Optimation of microorganism level of semi-solid emergency food based on denaturated whey protein concentrate (WPC) with different mineral concentration and sterilization levels

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Abstract. When post-disaster conditions occur, the problem of food procurement becomes a critical factor which able to disrupt the fulfillment of nutrition for the society, especially children. Procurement of emergency food products is one solution that can be offered to meet daily energy for the victims in need. The raw material for making emergency food products that can be used is denatured Whey Protein Concentrate (WPC), as a source of protein, sweet potato flour as a source of carbohydrates and mineral mix as a determinant of the texture and power of product acceptance. Emergency food products are also incidental, so they require a long shelf life, and sterilization is one way that can be done to extend the shelf life of food products. The factors tested in this study included protein levels, mineral levels, and the level of sterilization with the number of microorganisms as the response variable. The optimal combination of factors tested will produce the minimum number of microorganisms. This study aims to analyze and evaluate the combination of material formulations and sterilization rates using the analytical data from Response Surface Methode (RSM) with a validation of single objective optimization method in order to have a minimum number of microorganisms to meet the nutritional needs that are incidental. Based on the response surface analysis, the optimum formula of semi-emergency food was 11.6% protein content; added 4% mineral mix; and sterilized at 8.09 D log cycle.

1. Introduction
Indonesia is an archipelagic country that located in the Ring of Fire Zone or a meeting zone of tectonic plates and many volcanic mountains series, so that many active tectonic plates are found and often cause one of the adverse natural disasters, namely earthquakes [1]. When post-disaster conditions occur, food problems become a critical factor whose solution must be sought. Difficult access to the location will refer to the obstruction of fulfilling daily food needs that can interfere with the fulfillment of nutrition for the society, especially children, thus causing the nutritional status of the child to decline. To improve those in post-disaster conditions. Assistance via food that is ready to eat that can meet daily nutritional needs so as not to cause further disaster, could be one the solutions to assist those in need.

Semi-solid emergency food is one of the solutions that can be applied in overcoming this problem. Criteria for emergency food products to meet human daily energy when disaster conditions occurs is containing 35–45% fat, 10–15% protein and 40–50% carbohydrates [2]. One of the raw materials that
can be utilized in the manufacture of semi-solid emergency foods high in protein is denatured Whey Protein Concentrate (WPC) with a protein content of more than 25% [3]. As a source of carbohydrates in the manufacture of semi-solid emergency food products, that is sweet potato flour, where carbohydrates themselves are the main source of calories. In addition to fulfilling macro nutritional needs, the fulfillment of micronutrients such as minerals can be done by adding mineral mixes issued by the Indonesia National Agency of Drug and Food Control.

These emergency food products themselves are incidental, so the product must have a long shelf life so that it can always be available when needed. Production efficiency must be designed as well as possible so that emergency food products have a long shelf life. The shelf life of food products is very dependent on the penetration of the thermal process in the material, where the greater the penetration of heat that occurs in the material, the more likely the microorganisms are reduced, so that the shelf life of these food products will be longer. Shelf life also depends on the aw value contained in the material. Heat penetration and aw value will be determined by the composition contained in the food itself. In its application, at the thermal process, we must also paying extra attention to the impact on the characteristics and nutritional value of a product, so that the product produced remains of good quality.

Therefore, it is necessary to know the exact combination of material formulations and the level of sterilization used in making emergency food products so that they can have characteristics and shelf life that are in accordance with the criteria, so that they can meet the nutritional needs that are incidental.

2. Materials and methods

2.1. Material preparation
Denatured Whey Protein Concentrate (WPC) derived from sweet whey which is a by-product of mozzarella cheese making is processed according to the literature [4]. Sweet potato flour from PT. Galih Estetika. Mineral mix which is a product produced by the Indonesia National Agency of Drug and Food Control. Other raw materials include water, flavoring and granulated sugar.

2.2. Sample preparation
Semi-solid emergency food products are made by mixing the aforementioned raw materials, where water used as a solvent has a temperature of ± 80 °C. Food products made contain protein levels of 6.11- 11.6% protein with a mineral content of 2-4%. Semi-solid emergency food products are packaged using metallized packaging. Emergency food product samples were treated with thermal processes using autoclaves (Astel-Sci). The holding temperature of the autoclave is set at 110 °C with a length of time adjusted to the desired log cycle. The log cycle used is 5 D; 8.5 D; and 12 D. The temperature of the sample is recorded using a data logger.

