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Optimisation of pectin, solid content, and emulsifier for coconut milk set yoghurt analogue using response surface methodology

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Abstract

Non-dairy alternative products are one of the top promising food trends to serve the demands for plant-based products, either for health concerns or environmental responsibility. The present work aimed to investigate the effect of pectin, solid content, and emulsifier on the quality of coconut milk set-type yoghurt analogue (CYA). Response surface methodology was employed to facilitate experimental design and optimisation of these variables. Central Composite design (CCD) with a quadratic model was used to generate 20 experimental runs in order to investigate the effect of total soluble solids of the yoghurt mixture adjusted by sucrose (11.59 - 28.41°Brix), pectin concentration (0.4 - 1.4%), and emulsifier (CITREM) concentration (0.03 - 0.12%) on pH, titratable acidity, syneresis, viscosity, firmness, and overall liking score of CYA. The CYA was prepared by adding 0.02% yoghurt culture starter, and incubated for 6 h at 42°C until its pH was reduced to 4.5 - 4.6. It was found that the relationship models of independent variables studied with syneresis, viscosity, firmness, and overall liking score were significantly explained by the quadratic model ($p \leq 0.05$). However, only firmness and overall liking score provided a non-significant lack of fit model ($p > 0.05$). Amongst the three variables, only total soluble solids was a parameter significantly associated with syneresis, viscosity, firmness, and overall liking score of CYA ($p \leq 0.05$). Increasing total soluble solids resulted in increasing liking score, but decreasing syneresis, viscosity, and firmness. The optimised combination of these ingredients to achieve satisfactory CYA characteristics (minimum syneresis with maximum overall liking score) was found at 23°Brix total soluble solids, 1.02% pectin concentration, and 0.08% CITREM. The model validation yielded errors of prediction for firmness and overall liking score of optimised CYA of 7.17 and 0.28%, respectively. This result could be beneficial for CYA formulation and quality improvement of other alternative non-dairy-based yoghurt formulations.

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Introduction

Yoghurt is a fermented milk product using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Codex Alimentarius, 2003), widely consumed, and renowned for its health benefits (Fan *et al*., 2022). However, alternative non-dairy products have been developed and launched into the market to serve the current market for those who suffer from lactose intolerance or avoid animal products because of either health or environmental concerns (Raikos *et al*., 2020).

Amongst alternative non-dairy substitutes, coconut milk gains attention in this area due to its unique flavour and compatibility with dairy products (Janssen *et al*., 2016; Sebastiani *et al*., 2019; Raikos *et al*., 2020). The advantage of coconut milk is that it is free from allergic substances, as compared to other plant-based yoghurt from such as soy and nuts. Furthermore, coconut milk contains substantial amounts of fat, carbohydrate, and protein, potentially suitable for non-dairy yoghurt formulation. However, replacing cow milk with alternative plant-based materials often imparts unsatisfactory characteristics

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such as viscosity, syneresis, and sensory acceptability. To improve alternative non-dairy yoghurt characteristics, some variables contributing to key product properties need to be investigated and controlled such as solid contents, gelling agents, emulsifier, and hydrocolloids; to maintain or enhance yoghurt properties, including texture, mouthfeel, appearance, viscosity, consistency, and stability (Andic *et al*., 2013).

According to previous studies, the addition of a premix containing 10% coconut milk to soy milk improved the sensory characteristics and acceptability of soy yoghurts (Khubber *et al*., 2021). Incorporation of 25 or 50% coconut milk with cow milk and using ABT (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophiles*) culture produced bio-yoghurt with high nutritional value, and satisfactory colour, appearance, body, texture, and flavour (El-Kadi *et al.*, 2017). It was reported that the suitable conditions for fermentation of coconut milk yoghurt were at 37°C for 8 h with 3% (w/w) of starter culture (Mir *et al*., 2021). However, there is still a lack a report on ingredient optimisation of coconut milk yoghurt analogue, especially those contributing to key product characteristics such as texture and sensory acceptability. Therefore, the objective of the present work was to investigate the effect of total solids, pectin, and emulsifier on properties and sensory acceptability of coconut milk set yoghurt using response surface methodology. The optimisation of these three variables was subsequently performed to achieve a satisfactory coconut milk yoghurt analogue.

Materials and methods

Coconut milk preparation

White coconut meat (1 kg) combined with distilled water (50 mL) was pressed using a hydraulic machine to obtain coconut milk. It was subsequently filtered using a filter cloth, and measured for total soluble solids using a refractometer. The final coconut milk obtained was 9°Brix total soluble solids.

Gelling agent and emulsifier

Low methoxyl pectin and CITREM (citric acid ester) were used as a gelling agent and an emulsifier, respectively. Both ingredients were supplied from International Flavors and Fragrances (IFF, USA).

Starter culture

The yoghurt starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was obtained from International Flavors and Fragrances Incorporation (IFF, USA).

