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Optimization of Soymilk Fermentation Supplemented with Coconut Protein by *Lactobacillus plantarum* TISTR2084

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Abstract

Currently, there is an increasing demand for milk alternatives due to allergies to milk protein and lactose intolerance. Other milk alternatives such as soy milk, oat milk and corn milk, necessitating the development of plant-based yogurt. Additionally, plant-based protein substitutes have been used to improve the texture of yogurt. This research work aimed to optimize the levels of coconut protein concentration (CCP), *Lactobacillus plantarum* TISTR 2084 starter powder (STP) and sugar concentration (SUG) through incubation at 35 °C for 24 h, resulting in improved product characteristics. A response surface methodology (RSM) was used to determine the optimization of soy yogurt with the combined effects of STP (5 - 9 % w/v), SUG (3 - 7 % w/v), and CCP (3 - 7 % w/v). There were evaluated in terms of some quality criteria of plant-based yogurt namely: pH, titratable acidity (TA), lactic acid bacteria (LAB), and syneresis. The results revealed that soymilk yogurt exhibited average bio-physical-chemical properties. The experimental variables significantly affected TA and syneresis. The models of research were high value of R^2 for TA (0.9859) and syneresis (0.9760) which demonstrate the adequacy of RSM. When raising the CCP to an increase TA, decrease syneresis relative to other samples. It was possible to obtain high-quality yogurt. This product is in high demand for increasing protein level of regular yogurts. Soymilk yogurt products with 5 % CCP showed in higher LAB (11 log CFU/mL) and decreased syneresis, while 7 % CCP resulted in lower LAB (9 log CFU/mL) and increased syneresis. According to the optimal contents of 9 % STP, 7 % SUG and 5 % CCP were determined, with a desirability of 86.3 %. These STP and CCP findings provide critical information for future research into innovative plant-based yogurt substitutes.

Keywords: *Lactobacillus plantarum*, Soymilk, Plant based yogurt, Coconut protein, Probiotic, Fermentation

Introduction

Various foods, mainly dairy products are developed for the transportation of probiotics, as consumers regularly associate interest with fermented products. Traditional yogurt is typically prepared using whole milk and a starter culture of LAB, which undergo anaerobic fermentation. Starter culture consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which are commonly regarded as probiotics. They can digest lactose and alleviate allergies [1]. Probiotic foods are related to live bacteria with supplement of prebiotics. Research suggest that probiotics should contain at least 10^6 (1 million) CFU per gram. It possesses probiotic qualities that include building the anti-allergic barrier, protecting the intestinal epithelium, fighting infections and regulating the immunological activity of lymphoid tissues associated with the intestine [2]. According to The Panel on Dietetic Products (EFSA), starter cultures have a health claim since they can digest lactose and give health advantages to the host [3]. An investigation was conducted on a single culture of *Lactobacillus plantarum* (STP), isolated from rice cake starter (Loog pang Koawmak), used for the traditional Ka-nom Tuay-fu production of Thailand. It was tested using sucrose as a protective agent and rice flour as a carrier medium. After 90 days of storage at 4 °C, the LAB survival rate was 7.48 ± 0.15 log CFU/g, indicating a viable *L. plantarum* starter powder with low production costs [4]. Generally, *Lactobacillus* spp. has been stated that a competent starting culture should be capable of surviving stably at refrigerated temperatures usually 21 - 28 days during the storage period [1]. As sugar serves as the primary carbon source for *Lactobacillus* growth [6]. *L. plantarum* has been used in various research studies on fermented milk products, including soy, almond and coconut milk [6].

