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FULL PAPER

Formulation of low-fat cheese analogue from sweet corn extract using papain and lime extract as coagulant

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Abstract

Cheese is not only created using cow's milk and can also be made from a mixture of vegetable extracts, including corn extract. Cheese from corn extract has the advantages of low-fat and high-carotene. Notably, papain can be used as a coagulant in the production of cheese analogue, while maltodextrin functions to increase volume and total solids for greater yield. The objectives of the present study was 1) to optimize the formula composition between lime extract, papain, and maltodextrin to create a cheese analogue from sweet corn extract with high yield and protein as well as good sensory properties, 2) to study the physicochemical and sensory characteristics of the cheese analogue using the optimal formula, and 3) to compare analog cheese from corn milk to cow's milk cheese. The experimental design involved response surface methodology with three factors (lime extract, papain, and maltodextrin). The results of the study produced the optimal cheese analogue formula from corn extract with the addition of lime extract (2.283%), papain (0.022%), and maltodextrin (15%). The characteristics of this cheese analogue include a yield of 20.3%; pH of 5.4; 14°Brix soluble solids; water content of 65.3%; protein content of 13.5%; total-carotene of 544.4 ppm and of fat content 4.6%. The cheese analogue has sensory characteristics of soft texture, the ability to spread evenly, the typical color of cheese (i.e. yellowish-white), and was preferred by panelists. Cheese analogue has protein content of 7.1%, fat content of 4.55%, total carotene of 544.4 mg/g, cholesterol 0.02 mg/g; while commercial cheese from cow's milk has protein content 6.3%, fat content 24.53%, total carotene 5.32 mg/g and cholesterol 0.19 mg/g. Thus, sweet corn can potentially be used as a raw material for producing low-fat cheese analogues.

1. Introduction

Cheese is not only used in bakery products but also used in various other types of culinary items such as for appetizers, entrees, desserts, and various snacks. Grated, sliced, and liquid cheeses have been developed for various purposes in the modern food industry. A trend of increasing cheese popularity has led to an increased demand for cheese. In order to satisfy the increasing demand for cheese, products are developed from ingredients other than cow's milk. This product is called cheese analogue or imitation cheese. Cheese analogue is a type of cheese product, with fat, milk protein, or both being replaced in part or entirely by non-milk components, especially from vegetable ingredients (e.g. apricots, sunflower seeds, or other plants) (Cunha *et al*., 2010; O'Riordan *et al*., 2011; Mohamed and Shalaby, 2016). The fat originating from milk is replaced by vegetable oil or fat in cheese analogue production, resulting in saturated fatty acid levels being very low, thus reducing the risk of cardiovascular disease among consumers (Liu *et al*., 2008; Tuntragul *et al*., 2010). Sweet corn extract can be processed into yogurt (Aini, Prihananto, Wijonarko, Astuti *et al*., 2017); therefore, it is assumed that it can also be processed into cheese analogue. The production of cheese analogue from corn extract is not commonly performed; as such, there is room for development in cheese analogue production using corn extract. According to Aini, Prihananto, Wijonarko, Astuti *et al*. (2017), corn extract has low fat content and high beta carotene. The cheese analogue

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1. Introduction

Cheese is not only used in bakery products but also used in various other types of culinary items such as for appetizers, entrees, desserts, and various snacks. Grated, sliced, and liquid cheeses have been developed for various purposes in the modern food industry. A trend of increasing cheese popularity has led to an increased demand for cheese. In order to satisfy the increasing demand for cheese, products are developed from ingredients other than cow's milk. This product is called cheese analogue or imitation cheese. Cheese analogue is a type of cheese product, with fat, milk protein, or both being replaced in part or entirely by non-milk components, especially from vegetable ingredients (e.g. apricots, sunflower seeds, or other plants) (Cunha *et al*., 2010; O'Riordan *et al*., 2011; Mohamed and Shalaby, 2016). The fat originating from milk is replaced by vegetable oil or fat in cheese analogue production, resulting in saturated fatty acid levels being very low, thus reducing the risk of cardiovascular disease among consumers (Liu *et al*., 2008; Tuntragul *et al*., 2010). Sweet corn extract can be processed into yogurt (Aini, Prihananto, Wijonarko, Astuti *et al*., 2017); therefore, it is assumed that it can also be processed into cheese analogue. The production of cheese analogue from corn extract is not commonly performed; as such, there is room for development in cheese analogue production using corn extract. According to Aini, Prihananto, Wijonarko, Astuti *et al*. (2017), corn extract has low fat content and high beta carotene. The cheese analogue

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from corn extract produced the expected low in fat and have high levels of beta carotene as the benefits of its products.

