Effect of Incubation Temperature and Concentration
Innoculum Rhizopus Oligosporus Quality of Peanut Oncom Bungkil

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Abstract: The objective of this research was to study the effect of incubation temperature and inoculum concentration on "Oncom cake Peanut" characteristics. Benefit of this research was one of alternative beneficial "Peanut cake" to be a kind of food product such as "Oncom" can be preferable by publics. The experimental block design with a 3 x 3 factorial pattern and three times replications and was Followed Duncan's test was used in this research. Factors in this research consists of incubation temperature (A) and concentration of inoculum (B), with levels are a1 (26ºC), a2 (27ºC), a3 (28ºC) and b1 (0.12%), b2 (0.15 %), and b3 (0.18%), respectively. Organoleptic test conducted in this research by using ranking test method with the parameters were color, flavor, texture and taste. Chemical analysis were determined moisture, protein and fat content in "Oncom cake Peanut".

Keywords: Rhizopus oligosporus & Oncom cake.

1. Introduction

Oncom is a food that has a high nutritional value. In addition to high nutrient content, oncom also contain compounds that are beneficial to health, such as fatty acids, and vitamins isoflavin, Atmana [1]. Oncom widely circulated in the community is a food Indonesian specialties, especially in the area of West Java.

Oncom a mold fermentation product made from a mixture of peanut meal, pulp, pulp cassava (tapioca) the rest of the manufacture of tapioca and coconut pulp. Mold used is Rhizopus oligosporus that produces black or mold Neuspora oncom sithopila that produces orange pie, Sri Wulan [9].
Peanut (Arachis hypogaea L. Merr) is a good source of vegetable protein compared to other protein sources. This is supported by the essential amino acid composition sufficient and good digestibility values in groundnut cake which has undergone processing, Sitepu [11].

Oncom manufacture have been carried out by using Rhizopus oligosporus NRRL 2710, since molds of this type is a good mold (Steinkraus, 1983). According Titien Novianti [12], Rhizopus oligosporus NRRL 2710 is used in the manufacture of oncom produce oncom characterized by the formation of white mycelium and binding on the entire surface of groundnut cake.

This mold has the characteristics that is unique is that it can grow rapidly at a temperature of 30C - 37C, and the optimum temperature for 37C. During fermentation the mold will cover groundnut cake completely within 24 hours or more. At low temperatures the mold growth is inhibited, and the temperature is too high mold will die. Rhizopus oligosporus having a high lipolytic activity, and produce a powerful antioxidant, Hesseltine et al. [4]

Furthermore Hesseltine [4], states that Rhizopus oligosporus produce amylase enzyme after 48 hours of fermentation, but the activity of the decomposition of starch is very low compared to Rhizopus oryzae. The result of the breakdown of carbohydrates in peanut meal by enzymes produced by the fungus largely composed of cellulose and oligosaccharides that is simple, too hemicellulose can be dissolved in part so that the texture of the substrate becomes soft, and will facilitate digestibility oncom, Titien Novianti [12].

Based on this research, Sitepu [11], that the incubation temperature of 27C, the inoculum concentration of 0.08%, 0.10% and 0.12%, mold mycelial growth was uneven, off-white color tempe, tempe distinctive aroma, and appearance of tempe less attractive. At an incubation temperature 31C, the inoculum concentrations of 0.08% and 0.10%, mycelial growth was uneven, off-white color tempe, tempe distinctive aroma, and appearance of tempe less attractive. whereas the inoculum concentration of 0.12%, the growth of mycelium evenly, tempeh white color, aroma typical of tempe and tempe interesting sightings. At an incubation temperature 35C, the inoculum concentration of 0.08%, 0.10% and 0.12%, mycelial growth was uneven, off-white color tempe, tempe distinctive aroma, and appearance of tempe less attractive. While the fermentation temperature above the optimum temperature 39C, the inoculum concentration of 0.08%, 0.10% and 0.12%, the mycelium does not grow, because the condition of fermentation temperature is too high, so that the activity of Rhizopus oligosporus decreased.

