Food Research 6 (Suppl. 4) : 9 - 18 (2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Optimisation of soft cheese production conditions using papain as a plant-based enzyme by response surface methodology (RSM)

[1,2,*]Siti Roha, A.M., [1]Najihah, M.N., [1]Muhammad Yazid, A.H., [1]Noorlaila, A., [1,2]Siti Aimi Sarah, Z.A. and [1]Adi, M.S.

[1]*School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA 40450 Shah Alam, Selangor, Malaysia*
[2]*Malaysia Institute of Transport, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*

Article history:
Received: 2 February 2022
Received in revised form: 1 June 2022
Accepted: 2 August 2022
Available Online: 31 December 2022

Keywords:
Papain,
Soft cheese,
Physicochemical,
Response surface methodology (RSM)

DOI:
https://doi.org/10.26656/fr.2017.6(S4).002

Abstract

As the demand for cheese products increases, rennet enzyme supplied for cheese production is reduced due to the limited availability of ruminant stomach. In some countries, microbial coagulants have not been accepted for regular cheese manufacture because they are believed to result in a reduced yield and a lower quality product. Therefore, plant-based enzymes are one of the alternatives for animal-based enzyme substitution. Papain enzyme is chosen due to its capability of coagulating milk as it is a proteolytic enzyme. This study aimed to optimize the yield and viscosity of soft cheese by papain using response surface methodology (RSM) and to determine the physicochemical and textural properties of soft cheese. Experimental design was generated using RSM by MINITAB software version 19 with three selected variables including papain concentration (0.2 – 1 U/g), incubation time (1 – 5 hrs) and incubation temperature (30 – 50^{o}C). The optimum condition for yield and viscosity of soft cheese was achieved at a papain concentration of 0.26 g, incubation time of 1.30 hrs and incubation temperature of 34.82^{o}C, with the predicted yield and viscosity of 11.78 % and 4.77 Pa.s, respectively. The optimum condition was verified with the yield and viscosity obtained was 12.60% and 4.67 Pa.s, respectively. Since there was no significant difference ($p>0.05$) between the predicted and verified yield and viscosity values, thus it indicates that the predicted optimum condition by RSM can be accepted. Characterization of soft cheese produced using papain enzyme at optimum condition was also determined and compared with commercial soft cheese. There is a significant difference between these cheeses in terms of their physicochemical characteristics.

1. Introduction

Cheese is the fresh or ripened solid or semi-solid product in which 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 for the production of 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. As the demand for cheese products has increased by a factor of approximately about 3.5 since 1961, the enzyme used as rennet enzyme supplied for cheese production is reduced due to the limited availability of ruminant stomach (Shah *et al*., 2013). In addition, the cost of growing calf rennet has prompted the quest for alternative milk coagulant enzymes that would replace satisfactorily calf rennet in cheese production. Apart from this, some religious factors, such as the halal status of obtaining calf rennet for Muslims and others related to the vegetarianism of some consumers have greatly limited calf rennet use (Shah *et al*., 2013; Ben Amira *et al*., 2017)

Nowadays, most commercial rennet used in the cheese industry comes from recombinant sources or from microbial origin, and only 20%–30% comes from its

*Corresponding author.
Email: *sitiroha7796@uitm.edu.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

natural source (Abebe and Emire, 2020). Microorganisms including *Rhizomucor pusillus, Rhizomucor miehei, Endothia parasitica,* and *Irpex lactis* have been extensively used as sources of microbial rennets. Most of the coagulants from microbial sources that have been investigated appear to be extracellular in nature and thus these microbes are likely candidates for the elaboration of milk coagulants. Consumer acceptance of cheeses made with microbial rennets does not pose any problems as such products have been classified as "vegetarian" cheeses. Seeking an alternative new rennet substitute becomes demanding in order to increase global demand for diversified and high-quality cheese production. Plant rennets have become a subject of growing interest in the cheese industry, due to their easy availability and simple purification processes (Grozdanovic *et al*., 2013). Furthermore, the use of plant proteases in cheese manufacturing promotes greater acceptability of vegetarians and may improve their nutritional intake (Duarte *et al*., 2009). Plant extracts have been used long times as a milk coagulant agent in cheese production. The use of vegetable coagulant is found primarily in countries of the Mediterranean, West Africa and Southern Europe. Spain and Portugal have recorded the greatest variety and development of cheeses using *Cynara* spp. as the coagulant based on plants (Shah *et al*., 2013). Papain is one of the plant-based enzymes other than *Cynara* spp. which has been reported as an alternative to calf rennet. 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.

The papain is a proteolytic enzyme that can be obtained from papaya leaves, leaf latex, the stem and immature papaya fruit. It can be used to agglomerate protein, able to function properly in low concentration and resistant to temperature (Maskey and Shrestha, 2020). Most of the plant coagulants cause excessive proteolysis and result in lower cheese yield, defects in texture (softness) and bitter flavour (Ben Amira *et al*., 2017; Liburdi *et al*., 2019). Therefore, the present research was focused on the optimization of the papaya protease in the preparation of soft cheese and the quality characteristics of soft cheese.