The technique of making cheese through direct acidification can produce soft white cheese that can be consumed without going through the ripening process (González *et al*., 2018). This technique can produce soft cheese, high meltability, and good stretchability (i.e. forming fiber when stretched, making it suitable for use in making pizza and as cheese spread). However, the exploration of direct acidification techniques remains very limited. In this technique, the acidification stage is typically performed through the addition of organic acids, such as vinegar, lactic acid, or citric acid (Aini, Prihananto, Sustriawan *et al*., 2019). Research on direct acidification techniques remains very limited. High acidity fruit extracts can also coagulate milk in the production of soft cheese (e.g. lime and pineapple) (Sumarmono and Suhartati, 2016; Aini, Sustriawan, Prihananto *et al*., 2019).

The cheese production process typically involves using the enzyme rennet as a coagulant (Hashim *et al*., 2011). Rennet enzymes used as coagulants of cheese are very expensive, and their availability is limited; as such, an alternative to the rennet enzyme is required. According to (Mahajan and Chaudhari, 2014), in order to reduce dependence on rennet enzymes, vegetable extract can be used as a substitute. An ingredient that can be used as a substitute for rennet enzyme is papain (Aini, Prihananto, Sustriawan *et al*., 2019). The use of papain in cheese production is expected to replace the rennet enzyme in order to reduce production cost.

Moreover, the addition of fillers such as maltodextrin can increase the yield of cheese. According to (Moser *et al*., 2017), maltodextrin functions to increase the volume and raise the total solids of the material to increase yield. Thus, the addition of maltodextrin as a filler in cheese analogue can increase its yield (Aini, Prihananto, Sustriawan *et al*., 2019).

The objectives of this study were: 1) to optimize the formula composition between lime extract, papain, and maltodextrin to create a cheese analogue from sweet corn extract with high yield and protein as well as good sensory properties and 2) to study the physicochemical and sensory characteristics of the cheese analogue using the optimal formula.

2. Materials and methods

2.1 Materials

The main ingredients used in this study were sweet corn and lime obtained from the local market in Purwokerto, Indonesia; fresh cow's milk (moisture content of 86%, protein content of 3%, and fat content of 3.2%) from the Experimental Farm, Jenderal Soedirman University, Purwokerto, Indonesia; commercial papain (Paya); and *Lactobacillus casei* (in the form of liquid) from the Indonesian Institute of Sciences, Cibinong, Indonesia. The main tools used were blenders (Miyako), analytical balance (AND), an oven (Memmert), presses, a spectrophotometer (Shimano UV-VIS 1800), a refractometer (Atago), and a pH meter.

2.2 Formulation and characterization of the initial products

This research was performed in three stages, namely: formulation and characterization of the initial products, determine the optimal formula, and optimization stage. We followed the method of Aini, Prihananto, Sustriawan *et al.* (2019) with modification. The first stage aimed to determine the lower and upper limits of the concentration of the independent variables. Determination of the lower limits was based on trial and error, while the upper limits were determined through the organoleptic test. The experimental design of this study involved RSM with three independent variables (the concentrations of lime extract, papain, and maltodextrin). Each factor had a lower limit (1%, 0.018%, and 5%) and an upper limit (3%, 0.022%, and 15%). The determination of variations in formula combinations using the RSM method was assisted by Design Expert 11 software. Based on the results of Design Expert 11 calculations for three independent variables, 20 variations of formula were obtained. In this stage, each response was tested using quadratic model equations. Based on the test results of variance (ANOVA) when the p-value less than 0.05 indicates a real effect (significant), which means that the model represents the independent variable in influencing the response.

2.3 Determine the optimal formula

In the second stage, the optimization process was performed to determine the formula with the optimal response. In this section, it is determined criteria that include variables and each response that influences. The optimized components included the research factors (concentrations of lime extract, papain, and maltodextrin) and variables observed in stage I (moisture content, yield, soluble protein, soluble solids, pH, and fat). At this stage, the expected criteria for each variable are set by determining the level of importance and the target values. Yield, soluble solids and protein content have a higher importance level (5) than pH, water content and fat content (importance level 3).

