The use of soy protein isolate in meatballs and its effect on the quality and shelf life of the product

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Abstract
Beef meatballs are in great demand by the public because of their non-porous texture, juicy, and chewy characteristics, as well as their ability to be stable at cooking temperatures with a long shelf life. The use of Isolated Soy Protein (ISP) shapes the character of meatballs because of its functional properties, including good water holding capacity and emulsion stability formation in a mixture of processed meat products. This research aims to technically determine the multiple emulsion properties of ISP at doses of 0%, 2%, 4%, and 8% and obtain a meatball formulation with a long shelf life that suits the target consumer. The tests include the ISP emulsification, meatball peel formation, product stability, Arrhenius shelf life method, and the sensory evaluation of the hedonic test. Subsequently, data were processed in a completely randomized 1x5 and a 4x3 factorial design using SPSS. The emulsification properties of ISP were determined by producing OE (Oil Emulsion) and PG (Purine Gel). Furthermore, ISP was discovered to change the percentage of meat consumed by approximately 2% as the characteristics of meatball products desired by consumers were achieved. The best formulation of meatballs was achieved with 2% dry ISP in the first mixing and was conducted at a boiling point of 65°C.

1. Introduction

Meatballs are a traditional food favored by the Indonesian people (1) with an increasingly high demand yearly (2). These products are also considered highly nutritious (3) and are produced from beef and other livestock, which are then made into round shapes at the first mixing process (4). The meatball's quality is largely determined by its composition, including meat, preservatives, and flour (filler) (5). Furthermore, this research focuses on the panelist preference parameter, which affects the product's success in the market. Sensory attributes are a collection of data that describes the character of the food product. Consequently, the meatballs attributes enjoyed by Indonesian consumers include a non-porous, juicy, and chewy texture, along with stability at cooking temperatures, and a long shelf-life. (6). In this research, furthermore, knuckle meat with little fat content was used to obtain a non-porous sensory characteristic (7) due to the tendency of fat to melt at boiling temperature and increase porosity. Meanwhile, the juicy character of the meatball products is brought about by the amount of stored water from inspecting the first boiling process for the right temperature.
Conversely, springiness is obtained from the use of multiple ISP (8). Previous research shows that ISP is generally used in processed meat formulations that require emulsification stability, such as sausages, burgers, and smoked beef. (9)

Also, Sodium Tripolyphosphate (STPP) is used to maintain emulsion stability (10) due to the formation of cross-links with starch molecules (11). Elasticity is a character obtained from a high protein content of approximately 90% (12,13). Meanwhile, tapioca flour increases product elasticity because of its good adhesion induced by higher amylopectin content in comparison to amylose (14). BTM are food additives, which are safe (15) at a maximum use dose of 0.30% meat weight (16). Furthermore, due to the high water holding capacity (WHC), emulsion, and gelation power of ISP, it is used to obtain meatballs that are stable while boiling. Therefore, products with high WHC are stable at cooking temperatures and have a longer shelf life. The existence of a Standard Operating Procedure (SOP) is important in creating quality meatballs (17), where the production flow charts are carefully considered in detail as a reference for product quality standards. Therefore, this research aims to technically determine the multiple emulsification properties of ISP and obtain a meatball formulation with a long product shelf life suitable for the target consumer.

2. Research Methods

2.1. Research time

This research was conducted in 3 months between October and December 2020 at PT XYZ.

2.2. Materials

The materials included 5°C ice water, beef, salt, STPP, beef-flavored broth, white pepper, fresh garlic, and 5 types of ISP with an average protein content of 90% found in COA. Also, the required tools included trained panelists, bowl cutters, thermometer probe, meatball printing machine, texture analyzer machine with a blunt needle, boiling bowl with a thermometer, showcase chiller, incubator, and UF110 Memmert type oven. The IBM SPSS 22 operating system and the tiered shelf life method were used. Subsequently, Arrhenius, Anova, and Duncan Advanced tests were conducted with 3 replications using SPSS twice on a 1x5 RAL and 4x3 Full Factorial RAL.