Preparation of coconut milk yoghurt analogue (CYA)

Coconut milk was preheated to 60 - 65°C. Then, all ingredients including pectin and CITREM were added, and the desirable total soluble solids level was adjusted by sucrose addition based on each experimental run. The mixed liquid was well dispersed using a homogeniser (HG-15D and HG-15A models, Daihan Scientific, China) at 50 rpm. Next, the mixture was pasteurised at 85°C for 5 min, followed by cooling to 45°C, before 0.02% starter culture for yoghurt was added. The yoghurt solution (100 mL) was poured into plastic cups, and then incubated for approximately 6 h at 42°C, or until the pH was reduced to 4.5 - 4.6. Yoghurt samples obtained were kept at 10°C in the refrigerator.

pH and titratable acidity

The pH of each CYA sample was determined using a digital pH meter (MP 220, Mettler Toledo, Thailand).

The titratable acidity of each CYA sample was determined following the AOAC method (AOAC, 1995), and calculated using Eq. 1:

$$\text{Titratable acidity as lactic acid (\%)} = \frac{(N\ x\ V_1\ x\ 90.08\ x\ 100)}{(V_2\ x\ 1000)} \quad \text{(Eq. 1)}$$

where, N = normality of NaOH, V_1 = volume of NaOH at end point, V_2 = volume of sample, and 90.08 g/mole = MW of lactic acid.

Syneresis

The syneresis of each CYA sample was determined by centrifuging (3,000 rpm, 10 min, 8 - 10°C) 10 g of sample. The clear supernatant was then separated and weighed. The syneresis was calculated as the percentage of supernatant based on the initial weight of the yoghurt sample (Keogh and O'Kennedy, 1998) using Eq. 2:

$$\text{Syneresis (\%)} = \frac{Weight\ of\ supernatant\ (g)}{Weight\ of\ yoghurt\ sample\ (g)}\ x\ 100 \quad \text{(Eq. 2)}$$

Texture analysis

The texture of each CYA sample was determined for firmness using a texture analyser TA-XT 2i (Stable Micro System, Surrey, UK) with a cylinder probe size of 50 mm diameter. The depth of penetration was 15 mm with speed of 1 mm/s. The tested values were then recorded.

Viscosity

The viscosity of each CYA sample was determined using a rotational viscometer (Brookfield LVDV-I+, MA, USA) equipped with a helipath stand and spindle (S63) at a rotating speed of 4.0 rpm and sample temperature of 25°C. The apparent viscosity value was expressed in centipoise (cP).

Scanning electron microscopy (SEM)

The microstructure of each selected yoghurt sample was observed using SEM (JSM-IT300LV, JEOL, Japan). The CYA sample was prepared in a mould cup size of 5 × 5 × 5 mm before freeze drying for 48 h. The freeze-dried sample was subsequently defatted by soaking in hexane for 3 h. Then, the sample was taken and dried in desiccator for at least 24 h before analysis. Each sample was separately placed in a sample holder, coated with gold, transferred to SEM, and observed at 5 kV and 2,500× magnification.

Sensory evaluation

The yoghurt samples were evaluated by 50 untrained panellists using a 9-point hedonic scale (1 = extremely dislike; 5 neither like nor dislike; 9 = extremely like) for product attributes including appearance, taste, odour, texture, and overall liking. Only the overall liking score was evaluated for data analysis and optimisation by response surface methodology (RSM). The blind yoghurt samples in plastic cups (100 g each), under a controlled temperature of approximately 10°C, were 3-digit coded of which order were randomly served to each tasting panellist. The panellists were asked to rinse their mouths with drinking water before tasting the subsequent sample in order to prevent the carry-over effect from the previous tasting sample. Analysis of variance was performed following the randomised complete block design, where tasting panellists were treated as blocks. Mean scores were compared using Duncan's new multiple-range test. Data were analysed by XLSTAT (2006) Statistical Software for Excel (Addinsoft, France).

Probiotics determination

The CYA samples were analysed for the survivability of probiotics. Serial dilutions of CYA in duplications were prepared for the enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* on MRS agar incubated at 37°C for 4 d according to Demirci *et al.* (2020). *Streptococcus thermophillus* was cultivated in the selective M-17 agar, and incubated at 37°C for 2 d. Both bacterial counts were combined to represent total probiotics counts, and the values were reported in CFU/g.

Experimental design and data analysis

Central composite design (CCD) with three independent variables, including total soluble solids content (X_1), pectin concentration (X_2), and CITREM concentration (X_3), was employed for experimental design to achieve quadratic model construction for variable optimisation based on the response surface methodology (RSM). Five concentration levels of each ingredient were varied as coded values based on the CCD (-1.68, -1, 0, +1, +1.68). The Design Expert (6.0.5) software (Stat-Ease, USA) was employed for generating experimental runs with an assumed quadratic model, resulting in 20 treatment combinations. The actual values of each variable corresponding to coded values consisted of 11.59, 15, 20, 25, 28.41°Brix for X_1; 0.4, 0.6, 0.9, 1.2, 1.4% w/w for X_2; and 0.03, 0.05, 0.08, 0.1, 0.12% w/w for X_3. The full quadratic model used for generating experimental runs by the Design Expert software was as shown in Eq. 3:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \quad \text{(Eq. 3)}$$

where, Y_i = response variable i; i = 1,2,3,…6; β_0 = a constant indicating the Y-intercept; and β_1 - β_9 = coefficient values of independent variables.