The purpose of this study was to determine the effect of incubation temperature and optimum inoculum concentration on the characteristics of groundnut cake pie (Arachis hypogaea, L.Merr).

2. RESEARCH METHODS
2.1 The research material
Materials used are peanut meal, yeast tempeh (Rhizopus oligosporus) And tapioca flour, H2SO4 0.1N, concentrated H2SO4, Na2SO4, Se, HgO, Na2S2O3 0.1 N, H2O2 0.1N, 0.1N NaOH, 0.1N HCl, phenothalein, methilen blue, alcohol, and distilled water.

2.2 Research Tools
The tools used are the scales, basin, strainer, stirrer wood, stoves, steamers, printing, oven, analytical balance, an electric stove, a pH meter, Erlenmeyer flask, flask kjeldahl, pipette, measuring cups, beakers, test tubes and incubators.

2.3 Treatment Plan
The design of treatment used is: incubation temperature (A) and the concentration of inoculum (B), each factor consists of 3 levels. Taraf incubation temperature is 26C (a1), 27C (a2), and 28C (a3), while the addition of inoculum concentration level is 0.12% (b1), 0.15% (b2), and 0.18% (b3). To determine the temperature conditions and the optimum inoculum concentration, then tested the resulting organoleptic oncom include aroma, color, texture and flavor.
2.4 Design of Experiments

The experimental design used is the design of 3 x 3 factorial design in a randomized block design (RBD) with three replications, in order to obtain a total of 27 experimental units. Group Random Design (RAK) factorial pattern 3 x 3, Vincent [14].

To prove the difference in treatment effect and its interaction with all response variables were observed, then the data analysis with experimental model as follows:

\[ Y_{ijk} = K_k + A_i + B_j + (A\times B)_{ij} + \varepsilon_{ijk} \]

By using the above notations, then made table Variant Analysis (ANOVA) to obtain conclusions about the effect of the treatment. Furthermore, the hypothesis is determined as follows:

a. Ho accepted if F count larger than F table (at 5%)
b. H1 rejected if F count is smaller than F table (at 5%)

The conclusion of the hypothesis is accepted if there is a real difference of each treatment. The hypothesis is rejected if there is no significant difference or equal to each treatment, Vincent, [14].

2.5 Design Analysis

The design of the analysis carried out when there is a real difference between the average of each treatment (F count > F table) is to conduct further test using Duncan's multiple range test to determine the effect of treatment of the observed response, Vincent [14].

2.6 Draft Response

The draft response made to the groundnut cake pie in this research is the analysis of chemical, microbiological analysis, and product analysis.

2.7 Chemical analysis

a. Determination of water content by distillation method, Sudarmadji, [12].
b. Determination of protein content by the Kjeldahl method, Sudarmadji, [12].
c. Determination of fat content by Soxhlet method, Sudarmadji, [12].

2.8 Microbiological analysis

Total Plate Count analysis done by using PDA (Potatoes Dextrose Agar) and carried out in intervals of 3 hours for 48 hours, Heroine, [8]. Organoleptic test was conducted to determine the level of preference or consumer acceptance of oncom peanut meal, based on the sequence of the best (1) to the worst (9), so it can be known whether the product is liked and accepted by consumers or not. Organoleptic assessment done on that has not been fried pie that include color, aroma, and texture. While the assessment of the pie that has been fried including color, aroma, texture, and flavor.

3. RESULTS AND DISCUSSION

3.1 Research Introduction

Results of the analysis of the raw material is a mixture of groundnut cake and cassava flour is as follows: 34.4767% moisture content, protein content of 14.2160%, and fat content of 4.5796%.

The incubation temperature is done in the preliminary study is 27oC, 34oC and 40oC with inoculum concentration of 0.05%, 0.10% and 0.15%. During fermentation the calculation of growth \( \Sigma \) cells / ml Yeast and organoleptic test oncom peanut meal produced.