2.3. Research procedure

This research was divided into 2 stages:

2.3.1. First research.

2.3.1.1. Emulsification test.

i. 5 ISP formulations were made into the Oil Emulsion (OE) at a dosage ratio of ISP:oil:water = 1:5:4, using a bowl cutter of 2500 RPM minimum speed and stored for 1x24 hours in a cool temperature. The parameters of stickiness, aroma, and elasticity were observed by trained panelists. Then, the experimental 1x5 design data with 3 replications was calculated by SPSS using the Anova and Duncan's advanced test (18). Subsequently, 3 emulsified ISP were obtained to continue the meatballs production process.
ii. 5 ISP formulations were made into Purine Gel (PG) at a dosage ratio of ISP: water = 1:4, using a bowl cutter of 2500 RPM minimum speed and stored for 1x24 hours in a cool temperature. The parameters of stickiness, aroma, and elasticity were observed by trained panelists. Then, the experimental 1x5 design data with 3 replications was calculated by SPSS using the Anova and Duncan’s advanced test. Subsequently, 3 emulsified ISP were obtained to continue the meatballs production process.

2.3.1.2. The meatball peel formation test

The control (A1B1) and the other 11 meatballs (A1B2-A3B4) were produced at the first boiling process at 65, 75, and 85°C Flowchart of making control meatballs:

The frozen knuckles were thawed quickly, the meat chopped and then weighed along with other ingredients, such as tapioca flour, salt, STPP, and seasoning, which consisted of fresh garlic, white pepper powder, and beef-flavored broth (19). Subsequently, mix the ingredients in a bowl cutter with the steps in Figure 1:

![Flow Chart of meatballs A1B1 and A2B2-A3B4](image-url)
2.3.1.3. Stability test of the 12 types of meatballs (A1B4-A3B4):

i. Inflate power
   At 120°C for 180 seconds
   \( D_k = (D_{ak} - D_{aw}) \times 100\% \)
   \( D_k \) = Inflate power
   \( d_{ak} \) = final diameter
   \( d_{aw} \) = initial diameter

ii. Wrinkle power
    At temperatures of 5, 15, 25, 35, and 45°C
    \( D_{kr} = (b_{ak} - b_{aw}) \times 100\% \)
    \( D_{kr} \) = wrinkle power
    \( b_{ak} \) = final weight
    \( b_{aw} \) = initial weight

iii. Crack power
     This was performed until the meatballs cracked

iv. Texture Analyzer
   Until it cracks using a texture analyzer machine with a blunt needle tip (Kusnadi et al., 2012).

v. Elasticity
   The texture analyzer engine graphics start and end within seconds.

vi. Water level test
    (SNI 3818:2014)

2.3.2. The Second research

2.3.2.1. The shelf life of the product using the Arrhenius method.

The drained meatballs were placed in vacuum packaging (20) using the plastic-type NYL15/LDPE60 (75 mic) (21). Subsequently, the product was stored at temperatures of 5, 15, 25, 35, and 45°C. The tiered stage method and scoring points on a scale of 1-7 were used by trained panelists to observe the damage (22).

\[ k = k_0 \cdot e^{-\frac{E_a}{RT}} \]

Where:
- \( k \): quality loss constant
- \( k_0 \): constant (does not depend on the temperature)
- \( e \): base logarithm (2.718282)
- \( E_a \): activation energy
- \( T \): absolute temperature (°C + 273)
- \( R \): gas constant, 1,986 cal/mol

The stages of determining shelf life using the ASLT (Accelerated Shelf Life Testing) and Arrhenius method (23):