The error of prediction of each response variable was calculated from the difference between the observed value and its predicted value obtained from the predictive quadratic equation (Eq. 3) as shown in Eq. 4:

$$\text{Error of prediction} = \frac{|\text{Observed value} - \text{Predicted value}| \times 100}{(\text{Observed value})} \quad \text{(Eq. 4)}$$

Results and discussion

Physico-chemical properties of CYA samples prepared based on CCD experimental runs including pH (Y_1), titratable acidity or TA (Y_2, %), syneresis (Y_3, %), viscosity (Y_4, cP), firmness (Y_5, g), and overall liking score (Y_6) are presented in Table 1.

Table 1. Response variables of experimental CYA sample run generated from central composite design.

Sample run	Independent variable			Response variable					
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
1	11.59	0.9	0.08	4.45	0.79	60.26	19900	180.49	2.0
2*	20	0.9	0.08	4.55	0.77	28.90	30170	373.78	6.6
3	20	1.4	0.08	4.42	0.67	18.58	30100	376.76	6.4
4	15	1.2	0.05	4.62	0.71	36.72	27240	320.37	3.9
5*	20	0.9	0.08	4.43	0.78	26.91	30652	301.05	6.9
6	20	0.4	0.08	4.52	0.75	51.72	32788	382.51	5.0
7*	20	0.9	0.08	4.65	0.65	26.53	30612	371.05	7.0
8*	20	0.9	0.08	4.60	0.75	26.35	30832	365.07	6.9
9	15	0.6	0.05	4.40	0.78	43.60	27750	200.20	3.3
10	20	0.9	0.03	4.49	0.76	24.55	30100	266.19	6.5
11	20	0.9	0.12	4.45	0.79	26.04	30477	315.21	6.8
12	25	1.2	0.10	4.75	0.62	12.42	32788	395.70	5.4
13*	20	0.9	0.08	4.59	0.67	24.86	30578	380.76	6.9
14	28.41	0.9	0.08	4.69	0.65	36.68	33102	420.00	6.2
15*	20	0.9	0.08	4.45	0.79	28.90	30751	322.22	6.7
16	25	0.6	0.10	4.56	0.78	21.61	32700	377.01	6.4
17	15	0.6	0.10	4.60	0.68	39.00	26800	222.51	4.3
18	25	1.2	0.05	4.82	0.63	10.57	32888	399.18	6.5
19	25	0.6	0.05	4.53	0.67	37.23	30557	311.88	5.4
20	15	1.2	0.10	4.52	0.68	37.40	28090	236.86	4.6

X_1: total soluble solids (°Brix); X_2: pectin (%); X_3: CITREM (%); Y_1: pH; Y_2: TA (%); Y_3: syneresis (%); Y_4: viscosity (cP); Y_5: firmness (g); and Y_6: overall liking. (*) Replicated sample runs at centre points of three independent variables.

pH and titratable acidity (TA) of CYA

The pH and TA are vital quality parameters of yoghurt, and associated with its taste, textural characteristics, and consumer acceptance. The pH and TA of all samples were in ranges of 4.40 - 4.82 and 0.62 - 0.79, respectively. Both pH and TA in yoghurt are positively associated with total soluble solids (Lee and Lucey, 2010; Iguttia *et al*., 2011; Delikanli and Ozcan, 2017; Huang *et al*., 2020). Yoghurt with higher total soluble solids provides more available sugar for lactic acid bacteria to produce more acid, thus increasing TA and lowering pH. The addition of low methoxyl pectin has been previously reported to increase the total soluble solids in low-fat set yoghurt (Kim *et al*., 2020). A recent study demonstrated that an increase in the almond gum concentration resulted in a decrease in the TA of yoghurt (Lee and Lucey, 2010). The variation in TA and pH could be due to the amount of pectin and sugar in yoghurt. In the present work, variations of these values were in narrow ranges because the yoghurt incubation was under a controlled pH of 4.5 - 4.6. Hence, slight variation in TA was observed, but negligible. Based on the analysis of variance (Table 2), the model explaining the relationship of either pH or TA to the three independent variables studied was not significant ($p > 0.05$). Therefore, these two response variables were not included in the RSM model analysis.

Table 2. Analysis of variance of independent variables for model construction of each CYA properties (in terms of actual values).