Target values can be minimized, maximized, or in

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range, depending on the desired value. The components of maltodextrin, papain, and lime extract were optimized with a target range so that the values at the lower and upper limit concentrations have the same opportunity to be selected in determining the optimum formula. Moisture content is optimized in ranged because the moisture content of cheese analogues ranged between 63.5 and 68.3%, which is within the range of moisture content for fresh cheese (55-80%) (Lee *et al.*, 2015). Expected texture in spreadable cheese analogue is semi-solid. Moisture content that is too low can produce a hard texture, while moisture content that is too high cause the texture of the cheese to be runny and difficult to solidify when applied. The yield and protein content were optimized for a maximum target range. The fat content of cheese analogue was optimized for the minimum targets because it was expected to contain less fat. Also, pH was optimized for the target range so that the produced cheese analogue would be less sour, but remain within the isoelectric point range to obtain the maximum yield.

2.4 Optimization stage and verification

From the previous stage, 10 optimum formulas are obtained, but only three optimum formulas that have the highest desirability values are taken for this stage. Optimization was performed in order to achieve the optimal cheese analogue formula (Bezerra *et al*., 2008). At the optimization stage using Response Surface Methodology also obtained predictive value for each variable.

The three optimum formulas were verified in this stage. The verification phase is done by comparing the results of analysis in the laboratory with the predicted value of the response generated from the response surface methodology method. The three optimum formulas were also analyzed for sensory properties using ranking test. The tests were performed by twenty trained panelists using a ranking test based on sensory properties. Panelists are asked to rank products based on sensory qualities (color, aroma, taste, ease smeared and preferences). Products with the best sensory quality are ranked first. Rank test results were analyzed using ANOVA and when significant difference followed by the least significant difference test level 5%.

Three formulas obtained were then analyzed for effectiveness index to get the best product. Determining the best optimum formula is based on the chemical and sensory performed using the index effectiveness (DeGarmo *et al*., 2003). The best products are then analyzed levels of protein, fat content, total carotene, and cholesterol. The same analysis was also conducted on spreadable cheese from cow's milk. The two products were compared using LSD test.

2.5 Cheese analogue production

Cheese analogue was prepared based on previous research by Aini *et al.* (Aini, Prihananto, Sustriawan *et al.*, 2019) with slight modifications. Corn extract is made from corn and water in a ratio of 1: 2 which is mashed using a blender for 3 minutes, then filtered. Corn extract then mixed with cow's milk (ratio 1: 1) pasteurized at 72°C for 30 minutes, after which it was cooled to 30°C. The next step is the addition of 1 mL starter (in liquid form), 2 grams of $CaCl_2$ and 30 grams of sugar per 500 mL of corn extract and cow's milk. Subsequently, the mixture was incubated at 30°C for 2 hours. The next step is the addition of lime extract, papain and maltodextrin and stir until homogeneous, then allowed to stand 30 minutes. The mixture of materials that have undergone coagulation is inserted into the filter cloth and pressed. Pressing is done at 30°C until all whey and curd are separated. The next step is the addition of salt (1%) and sugar (6%) and then do the blanching at 40°C for 15 s. Cheese analogue from corn extract is ready to be analyzed.

2.6 Analysis of cheese analogue

The cheese analogue was analyzed for yield (Aini, Sustriawan, Prihanato *et al.*, 2019), pH using pH meter (Oboh and Imafidon, 2018), soluble solids (Dhanraj *et al.*, 2017), moisture content (AOAC, 2005), protein content (AOAC, 2005), and fat content (AOAC, 2005). The three best products were analyzed for sensory properties using the ranking method. The best products are then analyzed levels of protein, fat content, total carotene using AOAC 970.64 (Hoffmann *et al*., 2017) and cholesterol

The yield is the ratio between the cheese analogues and the ingredients. The determination of actual yield requires measurement of the weight of production inputs and outputs (Badem and Ucar, 2016). The actual yield may then be calculated using the following equation:

$$\text{Yield} = \frac{\text{the weight of cheese}}{\text{the weight of ingredients}} \times 100\%$$

Moisture content was analyzed using the AOAC method (AOAC, 2005) as follows: 1-2 g of sample is weighed and placed in a bowl that has been dried. Then, the sample and bowl were dried in a 105°C temperature oven until a constant weight was obtained. Moisture content is the difference between the weight of the starting material and the weight of the final material after drying.

The pH was assessed out using pH meter (Oboh and Imafidon, 2018). The pH meter was standardized using a

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buffer solution with a pH of 4.0 and 7.0. pH readings were taken after 10 mins stabilization. Then, a 10% w/v suspension of the sample was prepared using distilled water. The mixture was mixed vigorously by manual shaking, and pH was measured using a functional pH meter (Extech instruments, model DO700, China).

Determination of soluble solids is carried out using a refractometer (Cunha *et al.,* 2010). The measurement method is as follows: the sample is stirred until homogeneous, then filtered through filter paper. The filtrate is collected, then taken and dropped on the refractometer prism. Read scale on the tool, and then converted to the value of the refractive index of soluble solids.

