
CHAPTER 3 – RESEARCH METHODS

3.1 Venue and Time

The study will be carried out for 6 months from February 2025 to July 2025. The research will be conducted in the Laboratory of Life Sciences and Technology at Swiss German University, Tangerang.

3.2 Materials and Equipment

3.2.1 Materials

The material used in this research was overripe tempe (Rhisoya, Indonesia), commercially available instant noodles (Indomie, Indonesia), instant noodles (Doroku, Indonesia), salt (Dolpin, Indonesia), sugar (Gulaku, Indonesia), garlic powder (Jay's Kitchen, Indonesia), yeast extract (Bouillon, China), isolate soy protein (Rui Qian Jia, China), chicken stock powder (Kuwa, Indonesia), oil (Fosserca, Indonesia), and sweet soy sauce (Indofood, Indonesia).

3.2.2 Equipment

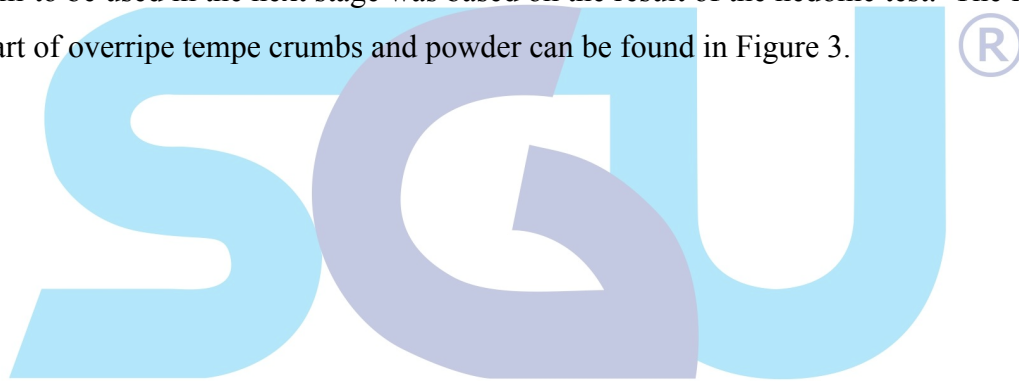
The equipment that was used in this research was an oven (Mettler, Germany), food processor (Panasonic, Indonesia), disc mill (Getra, Indonesia), grinder (Fomac, Indonesia), desicator, crucible, moisture analyzer (Ohaus, USA), and vacuum sealer (Dingye, China).

3.3 Experimental Design

This research was divided into two phases to develop a high-protein seasoning from overripe tempe. The first phase was dedicated to preparing the overripe tempe and determining the most acceptable form of dried overripe tempe: overripe tempe powder and overripe tempe dried crumbs. The selection was evaluated through sensory evaluation. The second phase focused on the formulation, optimization, and

confirmation of the final seasoning, which was based on the overripe tempe form selected in the first phase.

In the first phase, overripe tempe, obtained from PT. Pangan Bijak Indonesia was processed into two different forms. The preparation involved a two-stage drying process. The process began by grinding the overripe tempe into smaller pieces, which were then put through the first drying stage in an oven at 80°C for 4 hours. After cooling to room temperature, the overripe tempe was further ground using a food processor, followed by the secondary drying stage at 100°C for 30 minutes. This process resulted in dried overripe tempe crumbs. The dried crumbs were then milled using a disc mill to produce a fine powder. The selection of the dried overripe tempe form to be used in the next stage was based on the result of the hedonic test. The flow chart of overripe tempe crumbs and powder can be found in Figure 3.