This preliminary study was conducted to determine \( \Sigma \) cells / ml Rhizopus oligosporus, which was observed by using a microorganism growth curve, Heroine [8]. The complete results of the observation of cell growth Rhizopuz oligosporus mold, can be seen in Table 1 and Figure 1.
Table 1. Molds Cell Growth Rhizopus oligosporus (cells / ml) Based on Time

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>Cell Count (cells / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>19 X 10^7</td>
</tr>
<tr>
<td>6</td>
<td>40 X 10^7</td>
</tr>
<tr>
<td>9</td>
<td>75 X 10^7</td>
</tr>
<tr>
<td>12</td>
<td>74 X 10^7</td>
</tr>
<tr>
<td>15</td>
<td>74 X 10^7</td>
</tr>
<tr>
<td>18</td>
<td>73 X 10^7</td>
</tr>
<tr>
<td>21</td>
<td>70 X 10^7</td>
</tr>
<tr>
<td>24</td>
<td>68 X 10^7</td>
</tr>
<tr>
<td>27</td>
<td>54 X 10^7</td>
</tr>
<tr>
<td>30</td>
<td>43 X 10^7</td>
</tr>
<tr>
<td>33</td>
<td>31 X 10^7</td>
</tr>
<tr>
<td>36</td>
<td>26 X 10^7</td>
</tr>
<tr>
<td>39</td>
<td>19 X 10^7</td>
</tr>
<tr>
<td>42</td>
<td>9 X 10^7</td>
</tr>
<tr>
<td>45</td>
<td>4 X 10^7</td>
</tr>
<tr>
<td>48</td>
<td>2 X 10^7</td>
</tr>
</tbody>
</table>

Based on the evaluation of the above, it can be concluded that at the 9th cell Rhizopus oligosporus start entering a phase of logarithmic or exponential phase, which in this phase of the cell Rhizopus oligosporus have to adjust to the environment (substrate) given and began an increasing number of cell as shown on a growth curve. At the 9th Rhizopus oligosporus cells have reached the maximum number of cells that is approximately 75 x 10^7 cells / ml, Heroine [8]. In this phase the speed of cell growth is strongly influenced by the medium in which the growth of fungi such as pH, nutrient content, environmental conditions, temperature and humidity. In this phase, the cells require more energy than the other phases, besides the cells are most sensitive to environmental conditions, Heroine, [8].

3.2 Appearance Test Results

The preliminary study in the manufacture of peanut meal pie with organoleptic test using the "Test Ranking". Organoleptic tests carried out in the preliminary study is to determine the level of consumer acceptance of groundnut cake pie are fermented at a temperature of 27ºC, 34ºC, and 40ºC with inoculum concentration of 0.05%, 0.10% and 0.15%. Organoleptic test results are shown in Table 2.
Factors that affect a food ingredient, among others, the texture, color, flavor, and nutritional value. Before the other factors considered visually. Color factor is more influential and sometimes it determines a food is considered tasty, nutritious, and the texture is very good, it will not be eaten if it has a color that is not beautiful to look at, or to give the impression of having deviated from the color it should be, Winarno, [15].

The color values oncom groundnut cake given by the panelists, each providing varying values, namely the treatment a1b3 (27°C incubation temperature, inoculum concentration of 0.15%) at 23, treatment a1b2 (27°C incubation temperature, inoculum concentration of 0.10%) by 40, and the treatment a1b1 (27°C incubation temperature, inoculum concentration of 0.05%) is 47.

The aroma can be defined as one that can be observed with the sense of smell to the data to produce smells, the substance must be evaporated, slightly soluble in water and slightly soluble in fat. Smelling compounds up to a network of smell in the nose together with air. Sensing this way to popularize that smelling compounds are volatile, John M Deman, [7].

The test results organoleptic aromas groundnut cake pie in the treatment a1b3 (27°C incubation temperature, inoculum concentration of 0.15%) of 30, treatment a1b2 (27°C incubation temperature, inoculum concentration of 0.10%) of 33, and the treatment a1b1 (incubation temperature 27°C, inoculum concentration of 0.05%) at 40, this indicates that the sample among the most preferred is the treatment of a1b3.