Soluble protein was analyzed using the method of Lowry-Folin as follows: 0.1-1 g of sample was dissolved in 100 mL of distilled water. Then, 1 mL of the sample was taken and placed in a test tube. Then, 5.5 mL of copper sulphate was added and left for 10-15 mins at room temperature. Thereafter, 0.5 mL of Folin Ciocalteau was added to each of the test tubes, which were immediately beaten quickly and evenly. The solution was left for 30 mins until a blue color was formed. The intensity of blue was measured by its absorbance using a spectrophotometer at a wavelength of 600 nm. The calculation of soluble protein levels was determined using the standard bovine serum albumin curve.

Fat content measurements were performed using a soxhlet extractor as follows: 2-5 g of finely ground sample wrapped in filter paper was inserted into a soxhlet extraction tube. Then, a fat dish and extraction tube are installed on the distillation apparatus. The soxhlet extractor that was filled with solvents was then drained with cool water and the appliance was turned on. Extraction was performed for 4-5 hrs. Then, the solvent was separated from the fat, while the fat-filled dish was dried in an oven at 100-105°C for 30 mins. The residual weight in the fat dish is expressed as fat weight.

Total carotene was analyzed using AOAC 970.64 (Hoffmann *et al*., 2017). Samples of 2.5 g and 15 mL of a mixture of solution (hexane: acetone: ethanol: toluene in a ratio of 10: 7: 6: 7) were mixed using vortex for 1 min. After that, add 1 mL of potassium hydroxide in 10% methanol, and stir for 1 min. The next stage is heating the water bath at 56°C for 20 min. After that it is allowed to stand at room temperature for 1 hr, then add 15 mL of hexane and 29 mL of 10% sodium sulfate solution. After 1 hr, absorbance was taken at 450 nm. Standard curves are made using beta carotene (Sigma-Aldrich) dissolved in hexane.

Cholesterol was analyzed using Beyer and Jensen method (Osman and Chin, 2006). The sample was saponified using 2% alcoholic KOH for an hour. After that, the unsaponified fraction was extracted with 10 mL hexane twice. The extracts were collected and washed with 5 mL distilled water. The next, extract was heated in water bath at 45°C until dry. The dried extracts diluted in 3 mL glacial acetic acid and added with 2 mL of $FeCl_3$. The suspension is read on a spectrophotometer at 565 nm. The standard solution is prepared by making a solution of cholesterol at concentrations of 0, 0:03, 0:15, 0:30 and 0.60 mg/mL cholesterol than standard cholesterol (Sigma Chemical).

3. Results and discussion

3.1 Characterization of the initial product

Cheese analogue yields ranged from 17.1 to 22.9%. This yield is greater than the yield of cheese from cow's milk (Sumarmono and Suhartati, 2016), which is 7.75 – 10.29%. Based on quadratic models, the yield was a significant effect ($p<0.05$). Notably, increasing papain and maltodextrin concentrations increased the yield of cheese analogues (Figure 1). Papain is a protease enzyme that can break down casein in milk. According to Fernández-Lucas *et al*. (2017), papain interferes with kappa-casein to form para-kappa-casein. Papain cuts the peptide bond between phenyl and methionine in the kappa-casein, damages its structure, and produces para-kappa-casein—which has a hydrophobic portion. Then, when pH approaches the isoelectric point, casein joins and agglomerates. This casein can be joined due to the interaction of the hydrophobic portions of the kinetic kappa. The presence of calcium in milk aids the coagulation process as a bridge between micelles.

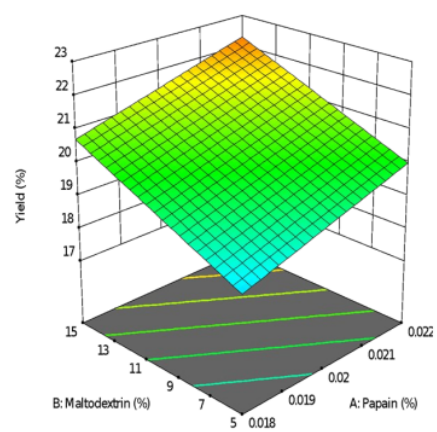

Figure 1. Cheese analogue yield was influenced by papain and maltodextrin concentration

The addition of maltodextrin increases cheese analogue yield because, according to (Jittanit *et al.*, 2010), maltodextrin serves as an enhancer and filler in

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order to increase yield. This result is similar to that of other studies (Dhanraj *et al*., 2017) highlighting that maltodextrin addition increases the yield of mozzarella cheese analogue. Cheese analogue has previously been produced from corn extract, and the addition of will increased its yield (Aini, Prihananto, Sustriawan *et al*., 2019).