2. Materials and methods

2.1 Materials

Raw fresh milk was purchased from the local market. Sekaki papaya (*Carica papaya*) was purchased from MARDI (Serdang, Selangor). Papaya fruit in the maturity stage of 3 was selected.

2.2 Extraction process

Papaya peels were crushed using a fruit juice processor with the ratio of papaya peel to purified water at 1: 1. The extract was filtered through a muslin cloth. Then, the papaya peel extract was centrifuged at 360×*g* for 10 mins. The clear supernatant was collected and used for analysis (Nadzirah *et al*., 2013).

2.3 Ammonium sulphate precipitation

Ammonium sulphate precipitations were carried out on enzymes crude extract, by adding 13.2 g of ammonium sulphate salt, pinch by pinch, to 30 mL crude extract with continuous stirring for 45 mins. The sample solution was incubated overnight at 4°C. After the incubation, the precipitated enzyme was centrifuged at 4,000 rpm for 30 mins. The pellet extracts were collected and dissolved in 10 mL of 10 mM Tris HCl buffer (Gautam *et al*., 2010). Then, the purified papain underwent salting process. The salt in the purified papain was removed by diafiltration using a diafiltrator machine. The desalted papain from peel was dried using a vacuum freeze dryer to produce papain powder (Nadzirah *et al*., 2012).

2.4 Preparation of cheese

Commercially available fresh milk was purchased from local supermarket (Shah Alam, Selangor). For each experiment, approximately 100 mL of the fresh milk was heated to 40-50°C, followed by the addition of 15 drops calcium chloride ($CaCl_2$) and then continuous stirring. The $CaCl_2$ helps to reduce milk bitterness and create a firm setting curd to make soft cheese easier to cut. The milk was allowed to stand for 10 mins before the addition of 0.2 g starter culture (Thermophile B cheese culture) and then continually stirring. The milk was allowed to ripen for 1 hr. Papain enzyme was added to the milk at a concentration of 0.2-1.0 U/g. Each papain enzyme was pre-weighed and dissolved in approximately 2 mL distilled water. Then, the papain was added into the ripened milk and stirred. Then, the milk was incubated at the desired temperature of 30-50^{o}C for 1- 5 hrs without stirring. The ripened milk was treated with different papain enzyme concentration, incubation temperature and incubation time. The separation of the whey and the curd was observed. The curds were poured into muslin cloth and let it drained for about 1 hr. The cheese was allowed to ripen for 1 week at the temperature of 7-8°C.

2.5 Experimental design

In this experiment, response surface methodology (RSM) was used in optimizing the enzymatic process parameter to achieve optimal yield and viscosity of soft cheese. The experimental design and statistical analysis

https://doi.org/10.26656/fr.2017.6(S4).002

were performed using MINITAB statistical software version 19. The experimental design was conducted based on central composite design (CCD). Three independent variables were used includes enzyme concentration (X_1), incubation temperature (X_2) and incubation time (X_3). The ranges for these three independent variables were enzyme concentration (0.2-1.0 U/g), incubation temperature (30-50°C) and incubation time (1-5 hr) as shown in Table 1.

Table 1. The coded and uncoded values used in optimisation of enzymatic condition

	-α	-1	0	1	+α
Enzyme concentration (U/g)	0.2	0.4	0.6	0.8	1
Incubation temperature (°C)	30	35	40	45	50
Incubation time (hr)	1	2	3	4	5

2.5.1 Determination of cheese yield

According to Mahajan and Chaudhari (2014)'s procedure, the yield of cheese was calculated by using the following equation:

$$\text{Yield (g/100 g of milk)} = \frac{W_1}{W_2 + W_3} \times 100$$

Where W_1 = weight of cheese, W_2 = weight of milk and W_3 = weight of enzyme

2.5.2 Determination of cheese viscosity

The viscosity of the cheese was determined using a rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) with a measuring system concentric cylinder (CC17) and a gap of 0.5 mm. The temperature was regulated by a Paar Physica circulating bath and a controlled peltier system (TEZ 150) with an accuracy of ±0.1°C. Shear rate of the cheese set up in the rheometer program was 1 to 100 s^{-1} (Ferrão *et al*., 2018).

2.6 Physicochemical analysis of cheese

The moisture content of cheese was determined by the oven-drying method (AOAC 977.11, 2005). The quantification of total solid content in cheese was determined using oven-drying method (AOAC 925.23, 2005) while the protein content was determined by the Kjeldahl method (AOAC 991.20, 2005). The fat and ash content of cheese were determined using AOAC 933.05 (2005) and AOAC 935.42 (2005) respectively.

2.7 Determination of firmness in cheese

Cheese textural properties were analysed at room temperature using a TA.XT2plus texture analyser (Stable Micro Systems, Godalming, UK). Sample for textural analysis were prepared by cutting the cheese into cube size. A texture profile double compression test was performed using a stainless-steel cylindrical probe with a diameter of 30 mins. A crosshead speed of 1.5 mm/s was applied to compress the cube samples (15 mm side) to 60% strain to determine the firmness of cheese (Alinovi and Mucchetti, 2020).