Texture is the quality factor of the most important food, thereby giving satisfaction to our needs. Therefore we want foods that taste and texture that suits your tastes we expected, so that when we buy food, hence the importance of the nutritional value is usually placed on the quality after the price, texture, and flavor.

Based on the test results of organoleptic texture groundnut cake pie texture values obtained oncom peanut meal, which varies based A panelist. The results obtained are a1b3 treatment (incubation temperature of 27°C, inoculum concentration of 0.15%) at 23, treatment a1b2 (27°C incubation temperature, inoculum concentration of 0.10%) at 39, and the treatment a1b1 (incubation temperature of 27°C, the concentration of inoculum 0 , 05%) of 43, it can be concluded that the most widely preferred treatment is a1b3, due oncom peanut meal is well established, in which the mold pie has grown across the surface of pie with the fermentation process.
4. Sense

The taste is a factor that is important from a food product. Components that can lead to the desired flavor depends on the constituent compounds. Generally food is not only composed of one kind of unified sense of giving rise to taste the food intact. Differences panelist ratings of the flavor can be interpreted as acceptance of flavor or taste that is produced by the combination of materials used.

Based on the results of organoleptic test showed that the value of groundnut cake pie taste the most preferred panelist is oncom peanut meal with a treatment a1b3 (27ºC incubation temperature, inoculum concentration of 0.15%) with a value of 24, compared to the treatment a1b2 (incubation temperature of 27ºC, inoculum concentration of 0.10%) with a value of 32, and the treatment a1b1 (27ºC incubation temperature, inoculum concentration of 0.05%) at 44, this is due to the fermentation and the addition of tapioca enough then the taste of peanut meal oncom be more savory so much preferred by the panelists.

3.3 Primary Research

1. Water

Water is an important content of many foods. Water can be a component of the cell intra- and extra-cells. The presence of water affects the quality of the food chemistry and microorganisms, John M Deman [7].

From the calculation of Variance Analysis (ANOVA) on water content oncom peanut meal showed that incubation temperature and concentration of inoculum give a real difference on water content oncom peanut meal produced at the level of 5%, while the interaction between the treatment does not give a real impact on oncom peanut meal produced. The results of the analysis of water content oncom peanut meal can be seen in Table 3.

<table>
<thead>
<tr>
<th>Incubation temperature</th>
<th>Water content (%)</th>
<th>The real level of 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a3 (28º C)</td>
<td>32.437%</td>
<td>a</td>
</tr>
<tr>
<td>a2 (27º C)</td>
<td>33.386%</td>
<td>a</td>
</tr>
<tr>
<td>a1 (26º C)</td>
<td>34.377%</td>
<td>ab</td>
</tr>
</tbody>
</table>

Description: Every letter followed by different letters indicate significant differences according to Duncan test at 5% level.

Table 3 shows that the effect of incubation temperature on water content oncom peanut meal is a1 treatment incubation temperature of 26ºC produces peanut meal pie with the highest water level that is equal to 34.377%, while the incubation temperature of 28ºC a3 treatment produces peanut meal pie with a low moisture content that is equal to 32.437%. This shows the lower temperature of incubation, the higher the water content oncom peanut meal produced, and the higher the temperature of incubation is done, the lower the water content oncom produced groundnut cake.

The results showed that at 0 hours to 24 hours has not happened fermentation, which has not happened metabolism to form H2O products of metabolism, while the fermentation over 24 hours of degradation compounds - organic compounds by the activity of enzymes that produce simple compounds also another result of the process metabolism is H2O, the energy in the form of heat and material - other materials. With the formation of heat during the fermentation process the temperature of the material will increase and the water produced during the fermentation process will evaporate resulting in decreased water levels.

The analysis results inoculum concentration on water content oncom peanut meal can be seen in Table 4.
Table 4. Effect of inoculum concentration Against Moisture Oncom Peanut cake

<table>
<thead>
<tr>
<th>The concentration of inoculum</th>
<th>Water content (%)</th>
<th>The real level of 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>b3 (0.18%)</td>
<td>32.493%</td>
<td>a</td>
</tr>
<tr>
<td>b2 (0.15%)</td>
<td>33.617%</td>
<td>a</td>
</tr>
<tr>
<td>b1 (0.12%)</td>
<td>34.090%</td>
<td>ab</td>
</tr>
</tbody>
</table>

Description: Every letter followed by different letters indicate significant differences according to Duncan test at 5% level.