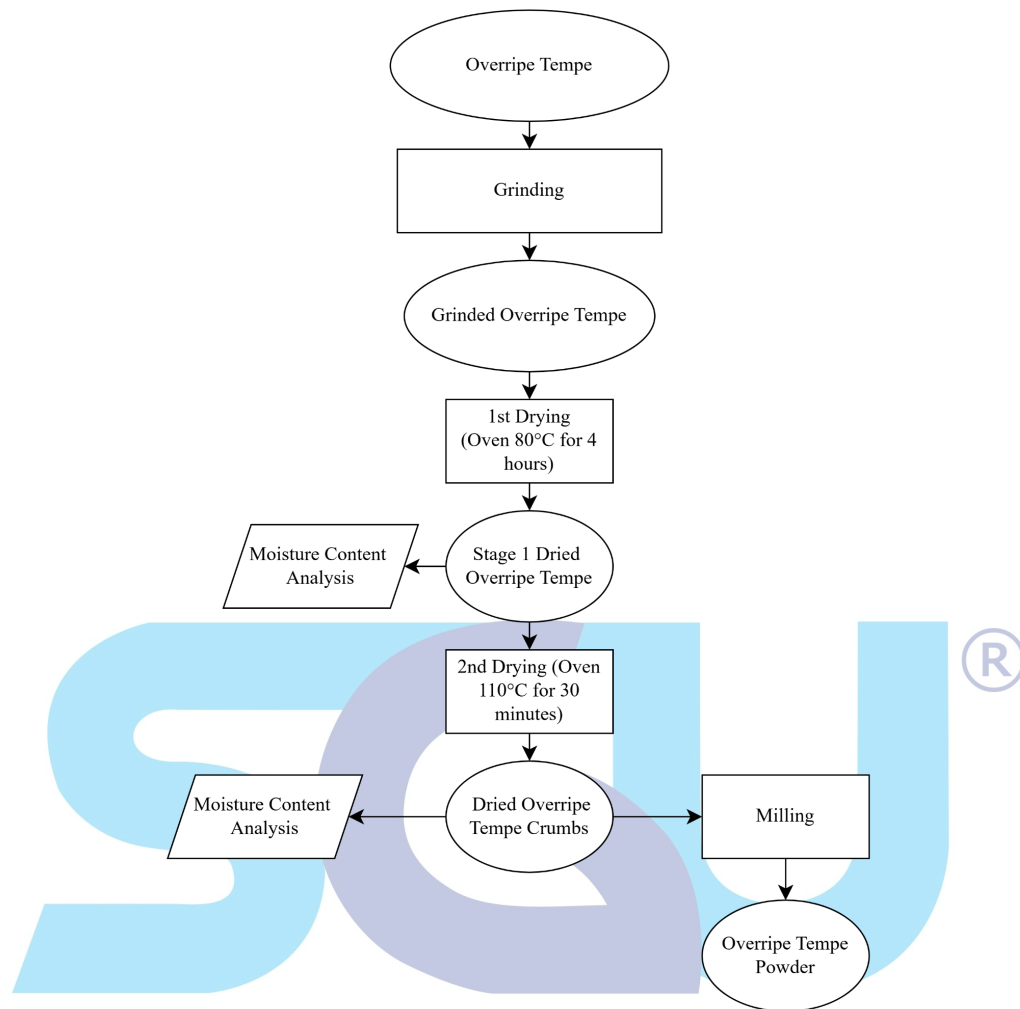


Figure 3. Overripe Tempe Crumbs and Powder Production

In the second phase, the selected form of dried overripe tempe was utilized as the key ingredient for the seasoning formulation. The formulation process was done using Design-Expert 13 ® software, which generated a series of formulas with different ratios of ingredients. Each of the formulas generated was evaluated using a hedonic test to obtain response data from panelists. The data was then used in Design-Expert 13 ® software to achieve an optimized formula. The optimized formula was then subjected to confirmation testing, which included another hedonic test to make sure the formula met the standard from Design-Expert 13 ® software. The first and second phases of the experimental design can be seen in Figure 4 and Figure 5.