2.8 Rheological properties of cheese

Rheological properties of soft cheese were measured using an MCR-300 rheometer and oscillatory dynamic small amplitude tests. Five grams of cheese were placed between a 40 mm diameter aluminium plate (0 angle) and the rheometer platform (gap = 2 mm). The linear viscoelastic area was calculated by strain sweep analysis at different frequencies before proceed to the stress sweep analysis. The following test parameters were used to conduct pressure sweeps: oscillated stress from 1 to 1000 Pa, logarithmic mode, frequency 1.5 Hz, and temperature of 5°C. The storage module (G') and loss module (G") are acquired from the linear viscoelastic region during stress sweeps (Gutiérrez-Méndez *et al*., 2019).

3. Results and discussion

In this research work, papaya protease was extracted from papaya peel. The impact of enzyme concentration, incubation temperature and time on yield and viscosity of soft cheese were optimized by response surface methodology. The prepared soft cheeses were analyzed for physico-chemical properties such as moisture, total solid, fat, protein and ash content. Firmness and viscoelastic properties of prepared cheeses were analysed also.

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 papain enzyme. The effect of the three independent variables of X_1 (papain concentration), X_2 (incubation temperature) and X_3 (incubation time) using five levels CCD on the yield and viscosity of soft cheese were determined using MINITAB software version 19.

The treatments with their respective actual variables level combinations and the obtained response were listed in Table 2 for percentage of yield and viscosity of soft cheese. The Table 2 showed the values of actual (experimental) and predicted response of each run. The highest actual and predicted soft cheese yield were 14.10% and 14.12%, respectively under specific condition of variable factors (papain concentration of 0.94 U/g, incubation temperature of 40°C and incubation time of 3 hrs), whereby the actual yield showed a slight difference from predicted value. On the other hand, the lowest actual and predicted soft cheese yield also

https://doi.org/10.26656/fr.2017.6(S4).002

Table 2. Factors and comparison between actual (Y) and predicted (FITS) responses

Run	Factors			Response Yield (%)		Response Viscosity (Pa.s)	
	X_1	X_2	X_3	Actual	Predicted	Actual	Predicted
1	0.4	35	2	10.60	10.42	2.69	2.91
2	0.8	35	2	9.70	9.62	0.25	-0.06
3	0.4	45	2	8.80	8.71	0.19	0.06
4	0.8	45	2	10.00	10.06	0.77	0.97
5	0.4	35	4	9.50	9.34	2.97	2.80
6	0.8	35	4	7.90	7.88	4.46	4.63
7	0.4	45	4	11.20	11.18	1.37	1.72
8	0.8	45	4	11.80	11.87	7.62	7.44
9	0.26	40	3	13.90	14.12	0.62	0.47
10	0.94	40	3	14.10	14.03	2.69	2.78
11	0.6	31.59	3	6.30	6.51	0.59	0.66
12	0.6	48.41	3	8.50	8.43	0.75	0.62
13	0.6	40	1.32	7.80	7.92	2.85	2.88
14	0.6	40	4.68	8.50	8.53	8.31	8.23
15	0.6	40	3	10.60	10.53	3.22	3.40
16	0.6	40	3	10.90	10.53	3.17	3.40
17	0.6	40	3	10.50	10.53	3.43	3.40
18	0.6	40	3	10.20	10.53	3.70	3.40
19	0.6	40	3	10.40	10.53	3.55	3.40
20	0.6	40	3	10.60	10.53	3.35	3.40

Yield, R^2= 99.31 (0.9931), Adjusted R^2= 98.68 (0.9868), Predicted R^2= 97.20 (0.9720)
Viscosity, R^2= 99.26 (0.9926), Adjusted R^2= 98.60 (0.9860), Predicted R^2= 95.11 (0.9511)
Where: X_1= papain concentration (U/g), X_2= incubation temperature (°C), X_3= incubation time (hrs)

displayed slight differences, which were 6.30% and 6.5%, respectively at the predetermined variable factors condition (papain of 0.60 U/g concentration, incubation temperature of 31.59°C and incubation time of 3 hrs). This finding also supported by Rana *et al*. (2017) which also showed similar trend of changes for the actual and predicted value of RSM model and this variation could be due to difference in milk composition and processing technique.

The actual viscosity of soft cheese ranged from 0.29 to 8.31 Pa.s with different variables combinations. The range was wider compared to the predicted values ranged from -0.06 to 8.23 Pa.s. The highest actual and predicted viscosity was obtained when the variables are at concentration of papain 0.6 U/g, incubation temperature set at 40°C and incubation time of 4.68 hrs (Run 14) and the lowest actual viscosity was obtained when the variables are set at run 3.