1. Determine the quality parameters of the test meatballs product and their acceptance status by trained panelists, especially in terms of mucus, and aroma, conduct a storage test at temperatures of 5, 15, 25, 35, 45°C to accelerate product damage and properly observe quality degradation.
2. Determine the initial quality value (Q0) of the measured parameter.
3. Measure the product quality damage at every fixed time interval and set the unit of measurement as days.
4. Conduct a sensory evaluation of the test product during the storage test process to measure its acceptance status by trained panelists and obtain a critical quality value (Qc) for the product.
5. Plot the quality parameter measurement data (y = Qt) against time (x = t) and change the data to the form ln (y = ln [Qt]).
6. Create a linear regression from the results of plotting quality vs time parameter data, where the determination of zero or one order is seen from the value of the coefficient of determination (R2). The closeness of the reaction to the specified order increases with the value of R2:
   a. If R2 is higher in the plot where y = Qt, then the reaction is zero-order; and
   b. If R2 is higher in the plot where y = ln(Qt), then the reaction is first order.
7. From the obtained regression equation, determine the value of [Q]0 or ln([Q]0) and k, which refers to the following zero-order equation:
   \[ Y = a - b \cdot x \]
   \[ [Q]_t = [Q]_0 - k \cdot t \]
8. Change the value of k obtained to ln (k) and modify the temperature treatment data (T) to 1/T (conversion of °C to K)
9. Plot the data ln(k) against the temperature treatment (y = ln(k), x = 1/T) and create a linear regression.
10. Enter the desired temperature data to calculate the value of k after the ln k0 and activation energy (E) is obtained.
    Note: temperature is converted to K in increments of 273 to the value in degrees Celsius.
    Example: 30 °C = 30°C + 273 K = 303 K
11. Enter the formula to find the value of ts.
    \[ ts = (N_0 - N_t)/k \] (for a zero reaction rate)
    \[ ts = \ln(N_0 - N_t)/k \] (for a first-order reaction rate)

2.3.2.2 Sensory evaluation of hedonic test
   The attributes of color, taste, texture, and aroma were assessed by the panelists. Subsequently, the 4x3 experimental design data with 3 replications was calculated by SPSS using Anova and Duncan's further test.

3. Results and discussion

3.1. First Research

3.1.1. Emulsification test
   The protein content of COA cannot be the basis for the emulsification properties of ISP due to the presence of ISP D and E, which are sticky and do not form a chewy texture. Consequently, the emulsifying properties of ISP B, A, and C showed the best ratings.

3.1.2. Meatball Peel Formation test
   The best meatball peels were obtained at 65°C during the first boiling. These products had a juicy and crunchy character in every bite unlike those obtained at 75°C and 85°C. This was made evident by the water content test conducted at 65°C, 75°C, and 85°C,
where the percentages of the water stored were 62.88%, 54.90%, and 51.70%, respectively.

3.1.3. Meatball Peel Formation test

3.1.3.1. Inflate Test and Wrinkle Test

The test results showed that the meatballs without ISP did not inflate and were excessively wrinkled. Meanwhile, the opposite reaction was observed in the products containing this substance, where the best ISP doses in this test were 8%, 4%, and 2%.

<table>
<thead>
<tr>
<th>Code</th>
<th>Inflate power</th>
<th>Wrinkle power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dak</td>
<td>Daw</td>
</tr>
<tr>
<td>A1B1</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>A1B2</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>A1B3</td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>A1B4</td>
<td>2</td>
<td>2.35</td>
</tr>
<tr>
<td>A2B1</td>
<td>2</td>
<td>2.15</td>
</tr>
<tr>
<td>A2B2</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>A2B3</td>
<td>2</td>
<td>2.25</td>
</tr>
<tr>
<td>A2B4</td>
<td>2</td>
<td>2.28</td>
</tr>
<tr>
<td>A3B1</td>
<td>2</td>
<td>2.11</td>
</tr>
<tr>
<td>A3B2</td>
<td>2</td>
<td>2.14</td>
</tr>
<tr>
<td>A3B3</td>
<td>2</td>
<td>2.16</td>
</tr>
<tr>
<td>A3B4</td>
<td>2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