Variable	Estimated coefficient response				*p*-value			
	Y3	Y4	Y5	Y6	Y3	Y4	Y5	Y6
Model	Q	Q	Q	Q	0.0008*	0.0005*	0.0009*	< 0.0001*
Constant	146.48	3668.39	-539.33	-26.32	-	-	-	-
X_1	-8.60	2221.19	40.32	1.98	0.0002*	< 0.0001*	< 0.0001*	< 0.0001*
X_2	-34.82	428.49	187.10	14.48	0.0005*	0.4078	0.0811	0.0940
X_3	319.79	-57246.02	6330.73	104.6	0.4660	0.6039	0.5019	0.2322
X_1^2	0.24	-47.84	-0.85	-0.03	0.0017*	0.0045*	0.0307*	< 0.0001*
X_2^2	15.30	-1116.55	74.90	-5.05	0.3574	0.7659	0.4452	0.0044*
X_3^2	3390.14	3.98	-39523	-184.9	0.1687	0.4658	0.0155*	0.3740
X_1X_2	-2.28	211.58	-2.37	-0.07	0.1046	0.4880	0.7605	0.4925
X_1X_3	-9.85	1243	122.85	-1.71	0.5351	0.7318	0.2071	0.2287
X_2X_3	379.16	-22383.3	-2907.16	-40.16	0.1658	0.7113	0.0845	0.1009
Lack of fit	-	-	-	-	0.0019*	0.0007*	0.5218	0.0852
R^2	0.89	0.90	0.89	0.94	-	-	- -	

X1: total soluble solids (°Brix); X_2: pectin (%); X_3: CITREM (%); Y3: syneresis (%); Y_4: viscosity (cP); Y_5: firmness (g); Y_6: overall liking score; Q: quadratic model; and (*) $p \leq 0.05$.

Syneresis of CYA

Syneresis is an important factor in indicating the quality of set-type yoghurt due to the loss of water-holding capacity in the gel network. The syneresis values of CYA samples are presented in Table 1. It was observed that as the pectin amount increased, the syneresis decreased. Similar results were found for total soluble solids. This finding was in agreement with the previously reported study of low-fat yoghurt gel (Khubber *et al*., 2021). This indicated the positive effect of pectin content and total soluble solids that promoted the gel network, thus increasing the water-holding capacity (Kim *et al*., 2020). Moreover, the decrease in syneresis of yoghurt could be related to increasing viscosity in relation to pectin addition. The gel of low methoxyl pectin used in this experiment is formed in the presence of divalent cations such as calcium ions (Celus *et al*., 2018; Chen *et al*., 2021). Pectin gel provides good water-holding capacity through its macromolecular network connected by the ion junction zones, in which a large amount of water is immobilised in the form of physical interception (Einhorn-Stoll, 2018). Water mobility was also limited by the increasing amount of soluble solids, mainly from sugar, which also exhibited kosmotropic properties, thus enhancing gel network association (Braga and Cunha, 2005). Therefore, increasing the amount of pectin and soluble solids resulted in decreased water separation from the gel network, and consequently lowered syneresis. The result indicated negative correlation of CYA syneresis with total soluble solids and pectin concentration. Increasing total soluble solids and pectin concentration resulted in a decrease in syneresis. Based on the analysis of variance (Table 2), there was a significant effect of total soluble solids and pectin concentration ($p \leq 0.05$), whereas no significant effect was found for CITREM on the syneresis of CYA ($p > 0.05$). The response surface plot of syneresis in CYA as a function of total soluble solids and pectin concentration is presented in Figure 1a.

Viscosity of CYA

Viscosity is one characteristic associated with the textural property and consistency of set-type yoghurt (Jeske *et al*., 2018; McClements and Peng, 2019; Greis *et al*., 2020). Stabilisers are usually incorporated in order to improve textural properties and stability of yoghurt. As shown in Table 1, the viscosity of CYA varied based on total soluble solids, pectin, and CITREM. The response surface plot of the viscosity of CYA as a function of total solid content and pectin concentration is presented in Figure 1b.