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 coded values:

$$\text{Yield} = 10.5292 - 0.0448X_1 + 0.9605X_2 + 0.3051X_3 + 3.544X_1^2 - 3.056X_2^2 - 2.306X_3^2 + 1.520X_1X_2 - \quad (1)$$

$$\text{Viscosity} = 3.405 + 1.153X_1 - 0.018X_2 + 2.674X_3 - 1.778X_1^2 - 2.763X_2^2 + 2.148X_3^2 + 2.752X_1X_2 + \quad (2)$$

3.2 Analysis of variance

The p value is the probability of the 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 (Panwal *et al*., 2011). Datta *et al.* (2012) reported that a high 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 (Patel *et al*., 2011). Based on reports by few researchers (Panwal *et al.*, 2011; Patel *et al.,* 2011; Datta *et al.,* 2012), the regression model found in this study was highly significant as denoted by the large F and low p value of 159.01, 149.93 and 0.000, respectively. From 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 380.11 with a low p value of 0.000, each. As shown in Table 4, it was observed that the linear and quadratic (square) factors were highly significant compared to interaction factors

https://doi.org/10.26656/fr.2017.6(S4).002

Table 3. ANOVA for optimisation of yield for soft cheese using papain

Source	DF	Adj SS	Adj MS	F	P	Status
Regression	9	67.6254	7.5139	159.01	0	Significant
Linear	3	4.9139	1.6380	34.66	0	Significant
Square	3	53.8878	17.9626	380.11	0	Significant
Interaction	3	8.8237	2.9412	62.24	0	Significant
Residual Error	10	0.4726	0.0473			
Lack of Fit	5	0.1992	0.0398	0.73	0.6.31	Not significant
Pure Error	5	0.2733	0.0547			
Total	19	68.098				

Where 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 viscosity for soft cheese using papain

Source	DF	Adj SS	Adj MS	F	P	Status
Regression	9	9.4732	10.1637	149.93	0	Significant
Linear	3	40.9396	13.6465	201.31	0	Significant
Square	3	29.8727	9.9576	146.89	0	Significant
Interaction	3	20.6608	6.8869	101.6	0	Significant
Residual Error	10	0.6779	0.0678			
Lack of Fit	5	0.4767	0.0953	2.37	0.183	Not significant
Pure Error	5	0.2011	0.0402			
Total	19	92.1511				

Where DF = degree of freedom, Adj SS = adjusted sum of square, Adj MS = adjusted mean square, F = fischer, P = probability.

as indicated by the large F values of 201.31 and 146.89 with a low p value of 0.000, respectively.

The lack-of-fit test measures the variation of data with regards 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 showed any significance in the lack -of-fit test (Siti Nadiah *et al*., 2013). Myers and his co-workers (2016) 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. Patel *et al.* (2011) testified that an insignificant lack-of-fit indicated a good model. 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 value of 0.631 and 0.183. Thus, the insignificant p value indicates that the model is good and fits well with the experimental data.

The goodness of 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 total sum of squares (Swamy *et al*., 2014). The R^2 lies in the interval range from 0 to 1 (Bradley, 2007) 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 yield and viscosity regression model was satisfactory (0.9931 and 0.9926, 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 (0.9868 and 0.9860) was slightly smaller, but reasonably closer to R^2. The predicted R^2 explains the variability in predicting new data sets. The predicted R^2 value (0.9720 and 0.9511) was in reasonable agreement with the adjusted R^2, indicating that the overall predictive capability of the

https://doi.org/10.26656/fr.2017.6(S4).002

model was satisfactory. Therefore, the overall model is highly significant.

A numerical response optimization technique was applied to determine the optimum condition of papain enzyme concentration, incubation temperature and incubation time for the yield and viscosity of soft cheese (Table 5). It was found that the optimum conditions for the target and maximum goal with a papain concentration of 0.26 U/g, incubation temperature of 34.82°C and incubation time of 1.32 hrs were feasible to be carried out. Meanwhile, the optimum condition for the minimum goal with a papain concentration of 0.26 U/g, incubation temperature of 48.41°C and incubation time of 1.6 hr was not feasible to be carried out.

3.3 Surface plots

Surface plots for yield and viscosity 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 papain concentration and incubation temperature, at a specific incubation time while maintaining other factors at a fixed level. In this study, the 3D plots revealed that yield and viscosity of cheese varied significantly upon the changing of initial 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 yield and viscosity up to an optimum point. Further increase of the variable levels beyond the point decreased the yield and viscosity. This explains the statistically significance of the quadratic terms. The linear, quadratic and interaction terms gives significant effect on the yield and viscosity of cheese.

Validation for the optimum condition of yield and viscosity of soft cheese was performed. The suitability of the model equation for predicting the optimum response value was evaluated for the optimum condition of yield and viscosity under conditions where the papain concentration was 0.26 U/g, incubation temperature was 34.82°C and incubation time was 1.32 hrs. The validation result obtained for yield and viscosity are 12.61% and 4.77 Pa.s, respectively. Optimization using actual experimental values was tested using the 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 can be used to predict the optimization of yield and viscosity of soft cheese produced from plant-based enzyme.