2.3. Experimental design and formulation optimization
Experimental design and formulation optimization using Response Surface Methode (Box-Behnken) with the help of Design Expert 10.0.3 software. This study used 3 treatment factors which included variations in protein levels, mineral levels, and sterilization rates. The response parameters tested are the total microorganisms contained in food products based on each predetermined formulation calculated using the Total Plate Count (TPC) method [5]. This research was conducted 17 runs with center points of 3. Table 1 shows the formulations tested based on the data inputted into the Design Expert (Box-Behnken).
Table 1. Experimental design.

| Run | Protein content (%) | Mineral mix concentration (%) | Thermal processing level (D) |
|-----|---------------------|------------------------------|-----------------------------|
| 1   | 11.6                | 2                            | 8.5                         |
| 2   | 8.86               | 3                            | 8.5                         |
| 3   | 8.86               | 3                            | 8.5                         |
| 4   | 8.86               | 3                            | 8.5                         |
| 5   | 8.86               | 4                            | 12                          |
| 6   | 8.86               | 4                            | 5                           |
| 7   | 11.6               | 3                            | 12                          |
| 8   | 11.6               | 4                            | 8.5                         |
| 9   | 8.86               | 2                            | 12                          |
| 10  | 6.11               | 2                            | 8.5                         |
| 11  | 8.86               | 3                            | 8.5                         |
| 12  | 8.86               | 3                            | 8.5                         |
| 13  | 8.86               | 3                            | 8.5                         |
| 14  | 6.11               | 4                            | 8.5                         |
| 15  | 6.11               | 3                            | 12                          |
| 16  | 8.86               | 3                            | 8.5                         |
| 17  | 11.6               | 3                            | 5                           |

3. Results and discussions

Semi-solid emergency food products developed in this study are made from denatured Whey Protein Concentrate (WPC), sweet potato flour, mineral mix, powdered chocolate flavor, granulated sugar, and water. The protein content produced by denatured Whey Protein Concentrate is 52.28%. The protein content value has met the standards set by U.S. Dairy Export Council, where WPC 50% has a standard protein content ranging from 50–52% [6]. High levels of protein in denatured Whey Protein Concentrate (WPC) are expected to improve the texture characteristics of semi-solid criteria, which are being developed in this study, so as to minimize texture hardening during storage, because of the high protein content contained in WPC, the amount of WPC is compared to the WPC with a small percentage of protein. The more raw material sources of protein added to the product will cause hardening of the product texture due to the interaction of protein-based bonds, which will lead to a more rigid structure that leads to hardening in the texture of a product.

The level of mixed minerals used as formulations in the manufacture of semi-solid emergency food products used is 2%, 3%, and 4%. Addition of mixed mineral content is used as a mineral nutrient source in this semi-solid emergency food product. The addition of the mineral mixture itself must be adjusted, because the addition of uncontrolled mixed minerals will cause a chalky, gritty sensation in the mouthfeel [7]. Semi-solid food products tend to have high aw (water activity) which causes the product to be susceptible to damage due to the growth of microorganisms. Efforts made to minimize damage due to the growth of microorganisms are applied to thermal process treatment. The thermal process needs to be controlled in its application in order to produce a product that has a long shelf life with minimal damage.

3.1. Thermal processing time

The adequacy of the normal thermal process is denoted by $F_0$, which is defined as the time (usually in minutes) needed to kill the target microorganism until it reaches a certain level at a certain temperature. The value of $F_0$ usually states the process time at standard temperature, namely at the sterilization temperature of 121 °C, while the value of F at the modification temperature is expressed by the value of $F_r$. The formula for determining the value of $F_0$ can be seen in the following equation (1).

$$F_0 = S \times D$$  (1)

Where $F_0$ is a thermal process sufficiency, $A$ is a logarithmic cycle, and $D$ is the time needed to kill C. *Botulinum* microorganisms (0.25 minutes) at a temperature of 121 °C.

In this study a modification of sterilization temperature was carried out which was only 110 °C to minimize product damage. The adequacy of the thermal process at the modification temperature was carried out using the trapezoid method, which is a calculation by measuring the area under the curve of
the relationship between the temperature of the thermal process and the process time with the total trapezoidal approach added. The results of trial and error show that at 110 °C sterilization temperature to be able to reach the level of 5 D sterilization; 8.5 D; and 12 D requires consecutive sterilization times of 9, 21 and 30 minutes.

