecting soft cheese production using bromelain enzyme. The effect of the three independent variables of X_1 (bromelain concentration), X_2 (incubation time) and X_3 (incubation temperature) using five levels of CCD on the MCA and yield of soft cheese were determined using MINITAB software version 15.

Table 2. Factors and comparison between actual and predicted responses.

Run	Factors			Response			
	X_1	X_2	X_3	MCA (SU/mL)	FITS1 (SU/mL)	Yield (%)	FITS2 (%)
1	0.40	2.000	47.5	33.33	32.44433	24.45	24.74580
2	0.80	2.000	47.5	30.86	31.09492	23.50	23.73843
3	0.40	4.000	47.5	55.38	55.70404	22.79	23.12567
4	0.80	4.000	47.5	56.16	58.15462	26.40	25.96330
5	0.40	2.000	52.5	50.00	50.19236	27.40	27.91608
6	0.80	2.000	52.5	49.59	51.45295	22.12	21.86371
7	0.40	4.000	52.5	44.44	46.39207	28.14	27.98096
8	0.80	4.000	52.5	48.38	51.45266	25.99	25.77359
9	0.26	3.000	50.0	40.98	41.09321	23.80	23.25050
10	0.94	3.000	50.0	47.42	44.21393	20.11	20.54723
11	0.60	1.320	50.0	34.64	34.85919	24.57	24.13614
12	0.60	4.680	50.0	57.73	54.41795	25.74	26.06159
13	0.60	3.000	45.8	52.19	52.25260	27.74	27.52069
14	0.60	3.000	54.2	64.70	61.54120	29.92	30.02704
15	0.60	3.000	50.0	61.77	63.75733	27.54	27.21488
16	0.60	3.000	50.0	61.95	63.75733	27.34	27.21488
17	0.60	3.000	50.0	63.91	63.75733	27.94	27.21488
18	0.60	3.000	50.0	66.04	63.75733	27.53	27.21488
19	0.60	3.000	50.0	66.04	63.75733	26.28	27.21488
20	0.60	3.000	50.0	62.31	63.75733	26.64	27.21488

X_1 (Bromelain concentration, %), X_2 (Incubation time, hr) and X_3 (Incubation temperature, °C), MCA (milk clotting activity, SU/mL), FITS1 and FITS2 (predicted responses)

The treatments with their respective actual variables level combinations and the response obtained were listed in Table 2 for MCA and percentage of yield of soft cheese. Table 2 shows the values of actual (experimental) and predicted responses for each run. The highest actual and predicted MCA were 66.04 SU/mL and 63.75 SU/mL, respectively, under specific conditions of variable factors (bromelain concentration of 0.6%, incubation time of 3 hrs and incubation temperature of 50°C). On the other hand, the lowest actual and predicted MCA were 30.86 SU/mL and 31.09 SU/mL, respectively, at the predetermined variable factors condition (bromelain of 0.8% concentration, incubation time of 2 hrs and incubation temperature of 47.5°C).

The highest actual and predicted yield were 29.92% and 30.02%, respectively, under specific conditions of variable factors (bromelain concentration of 0.6%, incubation time of 3 hrs and incubation temperature of 54.2°C). On the other hand, the lowest actual and predicted yield were 20.11% and 20.54%, respectively, at the predetermined variable factors condition (bromelain of 0.94% concentration, incubation time of 3 hrs and incubation temperature of 50°C).