3.4 Physicochemical of cheese

The physiochemical characteristic of papain enzyme soft cheese and commercial cheese were compared and evaluated based on its content of moisture, total solid, protein, fat, ash and firmness (Table 6). Papain enzyme soft cheese exhibited higher moisture content than commercial cheese which was 38.3% and 28.6%, respectively. Meanwhile, the total solid content of papain enzyme soft cheese was lower than commercial cheese. Previous study from Buffa *et al*. (2003) reported that the weight loss in cheese during ripening process has been ascribed closely to moisture loss. This was in line with the results obtained where moisture and total solid content of soft cheese are correlated to each other. The amount of protein found in commercial cheese was 10.8%. However, papain enzyme soft cheese produced contained lower protein content, which was 6.47%. The disagreement may due to higher amount of casein protein coagulation occurred in commercial cheese when

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

Goal		Lower	Target	Upper	Optimum condition			Predicted Response (FITS 1)	Predicted Response (FITS 2)	F/NF
					X_1	X_2	X_3			
Target	Yield (%)	6.30	14.09	14.10	0.2636	34.8187	1.3182	11.7778	4.7709	F
	FITS 1	6.51	14.11	14.12						
	Viscosity	0.19	8.30	8.31						
	FITS 2	-0.06	8.22	8.23						
Maximum	Yield (%)	6.30	14.10	14.10	0.2636	34.8187	1.3182	11.7778	4.7709	F
	FITS 1	6.51	14.12	14.12						
	Viscosity	0.19	8.31	8.31						
	FITS 2	-0.06	8.23	8.23						
Minimum	Yield (%)	6.30	6.30	14.10	0.2636	48.409	1.6008	6.1803	0.04	NF
	FITS 1	6.51	6.51	14.12						
	Viscosity	0.19	0.19	8.31						
	FITS 2	-0.06	-0.06	8.23						

Where X_1 = papain concentration (U/g), X_2 = incubation temperature (°C), X_3 = incubation time (hrs), FITS = predicted response (%), F = feasible, NF = not feasible

https://doi.org/10.26656/fr.2017.6(S4).002

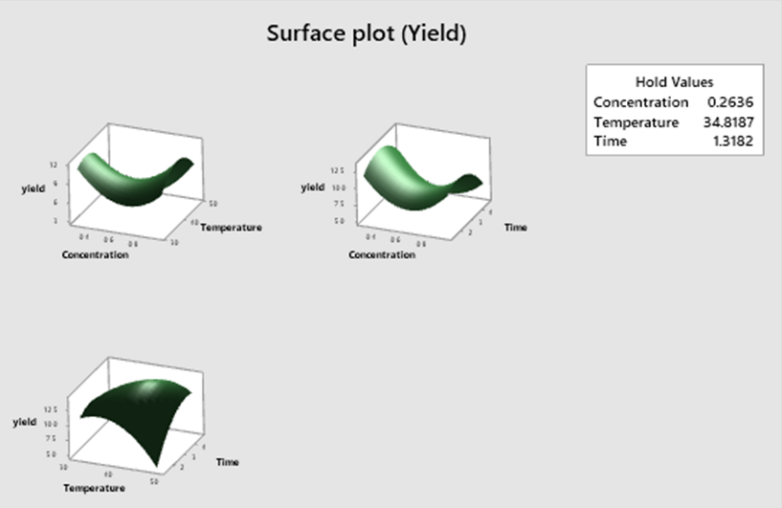

Figure 1. Surface plot of yield at feasible optimum conditions

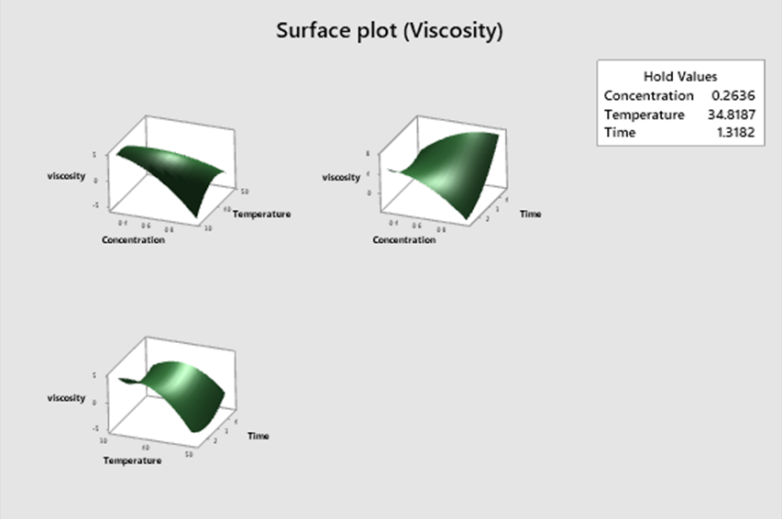

Figure 2. Surface plot of viscosity at feasible optimum conditions

compared to papain enzyme cheese. Among milk proteins, caseins are the main proteins in cheese which exists as aggregates after combined with colloidal calcium phosphate, known as casein micelles (Kwak *et al*., 2012). The fat content in papain enzyme cheese was 11.62%, which was higher than commercial cheese (8.67%) as the fresh milk was used as a starter material while the low fat was used in the commercial cheese production. Furthermore, difference types of milk used will display different fat percentage in the cheese produced. The ash content of commercial cheese was slightly higher than papain enzyme cheese, which was 3.74% and 3.53% respectively. Rana *et al*. (2017) reported that salt added into cheese will contribute to the ash percentage. In term of cheese firmness, it can be seen that there was a vast gap of firmness data between commercial cheese and papain enzyme soft cheese, which was 2280.98 g and 1350.31 g, respectively. This was corresponded to higher amount of casein protein coagulation occurred in commercial cheese, which contributes to stronger cheese structure. As reported by Liburdi *et al*. (2019), the composition and structure of casein micelles, as well as how the caseins are hydrolysed will influence the firmness of the curd.