According to Hjalmarsson (2015), the most important factor affecting yield is the composition of milk—particularly the concentrations of fat and caseins. Casein forms the continuous paracasein network and occludes the fat and moisture phases. Moisture contributes to cheese yield directly by weight and indirectly by containing soluble solids. The contribution from fat results in moisture content increasing with increased fat content. On its own, fat has low water-holding capacity, though it impedes syneresis when occluded in the casein network. Fat globules physically limit the aggregation of paracasein, resulting in less matrix contraction and reduced moisture expulsion (Al-Baarri *et al*., 2018).

According to Ogunlade *et al.* (2019), pH also has an influence on yield. This can be explained through the effect that pH has on the casein matrix. At pH values above 5.1, the casein matrix is in intact form, whereas at pH below 4.8, casein will be hydrolyzed. A disintegrated, looser matrix can influence the yield and the texture of cheese analogue because of high water levels can maintain texture. This cheese analogue has a pH of 4.8 to 6.0; as such, pH does not significantly affect yield in this case.

Increasing the concentration of lime extract decreases the pH of cheese analogue; however, the addition of papain and maltodextrin does not affect the pH value. The lowest pH value observed in this study for cheese analogue was 4.8, while the highest value was 6.3 (Figure 2).

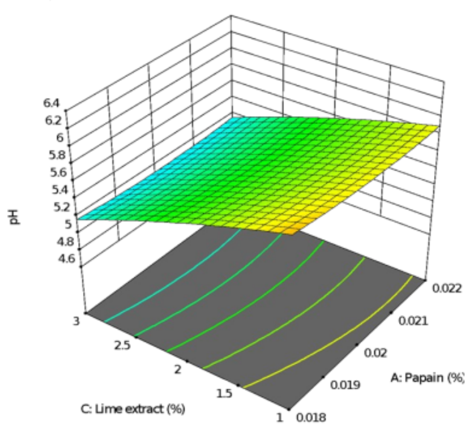

Figure 2. The pH of cheese analogue was influenced by the concentration of papain and lime extract

The effect of the lime extract on pH was lime contains citric and ascorbic acid. The citric acid content in lime has an acidic pH range of 2.48-2.5 (Chaudhari and Pathan, 2015). According to Grassi *et al.* (2014), if milk—which has a pH range of 6.5-6.8—and the lime extract is added in increasing concentrations, it will reduce the pH of the milk to near the isoelectric point. Decreasing the pH of milk causes the release of calcium ions from calcium caseinate due to the availability of increasing H ions, which can break down calcium phosphate compounds. The rupture of calcium phosphate compounds causes the stability of casein to falter so that coagulation occurs.

Maltodextrin has no effect on pH. According to Farimin and Nordin (2009), maltodextrin originates from oligosaccharide compounds that contain a large hydroxyl (OH) group capable of neutralizing acidic properties. If the pH value is neutral, the process of milk casein agglomeration will be inhibited because protein agglomeration can only occur under acidic conditions. However, in this study, pH values tended to be acidic; therefore, the casein protein could still agglomerate.

Soluble solids consist of sugars, pigments, organic acids, and proteins. The soluble solid range of cheese analogue was 11-18°Brix. The addition of maltodextrin was in line with an increase in soluble solids (Figure 3). Maltodextrin includes fillers that have a high total soluble solid value. According to Jittanit *et al*. (2010), the properties possessed by maltodextrin are rapidly dispersed and have high solubility that causes high levels of soluble solids. Therefore, an increase in the concentration of maltodextrin will increase soluble solids of cheese. This is congruent with results from Farimin and Nordin (2009) in the drying of purple sweet potato flour, which exhibited an increase in soluble solids due to maltodextrin being able to disperse in water.