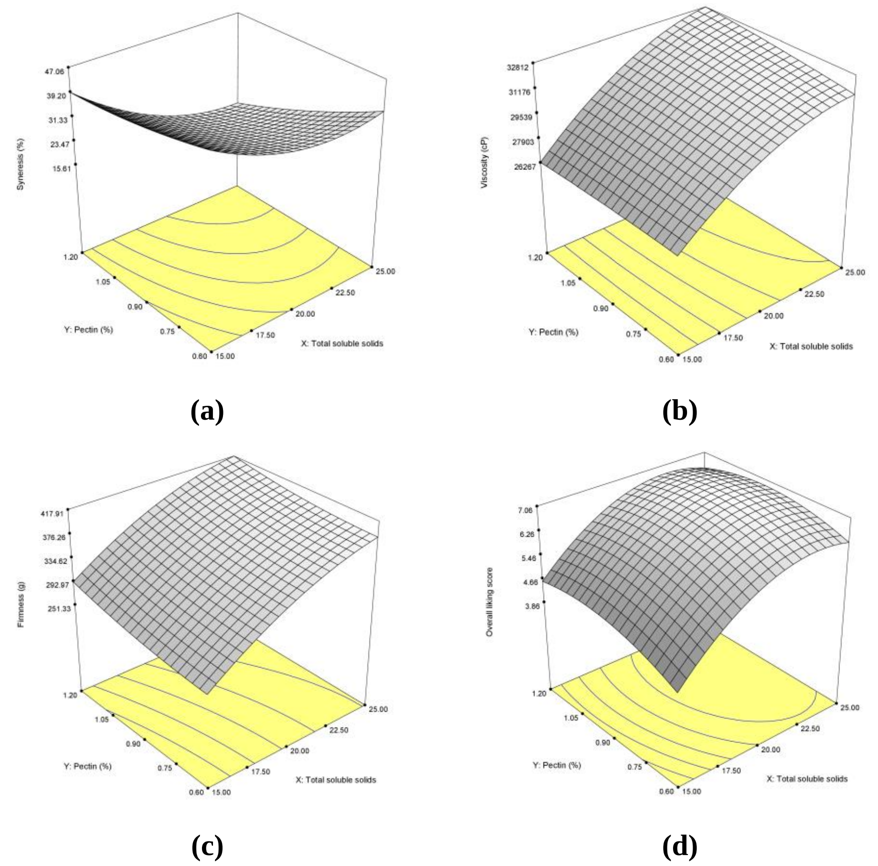

Figure 1. 3D-response surface plots showing effect of total soluble solids and pectin concentration on **(a)** syneresis of CYA, **(b)** viscosity of CYA, **(c)** firmness of CYA, and **(d)** overall liking score of CYA.

The maximum viscosity of CYA was observed at 28.41% total solids, 0.9% pectin, and 0.08% CITREM. A previous study revealed that the addition of hydrocolloids such as tapioca starch and psyllium (*Plantago ovata* Forsk) husk gum increased the viscosity of yoghurt (Ladjevardi *et al*., 2015; Khubber *et al.,* 2021). In the present work, the viscosity of CYA was found at maximum at certain levels of the combination of low total soluble solids, moderate concentrations of pectin, and CITREM acting as an emulsifier. This experiment demonstrated that increasing the amount of pectin resulted in increasing the viscosity of CYA. Under acid conditions like dairy yoghurt, it has been reported that negatively-charged pectin molecules are electrostatically adsorbed onto the positively-charged casein micelle particles, thus promoting emulsion system stability contributing to the textural characteristic of liquid yoghurt (Zhang *et al*., 2024). In the present work, pectin and CITREM contributed to emulsion stability and viscosity, playing an essential role in the texture of CYA. Total soluble solids in CYA indicated the amount of sugar that produced viscosity of CYA through hydration and reduced water activity (Benítez *et al*., 2009).

Firmness of CYA

Texture is the main attribute that indicates the quality of set-type yoghurt, and associated with its appearance and consumer acceptance. The firmness of CYA was measured in the present work to characterise its textural property, as presented in Table 1. The relationship of firmness of CYA with total soluble solids and pectin concentration is illustrated by response surface 3-D plots (Figure 1c). The highest firmness of CYA was observed at the highest level of total soluble solids of 28.41°Brix. The total soluble solids content and pectin concentration significantly affected yoghurt firmness. Similarly, it was reported that the addition of low methoxyl pectin increased the firmness of low fat-set yoghurt (Kim *et al*., 2020). This result suggested the capability of pectin in water holding capacity that promoted yoghurt gel formation and strengthening of set yoghurt (McCann *et al.*, 2011; Montemurro *et al.*, 2021; Meena *et al*., 2022). The presence of sugar or sucrose and calcium ions also affect the performance of both gel strength and gel elasticity of low methoxyl pectin gel (Wan *et al.*, 2019). Low methoxyl pectin gel is formed by fixing water through a network structure, which is dominated by ionic interaction between the dissociated carboxyl groups and cations (Kastner *et al.*, 2012). Sucrose promoted the gel strength by providing more hydroxyl groups to stabilise the junction zones, and promote hydrogen bonds to immobilise free water (Han *et al*., 2017). Similar results were also found for other natural sources of gelling agents addition such as aquafaba in oat-based yoghurt (Raikos *et al*., 2020), and apple and pineapple pomace in set-type yoghurt (Wang *et al*., 2019; Meena *et al.*, 2022).