Yogurt is 1 fermented milk production, that it was categorized as a functional food. It offers significant therapeutic benefits, including stabilizing gut microbiota, preventing against gastrointestinal infection, boosting immune system and reducing cholesterol levels [7]. Plant-based ingredients are increasingly utilized in yogurt production to meet the rising demand in the global vegan and non-dairy yogurt market. The plant-based ingredients, soy and coconut are the most popular ingredients for development yogurt product. Soymilk contains much lower saturated fatty acids than coconut as well as being lactose-free [8]. It contains excellent sources of protein, dietary fiber and phytochemicals such as saponins, phytosterols and isoflavones that enhance human health [9]. Thus, there might be important health benefits and soy milk has been applied to make yogurt. Soy yogurt is a good source of antioxidative, anticancer, antidiabetic, antihypertensive, immunomodulatory, bone-preserving properties, VLDL and LDL-cholesterol reducing in rats [10]. However, commercial soy yogurt without added protein enhancers had about 40 - 50 % and lower than dairy yogurt [11]. Likewise, the production of soy yogurt remains demanding, mainly related to appearance and texture. This is due to the fact that whey separation frequently causes stability problems with the product [12]. Approximately 80 % of the products investigated as stabilizers are hydrocolloids, with pectin being the most generally used hydrocolloid. Other stabilizers for plant-based yogurt whereby 73 % of the inspected products also contain corn and tapioca starch being the most used [13]. The addition of 1.0 % (w/w) tapioca flour enhances sensory properties, especially texture attribute. However, it did not affect the viability of LAB during storage at 4 °C [14].

In addition, plant protein fortification is used in a variety of food associated applications [15,16]. Protein content is a crucial role in the advancement of gelled products likewise yogurt. An alternative method to enhance the gel network structure is to increase protein content in yogurt [17]. Consumers are increasingly interested in plant proteins due to their numerous advantages, which are considered potentially supplementary to animal proteins and environmentally friendly [18]. Oludahunsi *et al.* [19] suggested that adding flavoring agents and fruit tastes might improve the acceptability of soy yogurt. Because of their nutritional value, coconuts (*Cocos nucifera* L.) are commonly utilized to make plant-based yogurt analogs [20]. CCP is the major by-product from the coconut milk industry. Coconut endosperm contains 59.8 to 71.62 % of fat, 8.80 to 10.2 % of protein, 6.90 to 24.3 % of carbohydrates, and 1.4 to 1.59 % of minerals, mostly potassium [21]. CCP consists of a high nutritional value including proteins (glutelin-1, glutelin-2, prolamine, globulin, and albumin), amino acids, polyphenolic compounds, dietary fiber and fatty acids [22,23]. CCP is mostly composed of globulin and albumin fractions, which account for 40 and 21 % of the total protein, respectively [24]. Furthermore, L-arginine is a precursor of nitric oxide; CCP could lower blood pressure and act as an antiplatelet agent, which has a hypolipidemic impact. The primary activity described is that of antioxidants [25]. Globulin, glutelin and prolamin fractions are effective at radical scavenging and ion chelation as well as protecting DNA from oxidative damage [24]. CCP serves as a natural emulsifier, mostly moderate the stability and firmness of coconut milk. Their activities including viscoelasticity, interfacial tension and interfacial adsorption are crucial for stabilizing emulsions [26]. Previously, some researchers investigated the addition of plant proteins to yogurt such as pumpkin, seed flour, hemp protein, pea protein, soya protein, and wheat gluten [27]. Currently, incorporation of STP and CCP in soy yogurt production has never been reported. Furthermore, studying the collective factors of ingredients to obtain the most suitable formula requires the use of the RSM program. Box-Behnken Design (BBD) also belongs to the RSM model. It focuses on the exploration of the design surface, often requiring fewer experiments than other methods and minimizing experimental risks. However, it differs from Central Composite Design (CCD) in that CCD explores a wider range of conditions, but it often requires more experiments and increases the risk of outliers or errors. In addition, OFAT tests 1 factor at a time while keeping others constant. This method fails to capture the interactions between factors and typically requires many more experiments to explore different combinations. Hence, the BBD design is suitable for optimizing this research.

Therefore, this research aimed to study the effect of fermented soy milk fortified with different CCP and SUG ratios on the survival of the *Lactobacillus plantarum* TISTR 2084. To prove our hypothesis with optimization the formulation ingredients response surface methods (RSM). We evaluated the effects of viability cells, pH, total acidity and syneresis. The developed yogurt products supplemented with CCP, present a new approach to this study of vegan milk alternatives.