A regression analysis was carried out to fit mathematical models to the experimental data aiming at an optimal region for the response studied. By applying multiple regression analysis, the empirical relationship between the input variables and the response variable can be expressed in the following quadratic, second-order polynomial equation (Equation 1 and Equation 2) in terms of uncoded values:

Equation 1

$$MCA = -1468 + 149.0\ X_1 + 178.8\ X_2 + 47.2\ X_3 - 186.5\ X_1X_1 - 6.760\ X_2X_2 - 0.388\ X_3X_3 + 4.75\ X_1X_2 + 1.31\ X_1X_3 - 2.706\ X_2X_3$$

Equation 2

$$Yield = 168.1 + 164.1\ X_1 - 6.25\ X_2 - 7.51\ X_3 - 46.99\ X_1X_1 - 0.748\ X_2X_2 + 0.0882\ X_3X_3 + 4.81\ X_1X_2 - 2.523\ X_1X_3 + 0.1685\ X_2X_3$$

3.2 Analysis of variance

The p-value is the probability of factors having very little or insignificant effect on the response. A larger F value signifies a better fit of the RSM model to the experimental data. A higher F value with a low p-value indicates the high significance of the regression model. However, the p-value should be lower than 0.05 for the model to be statistically significant (Zach, 2021). The regression model found in this study was highly significant as denoted by the large F value and low p value of 35.21, 32.61 and 0.000, respectively. From

Table 3. ANOVA for optimisation of MCA for soft cheese using bromelain.

Source	DF	Adj SS	Adj MS	F	P	Status
Regression	9	2303.05	255.895	35.21	0	Significant
Linear	3	577.68	192.558	26.49	0	Significant
Square	3	1348.63	449.544	61.85	0	Significant
Interaction	3	376.75	125.583	17.28	0	Significant
Residual Error	10	72.68	7.268			
Lack-of-Fit	5	53	10.6	2.69	0.15	Not significant
Pure Error	5	19.68	3.936			
Total	19	2375.73				

DF: degree of freedom, Adj SS: adjusted sum of square, Adj MS: adjusted mean square, F: Fischer, P: probability.

Table 4. ANOVA for optimisation of yield for soft cheese using bromelain.

Source	DF	Adj SS	Adj MS	F	P	Status
Regression	9	107.038	11.8931	32.61	0	Significant
Linear	3	20.879	6.9597	19.08	0	Significant
Square	3	64.621	21.5403	59.06	0	Significant
Interaction	3	21.538	7.1792	19.68	0	Significant
Residual Error	10	3.647	0.3647			
Lack-of-Fit	5	1.696	0.3393	0.87	0.559	Not Significant
Pure Error	5	1.951	0.3902			
Total	19	110.685				

DF: degree of freedom, Adj SS: adjusted sum of square, Adj MS: adjusted mean square, F: Fischer, P: probability.

Table 3, it was observed that the quadratic (square) factors were highly significant compared to linear and interaction factors as indicated by the large F values of 61.85 with a low p-value of 0.000, each. As shown in Table 4, it was observed that the quadratic (square) factors were highly significant compared to linear and interaction factors as indicated by the large F value of 59.06 with a low p value of 0.000, respectively.

The lack-of-fit test measures the variation of data with regard to the fitted model. It is one of the important aspects to check the suggested model fit with reliable data obtained. If the model does not fit the data well, the lack-of-fit test will be significant. A model should be rejected if the results show any significance in the lack-of-fit. Jensen (2017) explained that insignificant lack-of-fit is preferred because the significant lack-of-fit model would indicate the failure of the model to represent data in the experimental domain at points that are not included in the regression. The insignificant lack-of-fit indicated a good model (Kraber, 2022). The F value for the lack-of-fit can be obtained by dividing the lack-of-fit mean square by its pure error mean square. In this study, the selected model (linear, quadratic and interaction) showed a non-significant result ($p>0.05$) with a p values of 0.15 and 0.559. Thus, the insignificant p value indicates that the model is good and fits well with the experimental data.