Sensory evaluation of CYA

Sensory evaluation of CYA to determine the acceptability of the product was performed using a 9-point hedonic scale. Only the mean overall liking score was included in the model analysis as presented in Table 1. Sensory assessment of other attributes including appearance, colour, odour, texture, and taste was performed with statistical analysis. It was found that the highest overall liking score of CYA was 20% total soluble solids, 0.9% pectin, and 0.8% CITREM (7.00 ± 0.35) with high scores for all other attributes as well, including appearance (6.71 ± 0.51), colour (6.26 ± 0.49), odour (6.78 ± 0.55), texture (7.00 ± 0.40), and taste (6.10 ± 0.46). Nevertheless, this variable combination provided all attributes liking scores of CYA not significantly different from those at 20% total soluble solids, 0.4% pectin, and 0.8% CITREM ($p > 0.05$). The lowest overall liking score of CYA (2.02 ± 0.80) was found at combination of 11.59% total soluble solids, 0.9% pectin, and 0.08% CITREM (sample run no. 1), with low scores (below 6.0 and considered unacceptable) for all attributes evaluated, including appearance (3.00 ± 0.78), colour (5.10 ± 0.30), odour (2.22 ± 1.09), texture (2.76 ± 0.80), and taste (2.34 ± 0.66). The relationship of the overall liking score of CYA with total soluble solids and pectin concentration is illustrated by response surface 3-D plots as shown in Figure 1d. It was noticed that at pectin concentration above or below 0.9% with total soluble solids below 20%, the overall liking score of CYA tended to fall within unacceptable range. Both pectin and total soluble solids associated with sugar contributed to the textural characteristics of yoghurt (Benítez *et al*., 2009; Zhang *et al*., 2024). Therefore, a suitable amount of pectin and total soluble solids provided satisfactory textural characteristics, resulting in acceptable CYA.

Probiotics count

The purpose of the probiotics count performed in the present work was to ensure that the formulated CYA meets the standard of yoghurt. It was suggested by the USFDA that the probiotics count in functional foods should be at least 10^6 CFU/g (Tripathi and Giri, 2014). The results demonstrated that the total probiotics counts of all experimental CYA samples were in the range of 25×10^6 - 42×10^8 CFU/g without noticeable association with variables of this study ($p > 0.05$). Therefore, it was not taken as a response variable to be included in the model analysis and used for optimisation. However, all CYA samples met the standard concerning their number of probiotics viability.

RSM model analysis

Regression analysis of the polynomial equation with analysis of variance was performed, and the results are presented in Table 2. The quadratic models explaining the effect of total soluble solids (X_1), pectin concentration (X_2), and CITREM concentration (X_3) on syneresis (Y_3), viscosity (Y_4), firmness (Y_5), and overall liking score (Y_6) of CYA are demonstrated in Eqs. 5 - 8, respectively, as follows.

$$Syneresis\ (Y_3) = 146.48 - 8.60X_1 - 34.82X_2 + 319.79X_3 - 2.28X_1X_2 - 9.85X_1X_3 + 379.16X_2X_3 + 0.24X_1^2 + 15.30X_2^2 + 3390.14X_3^2 \quad \text{(Eq. 5)}$$

$$Viscosity\ (Y_4) = 3668.39 + 2221.19X_1 + 428.49 - 57246.02X_3 + 211.58X_1X_2 + 1243X_1X_3 - 22383.33X_2X_3 - 47.84X_1^2 - 1116.55X_2^2 + 3.98X_3^2 \quad \text{(Eq. 6)}$$

$$Firmness\ (Y_5) = -539.33 + 40.32X_1 + 187.10X_2 + 6330.73X_3 - 2.37X_1X_2 + 122.85X_1X_3 - 2907.16X_2X_3 - 0.85X_1^2 + 74.90X_2^2 - 39523.36X_3^2 \quad \text{(Eq. 7)}$$

$$Overall\ liking\ score\ (Y_6) = -26.32 + 1.98X_1 + 14.48X_2 + 104.58X_3 - 0.07X_1X_2 - 1.71X_1X_3 - 40.16X_2X_3 - 0.03X_1^2 - 5.05X_2^2 - 184.93X_3^2 \quad \text{(Eq. 8)}$$

It was observed that the relationship of three independent variables studied with each response variable followed the quadratic model ($p \leq 0.05$). Statistical models used to explain how independent variables affect the firmness and overall liking score of a product did not show a significant lack of fit. The *p*-value for the lack of fit test was greater than 0.05 ($p > 0.05$), which suggested that the models fit the data well enough, and were reliable for making predictions about firmness and overall liking scores. Considering the coefficient in the model (β_i), it was noticed that total soluble solids and pectin concentration were positively associated with viscosity, firmness, and overall liking score, whilst negatively associated with syneresis. CITREM had negative impact on the viscosity of CYA but positive impact on the rest. However, there was no significant effect of CITREM on all parameters (syneresis, viscosity, firmness, and overall liking score) ($p > 0.05$). Only total soluble solids significantly affected all response variables ($p \leq 0.05$), whereas pectin concentration significantly impacted syneresis of CYA ($p \leq 0.05$).