Increased papain concentration increases moisture content, as shown in Figure 4. The moisture content of cheese analogue ranged from 63.5 to 68.3%. According to Codex 275-2011, cream cheese has a high moisture content (over 67%). Papain cuts the peptide bonds between phenyl and methionine in the kappa-casein, thereby damaging the structure and producing para-kappa casein, which has a hydrophobic portion (Farimin and Nordin, 2009). This hydrophobic portion is insoluble in water; therefore, as the concentration of papain increases, water content should be lower due to the peptide bonds being increasingly cut. However, the work of the papain enzyme also depends on the substrate (casein). Therefore, if casein content is low, a small amount of cut peptide bonds causes the water content to be high. Water content can also be influenced by other

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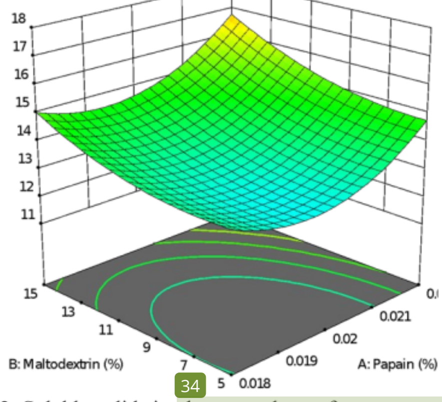

Figure 3. Soluble solids in cheese analogue from corn extract was influenced by the papain and maltodextrin concentration

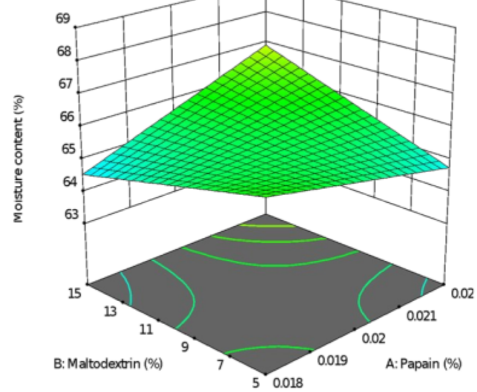

Figure 4. The moisture content of cheese analogue was influenced by papain and maltodextrin concentration

factors, namely pasteurization. According to Rizzolo and Cortellino (2017), the pasteurization process results in a decrease in moisture content during the process, as the water content in milk will evaporate.

Cheese analogues exhibited protein content ranging from 7.05 to 18.18%. Notably, increasing the concentration of lime extract increased the soluble protein content (Figure 5). Adding lime extract to protein caused cheese casein to agglomerate. When lime is added, the pH of the milk is reduced, and the casein coagulation process occurs. According to Shah *et al.* (2010), casein is easy to settle at the isoelectric point (pH 4.6-5.0). The addition of acid results in the addition of H^+ ions, which will neutralize proteins and achieve isoelectric pH, thus making the proteins hydrophobic (Ah *et al.*, 2015). At the isoelectric point, water is separated and protein will bind between its own contents, forming a fold and resulting in coagulation.

Protein solubility will increase if excessive acid treatment is performed, since according to Pang *et al.* (2016)—positive ions in acids that cause proteins (initially neutral or zero) to be positively charged. The further that the acidity of the protein solution is from the isoelectric point, the more soluble it will be. The observed protein level (22.9%) of cheese analogue produced by regulating the acidity of lime juice was lower than that of cheese produced from cow's milk (Razig and Babiker, 2009). This is because cow milk has a higher protein content of 16.5% (Colmenero and Broderick, 2006) compared to sweet corn (5.9%) (Almodares *et al.*, 2009). The greater the addition of maltodextrin, the more soluble protein will present. This is due to maltodextrin having good consistency and stability, as it can bind water-soluble proteins even in small amounts (Akhtar and Dickinson, 2007).

The lipid content of cheese analogue was 2.34 to 5.95%. Figure 6 shows that increasing the extract of lime will increase the fat content of cheese analogue. According to Seth and Bajwa (2015), the addition of acid during cheese production will increase fat-binding capacity. This is because the addition of acid can lead to the coagulation of the proteins that form fat globules. The protein coagulation process occurs in the presence of acids, resulting in an increased fat-binding capacity as

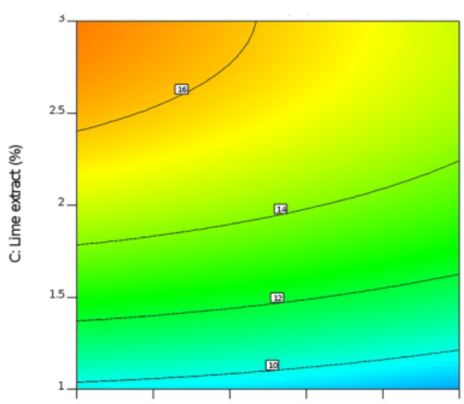

Figure 5. The protein content of cheese analogue was influenced by lime extract and maltodextrin concentration

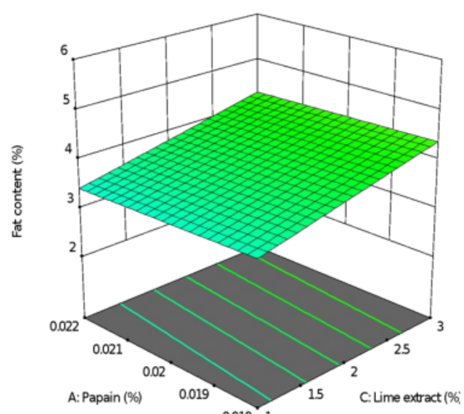

Figure 6. The fat content of cheese analogue was influenced by concentrations of lime extract and papain

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more acid is added.