Materials and methods

Materials

The materials selected for this research were obtained as follows:

1) The commercial soymilk powder product was obtained from the Morning Soy Milk Co., Bangkok, Thailand.

2) Coconut protein powder was gathered from One organic Co., Ltd, Prachuap Khiri Khan, Thailand with protein content of 55 % used in this study.

3) Sugar was acquired from Mitr Phol Sugar Corp., Ltd., Bangkok, Thailand.

4) Tapioca starch was obtained from Bangkok Inter Food Co., Ltd, Bangkok, Thailand.

Meanwhile, Dextrose monohydrate was employed as a protective agent in the growth medium to cultivate the LAB. which were obtained from Loba Chemie Pvt. Ltd., India. The potential probiotic strain *Lactobacillus plantarum* TISTR 2084 was derived from LAB, previously isolated by the method of Maneesri *et al*. [4]. Sodium hydroxide was obtained from Loba Chemie Pvt.Ltd., India. De Man Rogosa and Sharpe (MRS) agar was used for the analysis of LAB (HiMedia Laboratories Pvt. Ltd., India).

Culture preparation

Probiotic bacterial strains namely *Lactobacillus plantarum* TISTR 2084, was procured from Loog-pang Khaomak for the production of Ka-nom Tuay-fu was obtained from Pattani province, Thailand. These strains were collected at the Thailand Institute of Scientific and Technological Research (TISTR), subsequently sub-cultured every month on De Man Rogosa and Sharpe (MRS) agar respectively and before being stored at 4 °C for further use [4].

Dried starter powder preparation

L. plantarum TISTR 2084 was cultured in a 500 mL flask containing 300 mL of MRS broth and incubated at 30 °C with a shaking speed of 110 rpm for 48 h. The culture was collected from the process of centrifugation at 9,000× g for 10 min at 4 °C. The supernatant liquid was decanted, and the cell paste was resuspended in 30 mL of distilled water. This procedure was repeated 2 times to collect the cells for subsequent mixing with distilled water. Subsequently, a protective agent was added, prepared with a 15 % (w/v) D-glucose concentration in *L. plantarum* TISTR 2084 containing approximately 10^8 - 10^9 CFU/mL. A 5 mL culture suspension was transferred and mixed with 5 g of sterilized commercial rice flour in petri dish to make the starter culture powder. Starter culture was dried in a hot air oven at 40 °C. The starter culture powder of *L. plantarum* TISTR 2084 were filled into aluminum foil packets, approximately 10 g each, for storage before use [4].

Preparation of soymilk yogurt

The soymilk powder was prepared with a solid-to-water ratio 1:16 w/v. CCP (3 - 7 %, w/v) [28], SUG (3 - 7 %, w/w) [29], and 1 % tapioca starch were added to the soymilk according to mixing ratio to combine and designed by BBD (**Table 2**). Ingredients were mixed. Then, the suspension was pasteurized at 90 °C for 3 min and cooled to 43 °C for inoculum inoculation. The STP (5 - 9 % w/v) [30] ratio designed by BBD was added to soymilk. All ingredients were gently shaking and put on polypropylene cups in 100 mL portions and incubated in a 35 °C incubator (BINDER Model BD53, Germany) for 24 h. The curd will be kept at 4 °C to break it down and prevent further fermentation by the LAB.

Table 1 Code levels and variable of the BBD.

Code levels	Variable		
	A: STP (%)	B: SUG (%)	C: CCP (%)
–1	5	3	3
0	7	5	5
1	9	7	7

Table 2 Actual values of responses (independent variables) used in BBD for characteristics of soymilk yogurt.