Optimisation procedure and RSM model verification

The optimisation of total soluble solids, pectin concentration, and CITREM was performed by utilising Design-Expert software version 6.0.5. Based on model analysis in Table 2, the lack of fits of syneresis (Y_3) and viscosity (Y_4) was significant ($p \leq 0.05$), making them low accuracy for prediction. Therefore, firmness (Y_5) within the experimental range (180 - 420 g) and maximum overall liking score (Y_6) were two major criteria set for optimisation. The optimum combination of three variables was consequently predicted, and found at 23°Brix total soluble solid, 1.02% pectin, and 0.08% CITREM, respectively. In order to validate the prediction of the model, CYA with these optimised ingredients combination was prepared. Then, the selected key parameters contributing to the attributes of this yoghurt including firmness and overall liking score were evaluated. The results are presented in Table 3. The error of prediction of each response variable was calculated from the difference between the observed value and its predicted value (Eq. 4) obtained from the predictive quadratic equation (Eqs. 7 and 8) for firmness and overall liking score, respectively. Normally, the percentage error of prediction indicates the accuracy of forecasting, *i.e.* below 10 = high, 10 - 20 = good, 20 - 50 = reasonable, and above 50 = inaccurate (Moreno *et. al*., 2013). It was found that the error of the predictive model for either firmness or overall liking score was below 10%. Therefore, the predictions made by these models were highly reliable for prediction. Thus, the predicted optimum combination for CYA was considered satisfactory.

Table 3. Predicted values and observed values of optimised CYA for model validation.

Response variable	Predicted value	Observed value	Error of prediction (%)
Firmness (g)	391.26	363.18	7.17
Overall liking score	7.00	6.98	0.28

Microstructure and sensory evaluation of optimised and selected CYA

The desirable characteristics of CYA include satisfactory texture and taste with low syneresis and high sensory liking score. Based on the experimental results previously described, the optimised combination of total soluble solids, pectin, and CITREM was found at certain levels. In order to understand how gel characteristics contribute to CYA texture and syneresis, its microstructure was investigated. Microstructures of the optimised CYA sample (23.00°Brix total soluble solid, 1.02% pectin, and 0.08% CITREM) were observed through scanning electron microscope (SEM), and compared with selected experimental samples containing the lowest and the highest total soluble solids (11.59 and 28.41°Brix, respectively), at equal amount of pectin and CITREM (0.9 and 0.08%, respectively) to enable the comparison as influenced by a single main variable. The results are presented in Figure 2. Differences in microstructure characteristics of CYA samples containing different levels of total soluble solids associated mainly with sucrose amount were observed.

The gel microstructure of the lowest total soluble solid CYA (Figure 2a) exhibited the formation and distribution of cavities within the gel network where water was trapped. Increasing total soluble solids corresponding to increasing sugar concentration promotes cross-linkage of pectin molecules to create more cavity spaces and free-water immobilisation (Han *et al*., 2017). As a result, more water could be trapped within the gel network, hence