Furthermore, fat content tends to increase with the addition of papain (Figure 6). According to Fernández-Lucas *et al.* (2017), papain exhibits proteolytic activity during the milk coagulation process, which causes the phospholipid-protein layer to be damaged. As a result, fat globules will be trapped at the point of protein clumping and eventually unite with the curd.

According to Thorning *et al.* (2016), the fat content found in cheese is heavily dependent on the type of milk and other ingredients used as raw material for cheese production. The low-fat content of cheese analogue in this study is due to the basic ingredients of cheese that do use cow's milk but rather corn extract. According to Aini, Prihananto, Wijonarko, Astuti *et al.* (2017), sweet corn has a fat content of 1%, while milk fat content is 3.4%. This causes the fat content of corn-based cheese analogue to be relatively low. According to Mohamed *et al.* (2017), substituting milk fat with vegetable fat changes the nutritional profile of cheese analogue, making it lower in cholesterol and the composition of saturated and unsaturated fatty acids.

The cheese analogue in this study included low-fat cheese, which has lower fat content compared to full-fat cheese (Wang *et al.*, 2016). Based on wet weight, full-fat cheese in fresh form has a fat content of 24.5%. Based on the fat content of cheese analogue, it can be concluded that fat content is generally in accordance with the USDA (2001) suggestion (below 5%).

This cheese analogue has 472-1146 ppm of beta-carotene. Moreover, levels of maltodextrin, lime, and papain do not affect the levels of beta-carotene in cheese analogue. This level of beta-carotene is comparable to Kareish cheese, which is fortified with 1 to 1.5% of *Spirulina platensis*, which has beta-carotene levels of 600-1020 ppm (Mohamed and Darwish, 2017).

3.2 Formula optimization and verification of the optimum formula

Based on the optimization phase, three formulae were obtained that had the highest desirability value and according to the objectives of this study. Cheese analogue formula I was made with the addition of 15% maltodextrin, 2.283% lime extract, and 0.022% papain. Formula II was produced from sweet corn with the addition of 15% maltodextrin, 2.178% lime extract, and 0.022% papain. Formula III made with the addition of 15% maltodextrin, 2.39% lime extract, and 0.022% papain.

The testing accuracy results for these three formulae are presented in Table 1. Based on the verification results of Formula I, II and III, all responses were deemed appropriate and within the prediction interval range. The results of sensory testing are presented in Table 2. The cheese with a minimal yellow color was the best choice among panelists. Panelists chose formula II as the best color, since it has a yellowish-white color. According to Ben-Amira *et al.* (2017), cheese made from cow's milk without coloring will produce cheese that is yellowish-white. Yellow comes from fat-soluble carotene pigments. However, the fat content of cheese analogue is low, thus the fat-soluble carotene pigments levels are also low. Therefore, white is the more dominant color among the cheese analogues.

Products with the best aroma are those possessing the maximum or strongest distinctive cheese aroma. Based on the analysis of various rankings, results from tests of aroma were not significantly different among the three optimum formulae. The distinctive aroma of cheese originates from the fatty acids found in milk (Villalobos-Chaparro *et al.*, 2018). According to Everard *et al.* (2007), a change in dairy products such as cheese is caused by the fermentation of lactose, citrate, and other organic compounds into various acids, esters, alcohols, flavor-forming compounds, and volatile aromas. However, the use of lime ingredients can affect the distinctive aroma of spreadable cheese analogue.

Table 1. Verification results for the optimal formula

Response	Formula I			Formula II			Formula III		
	Actual data	Predictive value	Verification	Actual data	Predictive value	Verification	Actual data	Predictive value	Verification
Moisture Content (%)	65.692	66.33	Appropriate	65.329	66.388	Appropriate	62.083	64.001	Appropriate
Yield (%)	21.178	21.757	Appropriate	20.314	21.745	Appropriate	20.456	21.778	Appropriate
Soluble solids (°Brix)	14	16	Appropriate	14	16	Appropriate	14	16	Appropriate
Soluble protein (% db)	11.223	13.439	Appropriate	13.516	13.255	Appropriate	14.078	13.593	Appropriate
pH	5.4	5.4	Appropriate	5.4	5.5	Appropriate	5.3	5.4	Appropriate
Fat (% db)	4.344	3.718	Appropriate	4.553	3.6527	Appropriate	5.428	3.795	Appropriate