Run	STP (%)	SUG (%)	CCP (%)	Responses			
				pH	TA (%)	LAB (log CFU/g)	Syneresis (%)
1	5	3	5	5.20 ± 0.00	0.92 ± 0.01	9.92 ± 0.03	18.89 ± 2.68
2	9	3	5	4.86 ± 0.00	0.98 ± 0.00	10.18 ± 0.07	15.92 ± 1.29
3	5	7	5	4.86 ± 0.02	0.95 ± 0.01	9.60 ± 0.07	19.80 ± 1.29
4	9	7	5	4.77 ± 0.00	0.96 ± 0.00	11.22 ± 0.13	19.82 ± 1.05
5	5	5	3	5.17 ± 0.00	0.84 ± 0.03	9.60 ± 0.07	21.95 ± 2.62
6	9	5	3	4.77 ± 0.01	0.90 ± 0.00	9.62 ± 0.15	29.20 ± 5.94
7	5	5	7	4.98 ± 0.01	0.97 ± 0.00	9.90 ± 0.01	5.42 ±1.55
8	9	5	7	5.28 ± 0.06	0.90 ± 0.00	9.64 ± 0.19	8.18 ± 0.61
9	7	3	3	4.84 ± 0.00	0.85 ± 0.00	9.63 ± 0.06	28.56 ± 1.88
10	7	7	3	5.05 ± 0.00	0.74 ± 0.00	9.79 ± 0.19	21.36 ± 0.94
11	7	3	7	5.20 ± 0.00	0.97 ± 0.00	9.82 ± 0.07	35.00 ± 1.02
12	7	7	7	5.22 ± 0.00	0.94 ± 0.00	9.75 ± 0.08	10.48 ± 2.56
13	7	5	5	5.01 ± 0.02	0.92 ± 0.00	9.80 ± 0.18	27.79 ± 0.29
14	7	5	5	5.02 ± 0.02	0.94 ± 0.00	9.86 ± 0.09	27.87 ± 0.37

Experimental design

Fourteen experimental values, including 3 central points, were obtained according to the BBD using RStudio® (version 4.3.1, Posit Software, PBC, Vienna, Austria) for optimization of starter powder level (STP), sugar concentration (SUG), coconut powder concentration (CCP) for the soymilk yogurt recipe. The independent variable, namely STP (A), SUG (B), and CCP (C) were set at 5 - 9, 3 - 7 and 3 - 7 %, respectively. The factors and their concentration with actual values are summarized in **Table 1**. The BBD with the actual values and responses is presented in **Table 2**. The dependent of variables in this case were pH, TA, LAB, and syneresis. The 2nd-degree polynomial was fitted to each response using the following equation is represented by:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

pH and TA

The pH of the samples was determined using a calibrated pH meter (SI Analytics, Scientific Promotion Co., Ltd, Bangkok Thailand) and the pH for 50 mL of sample was observed and recorded at room temperature [31]. The total acidity of the sample was determined by the titration method [31]. During the acidification process of fermentation, 10 mL of sample was titrated using a 0.1 N NaOH solution. The percentage of TA can be calculated using the following equation:

$$TA(\%) = \frac{volume\ of\ titrant\ NaOH \times N\ NaOH \times E.F.}{weight\ of\ sample \times 1000} \times 100. \quad (2)$$

N NaOH = Molarity of sodium hydroxide (NaOH),
E.F. = Equivalent factor = 90.

Microbiological analysis

LAB were enumerated on MRS agar. A 10 g samples was added to 90 mL of sterilized water and vortex for 1 min. It was diluted in a 10-fold series. Diluted sample of 1 mL was used in the pouring plate technique with 20 mL of sterilized MRS agar and incubated at 30 °C for 24 - 48 h [31]. Colonies were counted after incubation, and the results were presented as log colony-forming units per gram of soy yogurt (log CFU/g). The countable plate has between 30 - 300 colonies.

Syneresis analysis

Syneresis was measured to the quantity of each sample by using a 30 g sample into centrifuge tubes and centrifuging at 1200 rpm for 20 min. The volume of whey extracted from a gel on the surface of the product was measured [32]. The % syneresis can be calculated using the following equation:

$$syneresis(\%) = \frac{weight\ of\ supernatant\ (g)}{weight\ of\ yogurt\ (g)} \times 100 \quad (3)$$

Statistical analysis

The results of soymilk yogurt were obtained each experimental for 3 times. The responses were determined at least once for each sample. The results were determined using One-Way Analysis of Variance (ANOVA) and Duncan's Multiple Rank Test (DMRT) was performed by IBM® SPSS® Statistics version 25. Additionally, RSM was conducted using the mean values, which were repeated 3 times for each response. These statistical analyses were conducted using BBD in RStudio® (version 4.3.1, Posit Software, PBC, Vienna, Austria) to assess the significance of the regression model ($p < 0.05$).