Table 6. Physicochemical properties of papain enzyme cheese and commercial cheese

Assay	Papain Enzyme Cheese	Commercial Cheese
Moisture content (%)	38.3±1.87^{a}	28.6±0.07^{b}
Total solid (%)	61.7±1.87^{b}	71.4±0.07^{a}
Protein (%)	6.47±0.05^{b}	10.8±0.12^{a}
Fat (%)	11.62±0.09^{a}	8.67±0.06^{b}
Ash (%)	3.53±0.19^{b}	3.74±0.08^{a}
Firmness (g)	1350.31±15.25^{b}	2280.98±11.09^{a}

Values with different superscripts within the same row are significantly different ($p<0.05$).

Monitoring the evolution of rheological properties is one of the means for measuring gel formation during coagulation. Parameters such as the elastic or storage modulus (G′), which is a measure of the energy stored per oscillatory cycle, and reflects the behaviour of the sample as an elastic solid; the viscous or loss modulus (G″), which is a measure of the energy dissipated per cycle and indicates the behaviour of the sample as a viscous liquid. Both storage and loss modulus were used to evaluate the viscoelastic properties of papain enzyme soft cheese and commercial cheese. Both papain enzyme

https://doi.org/10.26656/fr.2017.6(S4).002

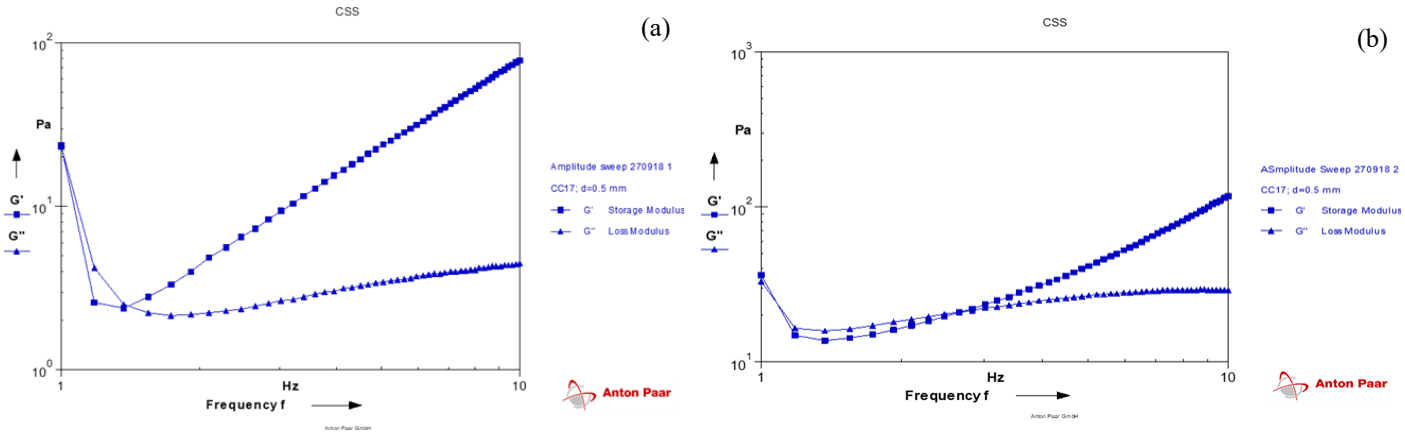

Figure 3. Amplitude sweep of (a) papain enzyme cheese and (b) commercial cheese

soft cheese and commercial cheese showed the behaviour of elastic solid like sample as shown in Figure 3.

The overall spectra for papain enzyme cheese and commercial cheese showing an increase in G' and G" as frequency increased. It was observed that, G' > G" and crossover modulus value occur at frequency of 1.3-1.4Hz and 2.3-3.0Hz for papain enzyme cheese and commercial cheese respectively. This response is a characteristic pattern of a viscoelastic solid, where frequency-dependent moduli are observed. The G′ value at the beginning of gelation by commercial cheese were recorded higher than those of papain enzyme as coagulants in soft cheese. This was due to differences between proteolytic activities of coagulants on casein, which may alter the degree of proteolysis on κ-casein. During gel formation, the hydrolysis of some additional bonds in casein particles may be accompanied by significant rearrangements of gel structure. The latter lead to the increase of strength and number of bonds between adjacent aggregates, resulting in a rapid increase of initial G′ values (Esteves *et al*., 2002).

4. Conclusion

Optimum yield and viscosity of soft cheese were obtained at papain enzyme concentration of 0.26 U/g, incubation temperature of 34.82°C and incubation time of 1.32 hrs. It was observed that papaya protease from papaya peel used for the production of soft cheese have comparable physicochemical and rheological properties to commercial cheese. The quality of the soft cheese made from papaya protease can be improved further by using concentrate pure enzyme.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research project was funded by the Fundamental Research Grant Scheme RACER/1/2019/WAB01/UITM//3).