Table 4 shows that the effect of inoculum concentration on water content oncom peanut meal is b1 treatment inoculum concentration of 0.12% has the highest water content that is equal to 34.090%, while treatment b3 inoculum concentration of 0.18% has the lowest water levels of 32.493%.

The higher concentration of inoculum the metabolic processes will be bigger than the small amount of inoculum concentrations. The results will be the formation of H2O metabolism and heat. With the heat and the water formed during metabolism will evaporate along the water in the material, as a result of water in the material decreases.

2. Levels of Protein

Protein is a nutrient that is essential for the human body, because these substances function as energy in the body, as well as builders and regulatory substances, Winarno [15]. Protein is also important for functional and structural purposes and for the purposes of the composition of the amino acids are very important building blocks of protein function. Therefore, the protein has a quality that varied depending on the extent to which the protein is able to provide the essential amino acids in adequate amounts, Buckle et al. [3]

Based on the analysis of variance (ANOVA) showed a significant effect of incubation temperature on water content oncom peanut meal, whereas the concentration of inoculum and interaction between treatments was not significant effect on water content oncom produced groundnut cake. The results of the analysis of protein content of peanut meal pie against incubation temperature can be seen in Table 5.

Table 5. Effect of Incubation Temperature Levels Of Protein Peanut cake Oncom

<table>
<thead>
<tr>
<th>Incubation temperature</th>
<th>Protein Content (%)</th>
<th>The real level of 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1 (26o C)</td>
<td>17.335%</td>
<td>a</td>
</tr>
<tr>
<td>a2 (27o C)</td>
<td>18.341%</td>
<td>a</td>
</tr>
<tr>
<td>a3 (28o C)</td>
<td>18.669%</td>
<td>ab</td>
</tr>
</tbody>
</table>

Description: Every letter followed by different letters indicate significant differences according to Duncan test at 5% level.

Table 5 shows that the effect of incubation temperature on the protein content of peanut meal pie is a3 treatment incubation temperature of 27ºC produces peanut meal pie with the highest protein content of 18.669%, while the incubation temperature of 26ºC a1 treatment produces peanut meal pie with the lowest protein content that is equal to 17.335%.

At a temperature of 28 ° C occurred metabolism resulting formed water, so the water evaporates along the water in the material, then the relative materials will be somewhat dry, so that the weighing certain will result in the amount of water that is weighted less, while most of it is material that contains protein. In addition to this, also the number of cells at 28 ° C becomes larger while the cells containing the protein.

Differences in levels of these proteins caused by the differences of temperature incubation of the protein content of peanut meal pie, so the higher the temperature of incubation is done will produce high levels of protein peanut meal oncom lower and lower. Protein is a nutrient that is very important because it is closely connected with the process of life. Protein molecules contain the elements C, H, O, and special elements that are not contained in the molecules of carbohydrates and fats are the elements
N, even in the analysis of foodstuffs that all elements of N ascribed to the protein. In addition, protein also contains phosphorus, sulfur, and metals such as iron and copper, Winarno, [15]. Determination of protein in food is generally determined is the total protein. Proteins are often considered sebagaii crude protein for the determination of nitrogen in the compound is not the determination of a specific protein, but the determination of total protein, Sudarmadji, [11]

3. Fat Content

From the analysis of variance (ANOVA) showed that the temperature of incubation and inoculum concentration gives a real difference to the fat content oncom peanut meal produced at the level of 5%, while the interaction between treatments do not provide a real impact on the fat content oncom peanut meal produced , The influence of incubation temperature on the fat content of peanut meal pie can be seen in Table 6.