3.2. Optimization of semi-solid emergency food production processes
Optimization of semi-solid emergency food production processes is carried out using ANOVA analysis which is listed on the Design Expert (Box-Behnken). The ANOVA analysis shows the following equation (2).

\[ Y = (243.12068 + 40.45913) A - 43.76786 B - 57.49575 C - (3.75994 \times 10^{-14}) AB - 2.86235 AC + 3.92857 BC - 0.44791 A^2 + 0.37500 B^2 + 2.88776 C^2 \] (2)

Where Y is the total microorganism in the product, A is the protein level, B is the mineral level, and C is the level of sterilization.
Based on the model of equation (2), it can be seen that the treatment factor variations in protein levels, mineral mix levels, and sterilization rates affect the total response variables of microorganisms, both independently and the influence of interactions between factors. Positive constants, such as protein levels will independently increase the total response value of microorganisms. In contrast, negative constants such as mineral levels and the degree of sterilization independently will minimize the total response value of microorganisms.
The influence of protein levels, mineral mix levels, and degree of sterilization on the total response value of microorganisms can also be seen through the three-dimensional curve as follows.
Figure 1. Graph of the Total Response of Microorganisms as Affected by Protein Level Factors, Mineral Mix Level, and level of sterilization (a) 5 D (b) 8.5 D (c) 12 D.

Figure 1 states the relationship between protein levels (A) and mineral levels (B) at three levels of sterilization (C) to the total response value of microorganisms. The color in the graph states the total amount of microorganisms in semi-solid emergency food products. The blue graph area shows the lowest total microorganisms, while the red graph area shows the high total response value of microorganisms. The graph is represented by A as the $x_1$ axis in the range 6.11 to 11.6 and B as the $x_2$
axis in the range 2 to 4. Based on these three graphs, it is known that the higher the protein and the lower minerals and the level of sterilization will increase the total level of microorganisms in the semi-solid emergency food product.

The success of microorganisms in products produced by thermal processes can be carried out by several factors, one of which is due to the number of thermophilic microorganisms contained in the product that produce poor quality raw materials and non-sterile processing. In addition, the presence of microorganisms left in the product after the thermal process can also be caused by the substandard thermal modification process, while the study uses a thermal modification process at the 5 D and 8.5 D sterilization stages.

Another factor besides the level of sterilization that can affect the total microorganism present in the product is the protein content even though the effect is not as large as the level of sterilization. The graph in figure 1 shows that as protein levels increase, the total microorganisms will also increase. Microorganisms, especially bacteria, will require nitrogen compounds in their growth, wherein these nitrogen compounds are obtained in the form of air or in the form of being bound to organic compounds such as amino acids [8]. As it is known that the raw material for semi-solid emergency food products in this study is denatured Whey Protein Concentrate (WPC), where denatured WPC used has a high protein content of 52.28% so that the use of denatured WPC plays a role in increasing the number of microorganisms on semi-solid emergency food studied.

Raw materials other than Whey Protein Concentrate (WPC) which are the treatment factors are mineral mix. The higher the mineral content, the more water can be trapped so that the water content produced in food products will also be smaller. This will cause total microorganisms to be reduced.

3.3. Optimization of processes and optimal condition validation

The criteria for treatment inputted into Design Expert 10.0.3 include maximum protein and mineral levels, as well as minimal levels of sterilization and total microorganisms. Design Expert provides several process optimization solutions with different levels of desirability, where the best process optimization desirability value is close to the value 1. The results of the process optimization proposed by Design Expert showed a protein content of 11.6% with a mineral content of 4% and a sterilization rate of 8.09 D. The desirability value of the model is 0.792. One factor graph for this solution can be seen in figure 2.

![One Factor Graph](image)

Figure 2. One factor graph optimization solution.

The optimal factor provided by the Design Expert software is then tested for validation to determine the accuracy of the predictions provided by the software. The results of the validation of the total
response of microorganisms showed a validity value of 77.21% where the predictive value was 65.632 cfu/mL and the actual value was 80 cfu/mL.

4. Conclusion
The formulation of semi-solid emergency food products in this study shows that the optimal product is produced by formulations with a protein content of 11.6% with a mineral content of 4% and sterilization rate of 8.09 D. The optimal formulation has a data validation rate of 77.21%. It is necessary to review the accuracy of the tools used in research so that the results obtained are more optimal. It should also be noted that the process and level of thermal sterilization are applied to the product so that the desired criteria and level of sterilization can be achieved.

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