Results and discussion

Fitness verification of RSM models

All main 2nd-degree polynomial equation and interactive effects were determined for the model. The *F* value, coefficient of correlation, lack of fit and p-value were evaluated to access the adequacy of the model (**Table 3**). The 2nd-degree polynomial demonstrated high value of R^2 for pH (0.8186), TA (0.9859), LAB (0.7280), syneresis (0.9760) which demonstrate the adequacy of RSM. The 2nd-degree polynomial model exhibited an insignificance lack of fit for pH, TA, LAB and syneresis. All the responses were insignificant in lack of fit tests. The adequacy of the model is accurate to predict association of independent variables within the studied ranges. This recommends that the achieve models can be utilized to regulate the correlative effects of the studied variables, facilitating the determination of optimum conditions integration for desirable responses as well as predicting values for other conditions.

Effect of soy yogurt on pH and TA

The effect of STP (A), SUG (B), CCP (C) on TA of the soy yogurt were analyzed (**Table 3**) The 2nd-degree polynomial model for TA can be expressed by the following equation:

$$TA = 0.8350 + 0.0300A + 0.0300B + 0.0975C + 0.0100AB - 0.0650A^2 - 0.0200B^2 + 0.0500C^2 \quad (4)$$

Yogurt was produced by starter powder with *L. plantarum* TISTR 2084 and incubated for 24 h. The pH and the TA were determined as indicators of lactic acid content. The pH and TA of soymilk yogurt ranged from 4.77 - 5.00 and 0.68 - 0.96 %, respectively (**Table 2**). pH is a determination of the acidity or basicity of a solution. Studies have shown that soymilk yogurt has a pH value of soymilk reducing it gradually to 5.1 cause lactic fermentation allowed gently generate acids and protons [18]. pH was not significantly affected by the STP, SUG and CCP with polynomial model (**Figure 1**). This result may be related to *L. plantarum* due to sugar consumption and lactate accumulation hence, acidifying the milk. The increase in sugar concentration in fermented drinks depends on increasing in acidity. Studies have shown that increasing sugar concentration on *L. salivarious* L105 can enhance L-lactic acid production and increase the amount of LAB [33]. It also causes the fermentation time to vary [34]. Nonetheless, the pH of soy yogurt due to the CCP, is classified as an alkaline forming food [35]. When the starter powder concentration increases, the acidity also significantly increases ($p \leq 0.01$). **Figure 2** shows the combination of variables related to the TA. This is consistent with previous research results on inoculum concentration for peanut yogurt products development [36]. In the fermentation process, *Lactobacillus helveticus* grows rapidly by converting fortified glucose into lactic acid-based organic acids [37]. LAB use sugar fermentation to convert starch or glucose in soymilk into lactic acid. Disaccharides undergo hydrolysis to become monosaccharide units. The glucose monomers are then degraded into lactic acid. Higher sugar content increase acidity [36]. Furthermore, STP produce from *Lactobacillus plantarum*, a facultatively heterofermentative (Group II) strain, was employed in this method [38]. This bacterium can produce both L-lactate and D-lactate dehydrogenase, as well as lactic acid, ethanol, acetic acid, and other carbon sources [39]. As a result, increasing sugar concentration causes increased acidity. An increase in TA was noted to increase CCP. The result was also concordant with those reported by [40]. In this paper, the fermentation technology of plant-based yogurt supplements with other proteins, including almond protein, pea protein and isolated soy protein. Considering the result of the pH and TA, increase protein concentration result in higher amount of lactic acid production [31]. The total acidity of dairy product is reported in the range of 0.5 - 1.0 % lactic acid [36].

Table 3 Design regression model for dependent variables of the soymilk yogurt and significance.