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Table 2. Test rank results for the three best cheese analogue formulae

Formula	Attribute[a]				
	Color	Aroma	Taste	Spreadability	Preference
I	2.75[b]	1.85[a]	2.15[a]	2.2[a]	2.3[a]
II	1[a]	1.75[a]	1.65[a]	1.35[a]	1.2[a]
III	2.25[b]	2.58[a]	2.2[a]	2.45[a]	2.5[b]

Values in the same column followed by different letters indicate a significant difference in the level of 5% LSD test.

According to Chaudhari and Pathan (2015), lime has a strong aroma and distinctive flavor. In optimum formula II, the concentration of lime is the lowest; thus, the panelists assessed the distinctive cheese aroma as stronger compared to the other optimum formulae.

The products with the best taste are those with the least or lowest sour taste. Based on the analyses on cheese analogue taste, the formulae were not significantly different. However, formula II was ranked as having the best taste. The lime extract contains a distinctive and strong sour taste. In formula II, the concentration of lime is lowest; therefore, the typical taste of lime in this cheese analogue is lower. The effect of the lime extract is not only sour taste, but it also causes a slightly bitter taste. According to Ben-Amira *et al.* (2017), the bitter taste in oranges is influenced by the presence of two compounds, namely flavonoids and limonoids. The main component of flavonoids is naringin, which gives the impression of an astringent to bitter taste. Therefore, the panelists tended to rank optimum formula II as having the best taste.

Products with the best spreadability performance are those possessing the best ease of spreading when applied. Based on the analysis of various ranking tests on the applicable performance of cheese, no significant differences were observed. However, the panelists tended to assess optimum formula II as having a softer texture, making it easier to spread than the other two formulae.

Based on the complete ranking test, panelists judged optimum formula II as the preferred spreadable cheese analogue. This preference was based on various sensory attributes, for which most panelists chose optimum formula II as being optimal. Determination of the optimal formula based on chemical and sensory properties was performed using the effectiveness index. Based on this index, the total value of each formula is presented in Table 3. With the highest total value of 1.504, optimum formula II was chosen as the best formula. Formula II has the lowest concentration of lime extract, an extract that results in a distinctive taste that can reduce consumer acceptance of cheese analogue. Therefore, the selection of formula II is appropriate since the taste of lime in regular cheese is low, while other physicochemical and sensory properties are as expected.

Thus, sweet corn extract has the potential to be developed as a raw material for the production of cheese analogue.

Table 3. Total values for optimum formula

Characteristics	Formula I	Formula II	Formula III
Chemical properties	0.63	0.504	0.406
Sensory properties	0.271	1	0.037
Total	0.901	1.504	0.443

The advantage of cheese analogue from sweet corn is lower fat and cholesterol content compared to cow's milk cheese, as shown in Table 4. Another advantage of analog cheese from corn is that it has higher carotene (544.4 mg/g) than commercial cheese (5.32 mg/g). The protein content of products has the same protein content, which is 7.1 and 6.3 %. These products can be designed to fulfill special dietary needs through changes in the formulation (e.g. lactose-free, low calorie, low saturated fat, and low cholesterol) (Chavan and Jana, 2007).

Table 4. Characteristics of selected spreadable cheese analogue and s commercial spreadable cheese (from cow's milk)

	Cheese analogues	Commercial cheese
Protein (%)	7.1	6.3
Fat (%)	4.55[b]	24.53[a]
Total carotene (mg/g)	544.4[a]	5.32[b]
Cholesterol (mg/g)	0.02[b]	0.19[a]

Values in the same column followed by different letters indicate a significant difference in the level of 5% LSD test.

4. Conclusion

The optimal formula for cheese analogue used 2.178% lime extract, 0.022% papain, and 15% maltodextrin, and provided the highest-ranked responses for chemical and sensory properties. This cheese analogue has yields of 21.178%, pH of 5.4, 14% of total soluble solids, moisture content of 65.3%, protein content of 7.1%, total carotene of 544.4 mg/g, fat content 4.55%, and cholesterol 0.02 mg/g. This cheese analogue has minimal yellow sensory properties, a distinctive strong cheese aroma, a minimal sour taste, while it was also the easiest analogue to apply due to its soft texture. Thus, sweet corn has the potential for use as a raw material for producing low-fat cheese.

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Conflict of Interest

The authors declare no conflict of interest.

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