3.1.3.2. Meatball Crack, Texture, and Elasticity Test, and Meatball Moisture Content

The results of the meatball crack and hardness tests using a texture analyzer machine prove the products without ISP crack faster and were very difficult to test due to their hardness. These meatballs had a lower water content value and were proven to save less amount of water (low WHC levels). In addition, these results showed that more water is absorbed as the dose of ISP increased and according to SNI 3818:2014, a good meatball contains a maximum of 70% water content. Furthermore, products with an 8% ISP dose did not meet the required standards as 4% and 2%, which are considered the best in this testing stage. This research was in agreement with a statement by Illanistyas et al., (2021), where the use of ISP was observed to increase WHC levels hence, forming stable emulsification and elastic properties. Also, following the statement by Ilma et al., (2019) (24), ISP assists in producing final products with a springy character.

<table>
<thead>
<tr>
<th>Code</th>
<th>Crack Test</th>
<th>Texture Hardness</th>
<th>Elasticity</th>
<th>Water content</th>
</tr>
</thead>
<tbody>
<tr>
<td>324</td>
<td>650</td>
<td>7,419</td>
<td>9</td>
<td>51.89%</td>
</tr>
<tr>
<td>345</td>
<td>690</td>
<td>5,722</td>
<td>3</td>
<td>61.00%</td>
</tr>
<tr>
<td>453</td>
<td>700</td>
<td>7,668</td>
<td>8</td>
<td>70.55%</td>
</tr>
<tr>
<td>567</td>
<td>680</td>
<td>6,836</td>
<td>9</td>
<td>73.99%</td>
</tr>
</tbody>
</table>
3.2. Second Research

3.2.1. Shelf Life Test

After assessing the results from trained panelists, the tiered stage shelf-life test data was inputted into the Arrhenius method to obtain the equations shown in Table 3.

<table>
<thead>
<tr>
<th>Code</th>
<th>Arrhenius Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1</td>
<td>( y = -14504x + 47.015 \ R^2 = 0.8926 )</td>
</tr>
<tr>
<td>A1B2</td>
<td>( y = -14121x + 45.75 \ R^2 = 0.8738 )</td>
</tr>
<tr>
<td>A1B3</td>
<td>( y = -13639x + 44.192 \ R^2 = 0.8693 )</td>
</tr>
<tr>
<td>A1B4</td>
<td>( y = -13571x + 43.979 \ R^2 = 0.8745 )</td>
</tr>
<tr>
<td>A2B1</td>
<td>( y = -14504x + 47.015 \ R^2 = 0.8926 )</td>
</tr>
<tr>
<td>A2B2</td>
<td>( y = -12960x + 41.491 \ R^2 = 0.8494 )</td>
</tr>
<tr>
<td>A2B3</td>
<td>( y = -13352x + 42.957 \ R^2 = 0.8167 )</td>
</tr>
<tr>
<td>A2B4</td>
<td>( y = -13571x + 43.979 \ R^2 = 0.8745 )</td>
</tr>
<tr>
<td>A3B1</td>
<td>( y = -14504x + 47.015 \ R^2 = 0.8926 )</td>
</tr>
<tr>
<td>A3B2</td>
<td>( y = -14121x + 45.75 \ R^2 = 0.8738 )</td>
</tr>
<tr>
<td>A3B3</td>
<td>( y = -13639x + 44.192 \ R^2 = 0.8693 )</td>
</tr>
<tr>
<td>A3B4</td>
<td>( y = -14487x + 47.242 \ R^2 = 0.8373 )</td>
</tr>
</tbody>
</table>

The table of average shelf life was presented in Table 4 and Figure 2.