The goodness of the fit of the regression model was defined by determining the coefficient R^2 and adjusted R^2 (multiple correlation coefficient, R), which provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interaction (Sudamalla *et al*., 2012). The coefficient of determination, R^2, measures the proportion of the total variation in the response expected by the model and is calculated based on the ratio of the regression sum of squares and the total sum of squares (Swamy *et al*., 2014). The R^2 lies in the interval range from 0 to 1 (Turney, 2022) and the closer it is to 1, the better the correlation between the observed and predicted values (Zou *et al*., 2013; Majd *et al*., 2014; Vijayaraghavan and Prakash Vincent, 2014; Liu *et al*., 2014). Thus, the smaller the value of R^2, the less relation that the independent variables in the model have in predicting the behaviour of the dependent variables (Majd *et al*., 2014). The R^2 value of the MCA and yield regression model was satisfactory (0.9694 and 0.9670, respectively) because a value of 0.80 and above indicates the aptness of the model (Saikia *et al*.,2015; Betiku and Taiwo, 2015).

Therefore, the adjusted R^2 corrects the R^2 value for additional terms and the sample size added to the regression, thus compensating for the over-fitting of the model (Swamy *et al*., 2014). A smaller adjusted R^2 than the R^2 value indicates that the additional terms are insignificant or the sample size is not very large (Potumarthi *et al*., 2008; Kadiri and Anand, 2016). In this present study, the adjusted R^2 (0.9419 and 0.9374) was slightly smaller but reasonably closer to R^2. The predicted R^2 (0.8190 and 0.8546) was in reasonable

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agreement with the adjusted R^2, indicating that the overall predictive capability of the model was satisfactory. Therefore, the overall model is highly significant.

A numerical response optimization technique was applied to determine the optimum condition of bromelain concentration, incubation time and incubation temperature for the MCA and yield of soft cheese (Table 5). It was found that the optimum condition for the target goal with a bromelain concentration of 0.53%, incubation time of 2.33 hrs and incubation temperature of 54.2°C was feasible to carry out. Meanwhile, the optimum conditions for the maximum and minimum goals were not feasible to be carried out.

In addition, research has been done on optimum conditions for milk clotting, for example, the optimum condition for crude papaya (*Carica papaya*) extract was, pH 6.5, temperature 70°C, enzyme concentration 1 g/1000 mL milk, by Maskey and Shrestha (2020), and for *Moringa oleifera* seed extract was, 1.5% concentration of extract, 55°C temperature, by Adesina *et al*. (2022).

3.3 Surface plots

Surface plots for MCA and yield of soft cheese at a feasible optimum condition are shown in Figures 1 and 2, respectively. The 3D surface plots are the graphical representatives of the regression equation used to illustrate the function of two (2) factors namely bromelain concentration and incubation time, at a specific incubation temperature while maintaining other factors at a fixed level. In this study, the 3D plots revealed that MCA and yield of cheese varied significantly upon the changing level of X_1 and X_2 variables. It was observed also from the plots, generally, an increase in the levels of either of the variables generally resulted in an increase of MCA and yield up to an optimum point. Further increase of the variable levels beyond the point decreased the MCA and yield. This explains the statistical significance of the quadratic terms. The linear, quadratic and interaction terms have significant effects on the MCA and yield of cheese. In addition, increasing the bromelain concentration followed by shorter incubation times reduces the yield of cheese, which may be due to the less bromelain enzyme

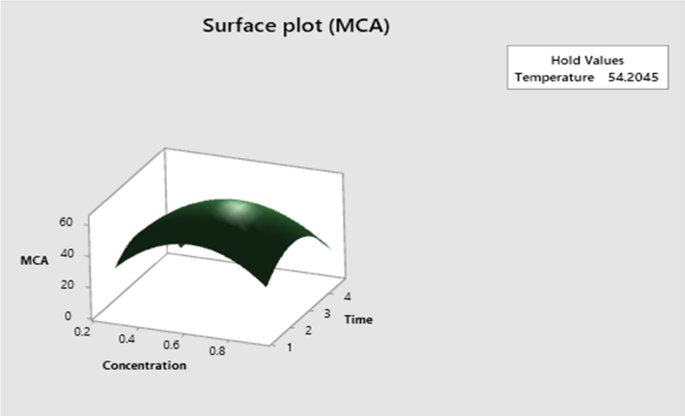

Figure 1. Surface plot of MCA at feasible optimum conditions.