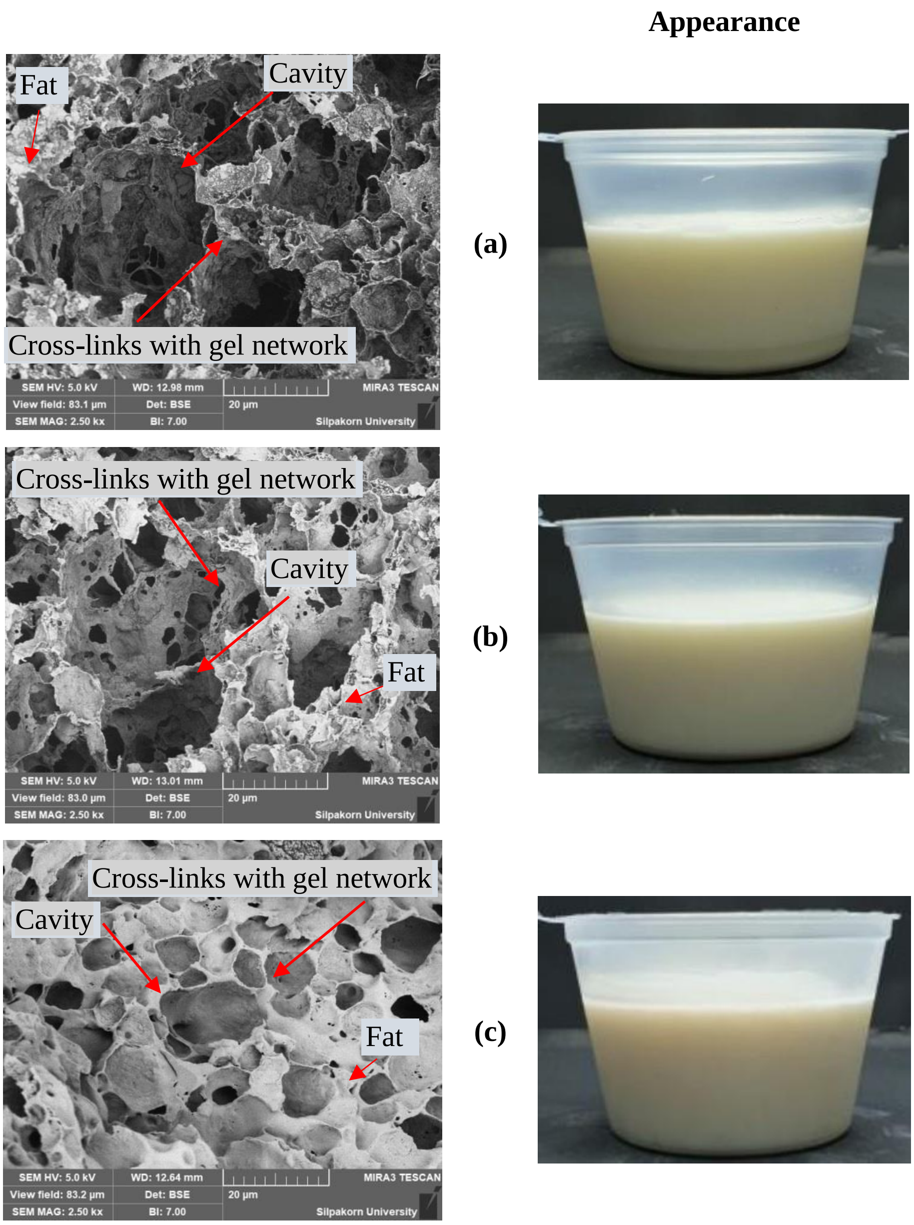

Figure 2. Scanning electron micrographs (2,500× magnification) illustrating the microstructure of CYA: **(a)** low level of total soluble solids (11.59°Brix, 0.9% pectin, 0.08% CITREM), **(b)** optimum combination (23°Brix, 1.02% pectin, 0.08% CITREM), and **(c)** high level of total soluble solids (28.41°Brix, 0.9% pectin, 0.08% CITREM).

lower syneresis and higher firmness could be anticipated as the amount of sugar increased. However, it appeared that at a high level of total soluble solids of 28.41°Brix, the amount of syneresis of CYA was higher compared to that of 23.00°Brix. It was possible that at higher levels of total soluble solids, more fat was also trapped within the gel network as observed in Figure 2c. At higher concentrations of sugar, larger molecular clusters of sugar-water hydration were formed and filled in the gel network cavity making the structure weaker and easily damaged resulting in loss of trapped water (Wan *et al.* 2021). Thus, at a certain amount of total soluble solids dominated by sugar beyond the holding capacity of the cavity, some water would be depleted from the gel network.

Sensory evaluation of these three CYA samples was performed using a 9-point hedonic scale with 50 untrained panellists. The results are presented in Table 4. It was found that the optimised CYA sample provided the highest scores of all attributes including overall liking as compared to CYA samples containing lower (11.59°Brix) and higher (28.41°Brix) total soluble solids ($p \leq 0.05$), with a given amount of CITREM of 0.08%. Therefore, this combination of optimised ingredients is considered suitable for CYA preparation for further non-dairy yoghurt application purposes. The findings from this study demonstrated the effective use of RSM as an experimental tool for food product optimisation.

Table 4. Liking scores of three CYA samples at various levels of total soluble solids, pectin, and CITREM (mean ± SD).

X_1	X_2	X_3	Liking score					
			Appearance	**Colour**	**Odour**	**Texture**	**Taste**	**Overall**
11.59	0.9	0.08	3.0 ± 0.8^{c}	5.1 ± 0.3^{bc}	2.2 ± 0.1^{c}	2.8 ± 0.8^{c}	2.3 ± 0.7^{c}	2.1 ± 0.8^{c}
23	1.09	0.08	6.7 ± 0.5^{a}	6.7 ± 0.5^{a}	6.92 ± 0.3^{a}	6.9 ± 0.3^{a}	6.8 ± 0.4^{a}	6.98 ± 0.1^{a}
28.41	0.9	0.08	5.3 ± 0.7^{b}	5.1 ± 0.4^{bc}	5.2 ± 0.5^{b}	5.1 ± 0.6^{b}	4.8 ± 0.4^{b}	5.3 ± 0.5^{b}

X_1: total soluble solids (°Brix); X_2: pectin (%); and X_3: CITREM (%).

Conclusion

The RSM was effectively and successfully applied to optimise total soluble solids, pectin, and emulsifier for coconut milk yoghurt analogue formulation. The desirable yoghurt quality prepared from the combination of predicted ingredients could be achieved with the required pH and titratable acidity, minimum syneresis, suitable viscosity, firmness, and satisfactory sensory score. The optimum CYA contained 23% total soluble solids, 1.02% pectin, and 0.08% CITREM acting as an emulsifier. The microstructure and sensory evaluation of optimised and selected CYA indicated the significant role of total soluble solids at a given amount of pectin and emulsifier.

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