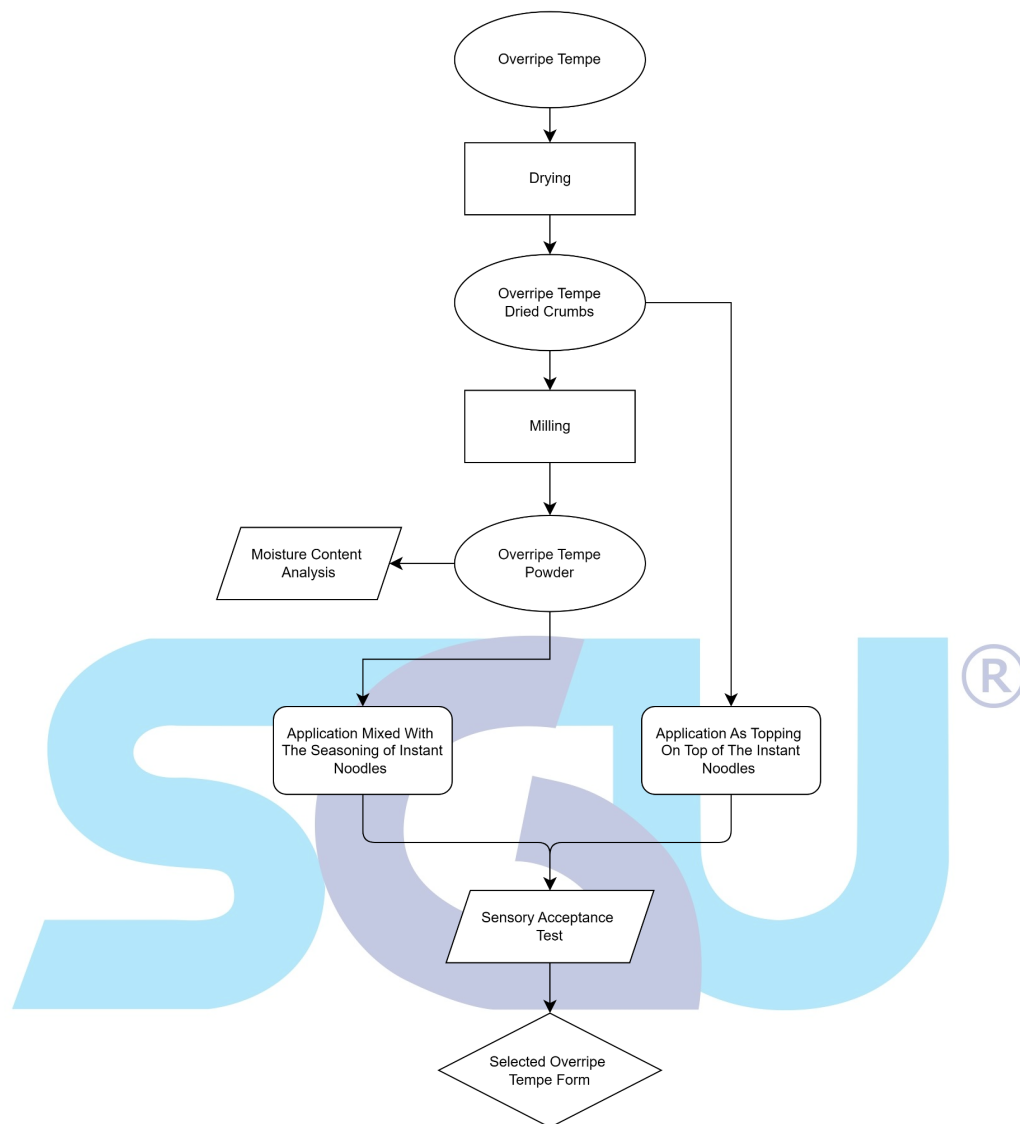


Figure 4. Experimental Design Phase 1

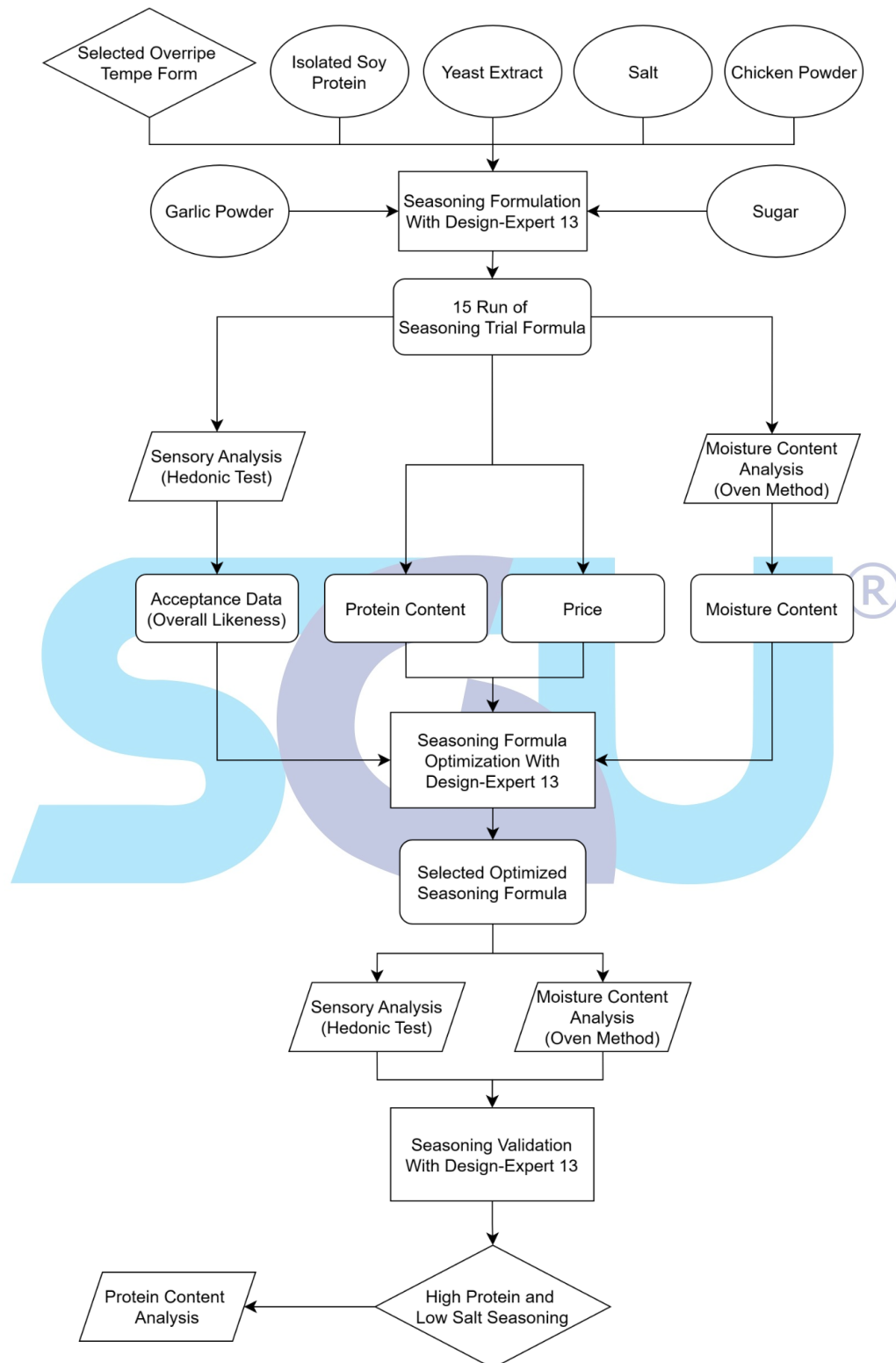


Figure 5. Experimental Design Phase 2

3.4 Experimental Procedure

3.4.2 Overripe Tempe Form Selection

The selection of the acceptable form of overripe tempe was done by a sensory acceptance test of instant noodles using the hedonic test. It was conducted using a commercially available instant noodle product with a noodle weight of 129 grams as the testing medium. Three samples were prepared, with all of the noodles being cooked in boiling water for 5 minutes. The first sample was a control and consisted of the original instant noodle that had no modification. The second sample featured the overripe tempe powder, which was mixed with the seasoning of instant noodle seasoning before being combined with the cooked instant noodle. The third sample was overripe tempe crumbs that served as a topping on top of the instant noodle. The amount of overripe tempe powder and crumbs utilized was determined based on the findings from Gunawan-Puteri research on the “Potential Application of Overripe Tempe Dried Powder as Plant Based Instant Stock”, where the selected proportion of overripe tempe utilized was 26.5% of the total seasoning. The panelist was then presented with two identical sets of the three samples to ensure a non-biased evaluation. The data obtained was then further analyzed using Microsoft Excel-Based SGU Internally Develop Programs (Courtesy of Abdullah Muzi Marpaung) to see whether or not there's a difference on tested samples.

3.4.3 Seasoning Formulation with Design-Expert 13 ®

To establish a baseline for seasoning formulation, two reference formulas were analyzed for preliminary trials. The ingredients used in the reference formulas were: salt, sugar, monosodium glutamate, chicken powder, and garlic powder.

In this formulation, yeast extract was utilized as a natural flavor enhancer, serving as a substitute for monosodium glutamate (MSG). Isolate Soy Protein was also added to enhance the protein content of the seasoning. The seasoning ingredient used for formulation consisted of salt, sugar, yeast extract, isolate soy protein, overripe tempe, chicken powder, and garlic powder.