Factor	**pH**	**TA (%)**	**LAB (log CFU/g)**	**Syneresis (%)**
Model	Polynomial	Polynomial	Polynomial	Polynomial
intercept	5.0150**	0.8350**	9.8300	14.8300**
A	–0.0660	0.0300*	0.2050	2.3825*
B	–0.0250	0.0300*	0.1013	–3.6138*
C	0.1063**	0.0975**	0.0588	–7.7488**
AB	0.0625	0.0100	0.3400	0.4975
AC	0.1750**	0.0000	–0.0700	–0.8725
BC	–0.0475	0.0000	–0.0575	–5.0800*
A^2	–0.0600	–0.0650**	0.1713	–1.5675
B^2	–0.0325	–0.0200	0.2288	5.0950*
C^2	0.0950	0.0500*	–0.3113	3.6750*
R^2	0.8186	0.9859	0.7280	0.9760
Adjust R^2	0.4105	0.9541	0.1159	0.9220
P value	0.2620	0.002389	0.4671	0.006734
Lack of fit	Insignificant	Insignificant	Insignificant	Insignificant

Note: $*p < 0.05$, $**p < 0.01$, A: Starter powder, B: Sugar and C: Coconut protein powder.

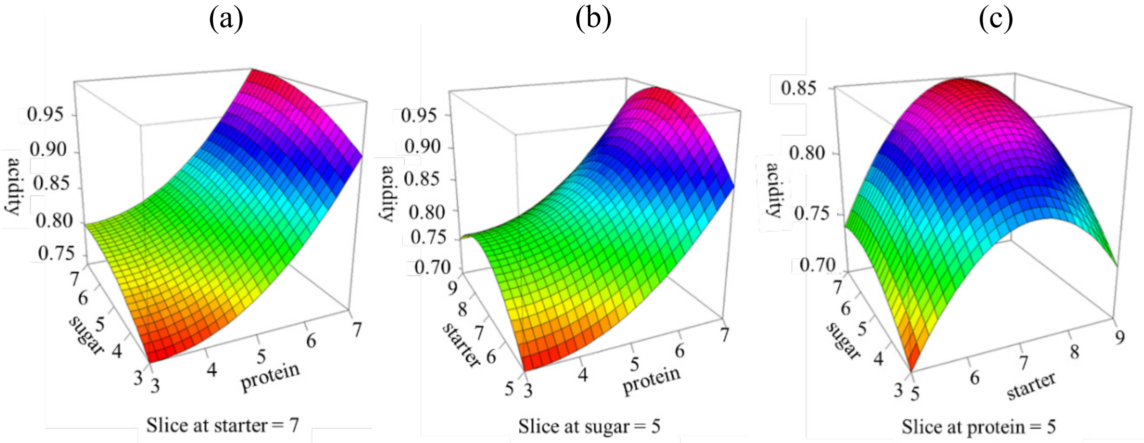

Figure 1 3D plot for acidity as an operation of (a) Sugar concentration and coconut protein at 7 % w/v starter powder, (b) Starter powder level and coconut protein concentration at 5 % w/v sugar and (c) Sugar concentration and starter powder level at 5 % coconut protein.