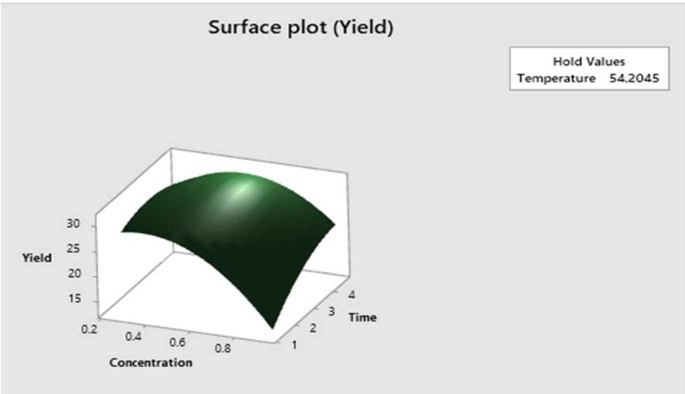

Figure 2. Surface plot of yield at feasible optimum conditions.

Table 5. Comparison values of target and predicted responses for different optimum conditions.

Goal		Lower	Target	Upper	Optimum Condition			Predicted Response		
					X_1	X_2	X_3	FITS1	FITS2	F/NF
TARGET	MCA	30.86	66.03	66.04	0.5287	2.3375	54.2045	60.812	29.8815	F
	FITS1	31.09492	63.75733	63.75733						
	YIELD	20.11	29.91	29.92						
	FITS2	20.54723	30.02703	30.02704						
MAX	MCA	30.86	66.04	66.04	0.5626	2.6433	54.2045	62.0897	30.0199	NF
	FITS1	31.09492	63.75733	63.75733						
	YIELD	20.11	29.92	29.92						
	FITS2	20.54723	30.02704	30.02704						
MIN	MCA	30.86	30.86	66.04	0.2636	2.949	45.7955	30.6217	20.0763	NF
	FITS1	31.09492	31.09492	63.75733						
	YIELD	20.11	20.11	29.92						
	FITS2	20.54723	20.54723	30.02704						

X_1: bromelain concentration, %, X_2: incubation time, hrs), X_3: incubation temperature, °C, FITS: predicted response, F: feasible, NF: not feasible, Y_1: predicted milk clotting activity, SU/mL) Y_2: predicted yield, %

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performance during the heating process. This condition results in less compact curd with higher moisture content (Arlene *et al*., 2015).

Validation for the optimum condition of MCA and yield of soft cheese was performed. The suitability of the model equation for predicting the optimum response value was evaluated for the optimum condition of MCA and yield under conditions where the bromelain concentration was 0.53%, incubation time was 2.33 hrs and incubation temperature was 54.2°C. From Table 6, the validation results obtained for MCA and yield were 60.16 SU/mL and 32.06%, respectively. Optimization using actual experimental values was tested using a t-test (SPSS). There was no significant difference ($p>0.05$) between predicted and verified values. Thus, it indicated that the model was significant and could be used to predict the optimization of MCA and the yield of soft cheese produced from plant-based enzymes.

3.4 Proteolysis of soft cheese

The proteolysis of soft cheese was done using the SDS PAGE method. In this study, the samples used were protein ladder, bromelain pineapple peel cheese, bromelain standard cheese and Laughing Cow cheese. The result of the proteolysis of soft cheese is shown in Figure 3. The protein ladder used has wide sizes ranging from 10 kDa to 220 kDa. The bands of the protein ladder can be seen on the gel with their appropriate molecular sizes. Also, the bands for the three soft cheese samples appeared on the gel at the protein ladder band size of 30 kDa. These bands can be pointed as the alpha (α), beta