Formulation was conducted using Design-Expert 13 ® software, which was utilized to generate a series of trial seasoning formulations based on the criteria that had been determined. The ranges for each ingredient are presented in Table 3 below.

Table 3. Ingredient Range in Design-Expert 13 ®

Ingredient	Units	Minimum	Maximum
Sugar	gram	1.49	2.79
Yeast Extract	gram	0.53	1.37
OTP	gram	0.14	9.98
ISP	gram	0	7.70
Total			12 gram

The total target weight for the seasoning was set at 12 grams, with the weight of the instant noodles set at 80 grams per serving, as this amount aligned with the standard portion size of regular instant noodles in Indonesia, which ranged between 80 to 90 grams. In the formulation, chicken powder and garlic powder were maintained at 0.55 grams and 0.34 grams, respectively. Salt was also used in a fixed gramation at 0.266 grams per serving, considering the recommended daily salt intake guidelines for 1 serving. This decision was made to make the seasoning lower in salt compared to the commercial instant noodle seasoning, which has a high salt content. The salt content of commercial instant noodle seasoning can be seen in Table 4.

Table 4. Salt Content in Commercialized Instant Noodles

Instant Noodles Brand	Salt (g/serving)
Brand A	1.70
Brand B	1.00
Brand C	0.70
Brand D	0.86
Brand E	0.87

All values are per serving

The number of formulas generated was 15 runs, where the series of formulas was then tested with a hedonic test using instant noodles as a medium to get responses in the form of attributes of overall likeness, appearance, texture, and taste. The 15 samples were then divided into 3 sets randomly to avoid bias. Moisture content analysis was also conducted on each formula and used as a response for optimization.

3.4.4 Optimization and Validation of Seasoning Formula

The optimization of the seasoning formula was performed using the feature in Design-Expert 13 ® Software. The response data used to determine the criteria for optimization was a response that has a correlation that affects the optimal seasoning criteria. Based on the collected response data, the potential optimized formulas were generated, each with a corresponding desirability score. The formula that achieved the highest desirability score was selected for validation.

In order to confirm the optimized formula, a validation test was performed using a hedonic test using instant noodles as a medium, where panelists were given five identical samples of the optimized formula without being informed that the samples were the same. Panelists were instructed to evaluate the overall likeness, appearance, texture, and taste. The sensory scores gathered from the validation test were then compared against the values predicted by Design-Expert 13 ® to validate the predictive accuracy of the optimized seasoning formula. Validation of high protein claim was also conducted referring to BPOM regulation No. 1 of 2022 on Control of Claims on Processed Food Labels and Advertisement in order to fulfill the regulatory requirements, namely high protein claim.

3.5 Analytical Method

3.5.1 Sensory Analysis

The sensory analysis used in this study was the hedonic test. The Hedonic test was conducted 3 times during the research. The first one is to assess the selection of overripe tempe form on commercially available instant noodles and an instant noodle seasoning with the addition of overripe tempe. The second one is to obtain response

data for optimization in Design-Expert 13 ® software, and the third one is to validate the optimized formula to confirm the tested formula in Design-Expert 13 ® software.

The sensory parameters to be evaluated included the level of overall liking, appearance, texture, and taste. The sensory test was initiated with a minimum of 30 naive panelists from Swiss German University. The panelists were given a hedonic scale of 1 (dislike very much) to 7 (like very much) on each parameter. The data collected from the sensory tests will be processed and analyzed using Microsoft Excel.

3.5.2 Moisture Content Analysis

Moisture content analysis for the seasoning formula was determined using a standard oven-drying method (SNI 01-2891-1992). All analyses were performed in duplicate. For each sample, 1-2 grams were weighed into a crucible that had been previously dried in an oven at 105°C for 1 hour and cooled. The sample was then dried at 105°C for 3 hours, followed by cooling to room temperature after removal from the oven. The sample was then reweighed, and the moisture content was calculated based on the weight loss. This analysis was applied to two sets of samples: the first analysis was conducted on 15 series of seasoning formula runs generated by Design-Expert 13 ® software, and the second analysis was conducted on the optimized seasoning formula. For the overripe tempe powder, the moisture content was measured using a moisture content analyzer.