<table>
<thead>
<tr>
<th>Incubation temperature</th>
<th>Fat level (%)</th>
<th>The real level of 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1 (26º C)</td>
<td>3.937%</td>
<td>a</td>
</tr>
<tr>
<td>a2 (27º C)</td>
<td>3.991%</td>
<td>b</td>
</tr>
<tr>
<td>a3 (28º C)</td>
<td>4.062%</td>
<td>c</td>
</tr>
</tbody>
</table>

Description: Every letter followed by different letters indicate significant differences according to Duncan test at 5% level.

Table 6 shows that the effect of incubation temperature on the fat content of peanut meal pie is a1 treatment incubation temperature of 26ºC produces peanut meal pie with the lowest fat content is equal to 3.937%, while the a3 perlakuana incubation temperature of 28ºC produces peanut meal pie with the highest fat content is equal to 4.062%. This indicates the higher the incubation temperature, the higher the fat content of peanut meal pie is generated, and the lower temperature of incubation, the lower the fat content of peanut meal oncom produced.

At a temperature of 28 °C occurred metabolism resulting formed water, so the water evaporates along the water in the material, then the relative materials will be somewhat dry, so that the weighing certain will result in the amount of water that is weighted less, while most of it is material that contains fat. In addition to this, also the number of cells at 28 °C becomes larger while the fat-containing cells. The analysis results inoculum concentrations on levels of fat pie peanut meal can be seen in Table 7.

<table>
<thead>
<tr>
<th>The concentration of inoculum</th>
<th>Fat level (%)</th>
<th>The real level of 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>b1 (0.12%)</td>
<td>3.980%</td>
<td>a</td>
</tr>
<tr>
<td>b2 (0.15%)</td>
<td>3.996%</td>
<td>a</td>
</tr>
<tr>
<td>b3 (0.18%)</td>
<td>4.014%</td>
<td>ab</td>
</tr>
</tbody>
</table>

Description: Every letter followed by different letters indicate significant differences according to Duncan test at 5% level.

Table 7 shows that the effect of inoculum concentration on the fat content of peanut meal pie is b3 treatment inoculum concentration of 0.18% has the highest fat content is equal to 4.014%, while the treatment of inoculum concentration of 0.12% b1 has the lowest fat content of 3.980%.

At a concentration of inoculum that more and more high number of cells and enzymes released by microorganisms thought to the more, so that these enzymes in the decomposition of the substrate on which micro-organisms will be maximized and product menghasilkann fat content with a high concentration.
3.4 Appearance Test Results Top Research

1. Color

Based on the ranking table on the number of treatments = 9 and the number of panelists = 15 with a significance level of 5% can know its value range is 50-100. From the total samples tested, indicating that the treatment a2b2 most preferred by the panelists.

2. Aroma

Based on the "Ranking Test" on the number of treatments = 9 and the number of panelists = 15 with a significance level of 5%, that the sample a2b1, is highly preferred sample, the sample while samples a3b2 is not preferable. It can be concluded that the samples most preferred is the treatment a2b1.

3. Texture

Based on the "Ranking Test" indicates that the sample is a sample a2b2 is preferred, while the sample a3b1, a3b2 and a3b3 is not preferred sample. It can be concluded that the samples most preferred is the treatment a2b2.

4. Sense

With the fermented protein content affects the taste of peanut meal pie. Improved taste peanut meal oncom mainly influenced by the protein and fat content.

In organoleptic taste, oncom peanut meal that will be served to panelists in advance fried, thus the taste test will not be affected by further changes due to the activity of fungi, although oncom peanut meal has finished. Rhizopus oligosporus will continue to actively degrade the nutrient content in peanut meal pie.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusion

a. The result showed that the highest protein content, fat content the highest and lowest water content obtained in the treatment of 28°C incubation temperature and concentration of inoculum 0:18%.

b. The results of organoleptic tests like the major studies of color and texture treatment incubation temperature is 27°C and inoculum concentration of 0.15%, while the aroma and flavor of the treatment temperature is 27°C incubation and inoculum concentration of 0.12%.

4.2 Recommendations

a. Further studies regarding oncom peanut meal with the addition of different raw materials.

b. For the artisans, it is advisable to develop this product, because of its nutrient content is very good including essential amino acids.

5. REFERENCES