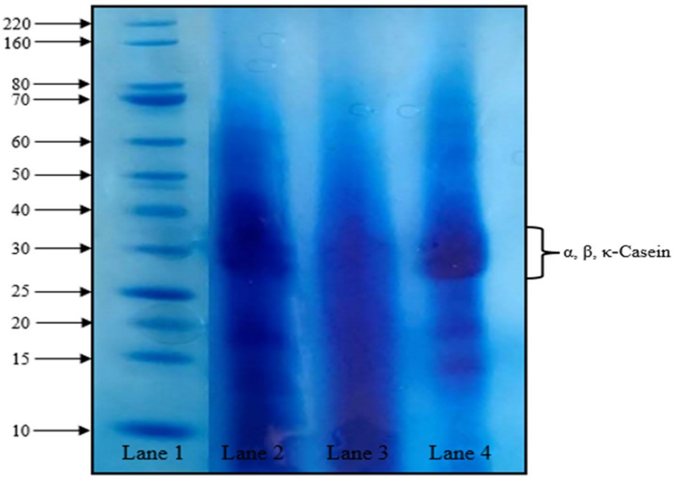

Figure 3. SDS PAGE for proteolysis. Lane 1: Protein Ladder, Lane 2: Bromelain pineapple peel cheese, Lane 3: Bromelain standard cheese, Lane 4: Laughing Cow.

(β) and kappa (κ) casein molecular structures. These bands appear as a result of the breaking down of casein micelles during the making of cheese by the coagulating enzymes. In addition, based on the similarity of the visible band on the gel, the purity of the bromelain pineapple peel enzyme was similar as compared to the bromelain standard enzyme even though it was partially purified.

According to Darwish (2022), research done on the proteolysis of low-fat Karish cheese with the addition of transglutaminase showed that the molecular weight of alpha α, beta, β and kappa, κ, casein band were about 31 kDa. Research done by Barac *et al*. (2019), on the proteolysis of white brine cheese made from cow and goat milk, showed that the molecular size of alpha α, beta β and kappa κ, casein was about 30 kDa, for both types of milk. According to Polak-Berecka *et al*. (2021), a study done on the proteolysis of casein by curly Kale leaf extract, showed that the molecular weight of alpha α, beta β, and kappa κ, casein was around 25 to 35 kDa.

According to Hassan *et al*. (2012), the proteolysis in the cheese contributes to the development of textural changes in the cheese curd, due to the breakdown of the protein network, decrease in water activity through water binding by liberated carboxy and amino groups and increase in pH. Second, the proteolysis resulted in a direct contribution to flavour and perhaps to off-flavour such as bitterness, of cheese through the formation of peptides and free amino acids. Third, proteolysis makes the liberation of substrates such as amino acids, for secondary catabolic changes including, deamination, decarboxylation, transamination, desulphuration catabolism of aromatic compounds such as phenylalanine, tyrosine, tryptophane and reactions of amino acids with other compounds. Fourth, proteolysis makes changes to the cheese matrix, which facilitates the release of the flavoured aromatic compounds.

In summary, the bromelain pineapple peel soft cheese showed similar purity of proteolysis band with the bromelain standard soft cheese. Thus, making the soft cheese comparable to the standard soft cheese.

4. Conclusion

Optimum milk clotting activity and yield of soft cheese were obtained at a bromelain enzyme

Table 6. Validation of Optimum condition for MCA and yield.

Optimum condition			MCA		Yield	
X_1	X_2	X_3	Predicted	Verified	Predicted	Verified
0.53	2.33	54.2	60.81±0.00^b	60.16±0.46^b	29.88±0.00^a	32.06±2.50^a

X_1 (bromelain concentration, %), X_2 (incubation time, hr), X_3 (incubation temperature, °C), MCA (milk clotting activity, SU/mL). Values with different superscripts are statistically significantly different at $p < 0.05$.

concentration of 0.53%, incubation temperature of 54.2℃ and incubation time of 2.33 hrs. For the proteolysis of soft cheese, the protein band that appeared has a molecular weight of around 30 kDa that shows the proteolysis products including the alpha (α), beta (β) and kappa (κ) casein.

Conflict of interest

The authors declare no conflict of interest.

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