References

Abebe, B. and Emire, S. (2020). Manufacture of fresh cheese using east African *Calotropis procera* leaves extract crude enzyme as milk coagulant. *Food Science and Nutrition*, 8(9), 4831–4842. https://doi.org/10.1002/fsn3.1765

Alinovi, M. and Mucchetti, G. (2020). Effect of freezing and thawing processes on high-moisture Mozzarella cheese rheological and physical properties. *Food Science and Technology*, 124, 109137. https://doi.org/10.1016/j.lwt.2020.109137

AOAC. (2005). Association of Official Analytical Chemists. Washington, DC, USA: CRC

Ben Amira, A., Makhlouf, I., Flaviu Petrut, R., Francis, F., Bauwens, J., Attia, H., Besbes, S. and Blecker, C. (2017). Effect of extraction pH on techno-functional properties of crude extracts from wild cardoon (*Cynara cardunculus* L.) flowers. *Food Chemistry*, 225, 258-266. https://doi.org/10.1016/j.foodchem.2017.01.040

Betiku, E. and Taiwo, A. (2015). Modelling and optimization of bioethanol production from breadfruit starch hydrolysate vis-à-vis response surface methodology and artificial neural network. *Renewable Energy*, 74(2015), 87-94. https://doi.org/10.1016/j.renene.2014.07.054

Bradley, N. (2007). The Response Surface Methodology (Master's Thesis). South Bend, Indiana, USA: Indiana University of South Bend.

Buffa, M., Guamis, B., Saldo, J. and Trujillo, A.J. (2003). Changes in water binding during ripening of cheeses made from raw, pasteurized or high-pressure -treated goat milk. *Lait*, 83(1), 89–96. https://doi.org/10.1051/lait:2002052

Datta, D. and Kumar, S. (2012). Modeling and optimisation of recovery process of glycolic acid using reactive extraction. *International Journal of Chemical Engineering and Applications*, 3(2), 141-146. https://doi.org/10.7763/IJCEA.2012.V3.175

Duarte, A.R., Duarte, D.M.R., Moreira, K.A., Cavalcanti, M.T.H., Lima-Filho, J.L.D. and Porto, A.L.F. (2009). *Jacaratia Corumbensis* O. Kuntze a new vegetable source for milk-clotting enzymes. *Brazilian Archives of Biology and Technology*, 52 (1), 1-9. https://doi.org/10.1590/S1516-89132009000100001

Esteves, C.L.C., Lucey, J.A. and Pires, E.M.V. (2002). Rheological properties of milk gels made with coagulants of plant origin and chymosin. *International Dairy Journal*, 12(5), 427-434. https://doi.org/10.1016/S0958-6946(01)00170-4

Ferrão, L.L., Ferreira, M.V.S., Cavalcanti, R.N., Carvalho, A.F.A., Pimentel, T.C., Silva, R., Esmerino, E.A., Neto, R.P.C., Tavares, L.M., Freitas, M.Q., Menezes, J.C.V., Cabral, L.M., Moraes, J., Silva, M.C., Mathias, S.P., Raices, R.S.L., Pastore, G.M. and Cruz, A.G. (2018). The xylooligosaccharide addition and sodium reduction in requeijão cremoso processed cheese. *Food Research International*, 107, 137-147. https://doi.org/10.1016/j.foodres.2018.02.018

Gautam, S.S., Mishra, S.K., Dash, V., Goyal, A.K. and Rath, G. (2010). Comparative study of extraction, purification and estimation of bromelain stem and fruit of pineapple plant. *Thai Journal of Pharmaceutical Science,* 34, 67-76.

Grozdanovic, M.M., Burazer, L. and Gavrovic-Jankulovic, M. (2013). Kiwifruit (Actinidia deliciosa) extract shows potential as a low-cost and efficient milkclotting agent. *International Dairy Journal*, 32(1), 46-52. https://doi.org/10.1016/j.idairyj.2013.03.001

Gutiérrez-Méndez, N., Balderrama-Carmona, A., Garcia-Sandoval, S.E., Ramirez Vigil, P., Leal-Ramos, M.Y. and Garcia-Triana, A. (2019). Proteolysis and Rheological Properties of Cream Cheese Made with a Plant-Derived Coagulant from *Solanum elaeagnifolium*. *Foods*, 8(2), 44. https://doi.org/10.3390/foods8020044

Kwak, H.S., Ganesan, P. and Hong, Y.H. (2012) Nutritional benefits in cheese. In Foster, R.D. (Ed.) Cheese: Types, Nutrition and Consumption, p. 269-289. New York, USA: Nova Science Publisher Inc.

Kadiri, D.D. and Anand, P.S. (2016). Optimization of Protease Extraction from Billbergia pyramidalis (Sims) Lindl. by Response Surface Methodology. *International Journal of Pharmaceutical Sciences Review and Research*, 36(48), 267-276.