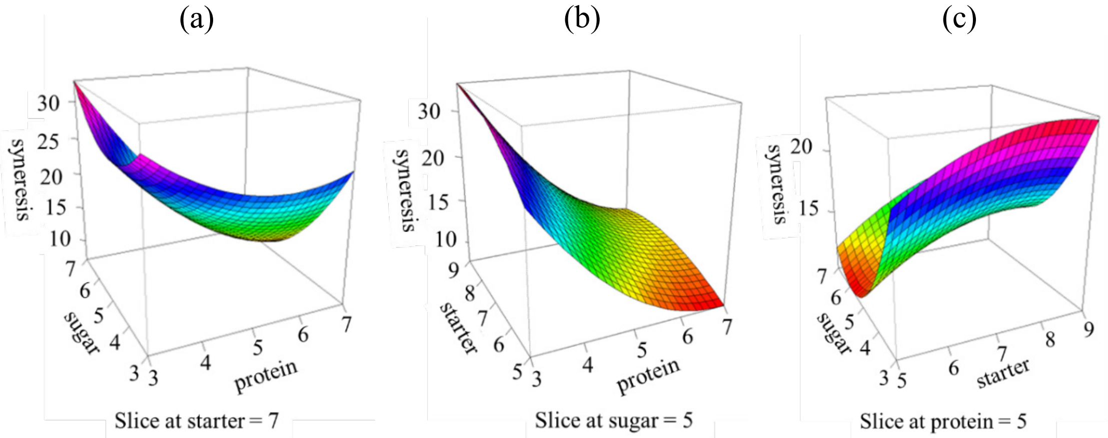

Figure 2 3D plot for syneresis as an operation of (a) Sugar concentration and coconut protein at 7 % w/v starter powder, (b) Starter powder level and coconut protein concentration at 5 % w/v sugar and (c) Sugar concentration and starter powder level at 5 % coconut protein.

Effect of soy yogurt on microbiological analysis

The 2nd-degree polynomial model fitted to the microbiological data was not significantly ($p \leq 0.01$) observed. The total counts of viable bacteria ranged from 9.60 to 11.22 log CFU/g. Starter powder is an essential factor in ensuring an adequate count of viable bacteria in the fermented sample. The maximum viable bacteria observed in yogurt supplemented with CCP was 11.22 log CFU/g at a starter powder concentration of 7 %. This implied that a STP from rice cake starter (Loog pang Koawmak) could meet the requirements for soy yogurt. As quoted from previous reported studies, the decrease in pH, along with the increase in the number of viability bacteria and total acidity, indicated that *Lactobacillus plantarum* strains have possible to improve the physicochemical properties of soy yogurt [41]. The number of viable cell increased with the increase in STP and CCP. In contrast, increased sugar concentration when viable cell decreased due to a *L. plantarum* is a strain with low sugar requirements [42], during the fermentation process in soymilk using probiotic strains, the concentration of glucose and fructose decreased obviously [43]. Sugar may restrict bacterial growth due to the inhibition of lactic acid on products or the consumption of amino acids poly and oligosaccharides and other growth factors, which are collectively referred to as nitrogen substrate [44]. In addition, there were research studies shown that after fermented 48 h, the levels of glucose, fructose and other monosaccharides of soymilk yogurt fermented with *Lactobacillus plantarum* KU985432 were all nearly depleted [45]. On the other hand, it was suggested that the initial sugar concentration intake did not limit or inhibit the early growth during the study period (9 - 90 g/L). Hence, medium sugar concentration result in high viable cell count. However, yogurt fortified whey protein powders exhibited higher results in promoting the proliferation of LAB [46]. The microstructure of yogurt depend on the denaturation of β-Lactoglobulin, protein content and total solids [47,48].

Effect of soy yogurt on syneresis analysis

The 2nd-degree polynomial model fitted to syneresis was significantly ($p \leq 0.01$) observed with a high F value of 18.08. The polynomial model for syneresis can be expressed by the following equation:

$$\text{Syneresis} = 14.8300 + 2.3825A - 3.6138B - 7.7488C + 0.4975AB - 0.8725AC - 5.0800BC - 1.5675A^2 + 5.0950B^2 + 3.6750C^2 \quad (5)$$