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Time and Temperature</th>
<th>Long Shelf Life</th>
<th>Average Shelf Life / Dayi</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1</td>
<td>Average morning temperature 22°C</td>
<td>4.81 day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average daytime temperature 35°C</td>
<td>0.6 day</td>
<td>3.41 day</td>
</tr>
<tr>
<td></td>
<td>Average night temperature 25°C</td>
<td>4.81 day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average morning temperature 22°C</td>
<td>4.65 day</td>
<td></td>
</tr>
<tr>
<td>A1B2</td>
<td>Average daytime temperature 35°C</td>
<td>0.62 day</td>
<td>3.31 day</td>
</tr>
<tr>
<td></td>
<td>Average night temperature 25°C</td>
<td>4.65 day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average morning temperature 22°C</td>
<td>4.31 day</td>
<td></td>
</tr>
<tr>
<td>A1B3</td>
<td>Average daytime temperature 35°C</td>
<td>0.61 day</td>
<td>3.08 day</td>
</tr>
<tr>
<td></td>
<td>Average night temperature 25°C</td>
<td>4.31 day</td>
<td></td>
</tr>
<tr>
<td>A1B4</td>
<td>Average morning temperature 22°C</td>
<td>4.24 day</td>
<td>3.03 day</td>
</tr>
<tr>
<td></td>
<td>Average daytime temperature 35°C</td>
<td>0.61 day</td>
<td></td>
</tr>
</tbody>
</table>
## Table 1: Temperature Conditions and Shelf Life of A2B2 Meatballs

<table>
<thead>
<tr>
<th>Code of Product</th>
<th>Average Daytime Temperature (°C)</th>
<th>Average Night Temperature (°C)</th>
<th>Average Morning Temperature (°C)</th>
<th>Average Shelf Life (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2B1</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>4.24</td>
</tr>
<tr>
<td>A2B2</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>4.62</td>
</tr>
<tr>
<td>A2B3</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>3.31</td>
</tr>
<tr>
<td>A2B4</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>2.56</td>
</tr>
<tr>
<td>A3B1</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>4.62</td>
</tr>
<tr>
<td>A3B2</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>3.31</td>
</tr>
<tr>
<td>A3B3</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>3.08</td>
</tr>
<tr>
<td>A3B4</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td>2.56</td>
</tr>
</tbody>
</table>

**Figure 2** The longest shelf life of A2B2 meatballs
The results showed A2B2 meatballs had a longer shelf life compared to other products. This is interesting because it agrees with the results, which show the emulsion stability of the products has an inverse relationship with the added ISP concentration (Kharisma et al., 2016). The decrease in stability caused the emulsion in the final product to decompose and expel its water content. In addition, the use of ISP has been shown to increase WHC in processed meat products (25).

3.2.2. Sensory Evaluation Test
The analysis results using SPSS with Anova and Duncan's tests showed A2B2 meatballs had the best aroma, taste, texture, and color as shown in the descriptions of the hedonic taste test data processing:

3.2.2.1. Sensory results taste evaluation
A distinctive and savory meatball taste with the right amount of salt, proper mouthfeel, and without a deviant or bitter aftertaste on the palate was obtained from the twelve trained panelists. Meanwhile, a tastier product was acquired from the use of an ISP, which agrees with Putri's 2018 statement (26).

3.2.2.2. Sensory results texture evaluation
The meatball texture obtained from the twelve trained panelists was unique, chewy, dense, crunchy, and juicy but not mushy or hard (medium texture) because of its water holding capacity. These properties were different from the other eleven products.

3.2.2.3. Sensory results color evaluation
The typical distinct meatball color was obtained from the twelve trained panelists.

3.2.2.4. Sensory results aroma evaluation
The aroma was distinctive with the absence of a distorted scent or smell.

4. Conclusions
The OE and PG methods were used to test the emulsification properties of ISP raw materials, where checking COA were insufficient. Meanwhile, the A2B2 meatball formulation was considered the best with an ISP dose of 2%, which was added dry at the first boiling temperature of 65°C. Therefore, ISP can serve as a substitute for meat because it results in a more stable meatball quality, with a longer shelf life, a juicy, crispy, and tasty product enjoyed by consumers.

References