Liburdi, K., Boselli, C., Giangolini, G., Amatiste, S. and Esti, M. (2019). An evaluation of the clotting properties of three plant rennets in the milks of different animal species. *Foods*, 8, 600. https://doi.org/10.3390/foods8120600

Liu, Z., Mei, L., Wang, Q., Shao, Y. and Tao, Y. (2014). Optimization of subcritical fluid extraction of seed oil from *Nitraria tangutorum* using response surface methodology. *LWT - Food Science and Technology*, 56(1), 168-174. https://doi.org/10.1016/j.lwt.2013.10.048

Mahajan, R.T. and Chaudhari, G. (2014). Plant latex as vegetable source for milk clotting enzymes and their use in cheese preparation. *International Journal of Advanced Research*, 2(5), 1173-1181

Majd, M.H., Rajaei, A., Bashi, D.S., Mortazavi, S.A. and Bolourian, S. (2014). Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (*Phlomidoschema parviflorum*) leaves using response surface methodology. *Industrial Crops and Products*, 57, 195-202. https://doi.org/10.1016/j.indcrop.2014.03.031

Maskey, B. and Shrestha, N.K. (2020). Optimization of crude papaya (*Carica papaya*) protease in soft-unripened cheese preparation. *Journal of Food Science Technology*, 12, 1 - 8. https://doi.org/10.3126/jfstn.v12i12.30139

Myers, R.H., Montgomery, D.C. and Anderson-Cook, C.M. (2016). Response surface methodology: process and product optimization using designed experiments. 4th ed. Hoboken, New Jersey, USA: John Wiley and Sons.

Nadzirah, K.Z., Zainal, S., Noriham, A. and Normah, I. (2012). Efficacy of selected purification techniques for bromelain. *International Food Research Journal,* 20(1), 43-46.

Nadzirah, K.Z., Zainal, S., Noriham, A., Normah, I., Siti Roha, A.M. and Nadya, H. (2013). Physico-chemical properties of pineapple variety N36 harvested and stored at different maturity stages. *International Food Research Journal,* 20(1), 225-231.

Panwal, J.H., Viruthagiri, T. and Baskar, G. (2011). Statistical modeling and optimization of enzymatic milk fat splitting by soybean lecithin using response surface methodology. *International Journal of Nutrition and Metabolism,* 3, 50-57.

Patel, S., Kothari, D. and Goyal, A. (2011). Enhancement of dextransucrase activity of *Pediococcus pentosaceus* SPAm1 by Response Surface Methodology. *Indian Journal of*

Biotechnology, 10, 346-351.

Potumarthi, R., Subhakar, C., Pavani, A. and Jetty, A. (2008). Evaluation of various parameters of calcium-alginate immobilization method for enhanced alkaline protease production by *Bacillus licheniformis* NCIM-2042 using statistical methods. *Bioresource Technology*, 99(6), 1776-1786. https://doi.org/10.1016/j.biortech.2007.03.041

Rana, M.S., Hoque, M.R., Deb, G.K., Nahar, T.N., Habib, R. and Siddiki, M.S.R. (2017). Preparation of cottage cheese from buffalo milk with different levels of papaya latex. *Bangladesh Journal of Animal Science,* 46(2), 128-133. https://doi.org/10.3329/bjas.v46i2.34443

Saikia, S., Mahnot, N.K. and Mahanta, C.L. (2015). Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry*, 171, 144-152. https://doi.org/10.1016/j.foodchem.2014.08.064

Shah, M.A., Mir, S.A. and Paray, M.A. (2013). Plant proteases as milk-clotting enzymes in cheesemaking: A review. *Dairy Science and Technology*, 94(1), 5-16. https://doi.org/10.1007/s13594-013-0144-3

Siti Nadiah, S., Noorlaila, A., Mohd Zahid, A., Norziah, M.H. and Normah, I. (2013). Optimization of Hydrocolloids and Maltodextrin Addition on Roselle -Based Fruit Leather Using Two-Level Full Factorial Design. *International Journal of Bioscience, Biochemistry and Bioinformatics,* 3(4), 387-391.

Sudamalla, P., Saravanan, P. and Matheswaran, M. (2012). Optimisation of operating parameters using response surface methodology for adsorption of crystal violet by activated carbon prepared from mango kernel. *Sustainable Environment Research,* 22, 1-7.

Swamy, G., Sangamithra, A. and Chandrasekar, V. (2014). Response surface modelling and process optimization of aqueous extraction of natural pigments from Beta vulgaris using Box–Behnken design of experiments. *Dyes and Pigments*, 111, 64-74. https://doi.org/10.1016/j.dyepig.2014.05.028

Vijayaraghavan, P. and Prakash Vincent, S.G. (2014). Medium optimization for the production of fibrinolytic enzyme by *Paenibacillus sp*. IND8 using response surface methodology. *The Scientific World Journal*, 2014, 276942. https://doi.org/10.1155/2014/276942

Zou, T.B., Jia, Q., Li, H.W., Wang, C.X. and Wu, H.F. (2013). Response surface methodology for ultrasound-assisted extraction of astaxanthin from *Haematococcus pluvialis*. *Marine Drugs*, 11(5), 1644-1655. https://doi.org/10.3390/md11051644

https://doi.org/10.26656/fr.2017.6(S4).002