Syneresis is the coagulum formed during fermentation, characterized by low structural stability, resulting in whey being extracted from the gel as it shrinks [40]. All 3 variables significantly affected syneresis (**Figure 2**). From a consumer perspective, the quality of fermented dairy production is an essential indicator to consider products. Dairy yogurt generally exhibits less syneresis than soymilk yogurt due to the presence of proteins, especially casein, which helps form a stable gel structure during fermentation. However, soymilk lacks casein and has a different protein structure. As a result, additional stabilizers or proteins are often added to soymilk yogurt to improve its texture and reduce syneresis [49]. The difference in pH between the coagulum and the syneresis reduces the quality of the products. Syneresis varied across yogurt samples within the range of 5.42 - 31.36 % as syneresis decreased with coconut protein and sugar increase. Due to the protein supplementation is main step in the yogurt industry, thus causing the increase of total solids, viscosity, firmness and reducing the capability to syneresis [48]. The implications of reduced syneresis can positively impact shelf life by improving physical stability, preserving the product's smooth texture, appearance, and uniformity over time, inhibiting microbial growth to enhance food safety and longevity, and increasing consumer appeal [49]. As a result, the addition of a small amount of CCP was found to lead to syneresis. In comparison to other experiments, CCP has been reported to posses higher emulsifying properties compared to other plant proteins [47]. Zhang *et al.* [51] indicated that glucose chains leached into the aqueous phase, leading to increased water absorption and viscosity. When the sugar content was increased, turbidity and syneresis decreased. On the other hand, syneresis increased when the STP increased. During fermentation, the STP produced lactic acid, causing the coagulum began and caused to form and the pH to decrease. It has been reported that during protein fermentation, the decrease in pH causes an increase in ionic strength, resulting in the stability of 11 S globulin [40]. During proteolytic fermentation, CCP is a plant protein that appears as a large globular protein folded into a tight and ordered structure. It contains a significant amount of ß-sheet content stabilized by disulfide connections, resulting in hydrophobicity, electrostatic forces, hydrogen bonds, and van der Waals forces. As a result, it is less susceptible to proteases, making the digestion of protein molecules challenging. Unlike milk proteins, which have a flexible micellar structure comparable to casein, the protein is readily available. As a result, plant-based dairy products tend to induce protein structures to congregate and degrade [38]. Syneresis occurs due to the resulting coagulation to the water holding capacity of CCP reduced after fermentation.

Optimization of level of independent variables

BBD of RSM was applied to determine the optimal combination of STP, SUG, and CCP in the soymilk yogurt formula. According to model fitting results, the 2nd-order polynomial regression model for pH, TA, LAB, syneresis were found to be insignificant due to the lack of fit ($p > 0.05$) in each of the models (**Table 3**). As a result, the effects of the 2nd order polynomial model on the soy yogurt were dependent on the specific response. Yogurt was prepared using the optimum conditions of independent variables as a confirmatory test to evaluate the adequacy model of the RSM model (**Table 4**). In numerical optimization, the desired goal was to maximize TA, LAB while minimizing syneresis. The objective were combined into an overall desirability function value of 0.863 which was obtained from the optimization procedure. The optimum values for yogurt with the highest desirability function was determined at 9 % STP, 7 % SUG and 5 % CCP. It has been confirmed that the results were similar to the predicted values of the optimum mixing ratio. Therefore, soy yogurt utilizing a single probiotic starter *Lactobacillus plantarum* TISTR 2084 was optimized using RSM. The achieved model could be used to determine the optimum condition whereby the procedure can be scale up production of probiotic soy yogurt fortified with CCP.

Table 4 Response of the optimum condition soymilk yogurt fortified coconut protein.

Response	Optimum conditions			Maximum value	
	Starter (%)	Sugar (%)	Coconut (%)	Predicted value	Observed value
pH	4.59	1.71	5.27	5.08	5.02 ± 0.04
TA (%)	7.59	6.65	3.05	0.80	0.73 ± 0.02
LAB (log CFU/g)	4.22	6.67	5.35	9.73	9.68 ± 0.34
Syneresis (%)	7.81	7.70	9.07	4.99	8.55 ± 0.63

Note: Value are mean of 3 determination (n = 3).

Conclusions

The current study documented the fermentation of soy milk containing STP, CCP and SUG by utilizing the usefulness gel network structure. The bio-physical-chemical properties of samples with different ingredients mixing ratios were evaluated to measure the BBD for optimizing the mixing ratio of the 3 independent factors affecting the quality parameters. The characteristics of the results were reduced syneresis, high cell viability, and increased total acidity. It shows that the results were as expected. The appropriate mixing ratio with 86.3 % desirability which was definite as 9 % w/v *Lactobacillus plantarum* TISTR 2084 starter powder, 7 % w/v sugar and 5 % w/v protein. The findings of this study could be used as a reference for the application of STP in the plant-based yogurt, which has the potential to pave the new way for commercialization use of plant-based yogurt products with plant-based proteins and single culture (STP) in the future.

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