FERMENTATION IN SALT SOLUTION TO PRODUCE JACK BEANS (Canavalia ensiformis L) SAUCE

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Abstract

The purpose of this research was to determine the proportion or optimal formulation so as to produce jack bean sauce best quality using Design Expert D-Optimal method. This research uses statistical data processing program for which the software Design Expert 7.0.0 with Mixture D-optimal design to design variable the jack bean (X1), concentration of salt (X2), Rhizopus sp (X3), garlic (X4), coriander (X5), anise (X6), turmeric (X7), bay leaves (X8), leaves of lemongrass (X9), galangal (X10), MSG (X11), brown sugar (X12), and keluak (X13). Analyzed response were cyanide acid content, protein content, water content, total dissolved solids, viscosity, flavor, color, consistency, and aroma. Selected jack bean sauce with optimal formulation is sauce with the following composition jack bean 12,5%; salt solution 20%; Garlic 1,1%; coriander 0,5%; anise 0,05%; turmeric 0,5%; bay leaf 0,6%; lemongrass leaves 0,6%; galangal 1,2%; MSG 0,4%; brown sugar 60,5%;keluak 2%. The formulation has been predicted by the program with levels of cyanide 13,79 mg/kg; protein content of 3,46%; water content of 28,16%; total dissolved solids 20.58 ^{o} Brix; viscosity 21.91 d.Pa.s; the flavor attribute scores 4.52; the color attributes score 4.55; the viscosity attributes scores on 3.96; and the aroma attributes score 3.63.

Keywords: Formulation; Jackbeans; Soy Sauce; Design Expert; D-Optimal.

Abbreviation:
MSG: Mono Sodium Glutamat

1. Introduction

In Indonesia, soy sauce is known as foods flavoring because soy can provide a distinctive flavor and aroma to food or cuisine. In general, soy sauce is a product processed or preserved from soybeans with a liquid texture (salty) and thick (sweet), blackish brown, and is used as a food flavoring ingredient (Turyoni, 2007).

Soy sauce is generally made from soy, but with today's soaring soybean prices cause unrest amongst the soy sauce industry that still relies on imported soybeans. Moreover, raw material requirements for the production of soy sauce have to compete with the industrial manufacture of tofu and tempeh so it takes the availability of alternative materials that can be continuous. One type of beans that can be used as a source of raw material for making soy sauce is jack bean.

Jack bean has a huge potential when viewed in terms of nutrition. Fundamental similarities when compared with black soy beans are the nutritional content, with this similarity; it can be used as a reason for the use of jack bean as a raw material in the manufacture of sauce.

Formulation development becomes very important in order to produce food products that can be accepted by society in terms of sensory. The use of spices in the formula will affect the characteristics of the soy sauce product.

One of the software can be used to determine the optimal formulation was Design Expert. Design Expert is used for process optimization in the primary response caused by several variables and the objective is the optimization of the responses (Bas and Boyaci, 2007).

However, the accuracy of the optimal formulation produced by design expert program with d-optimal methods still needs to be proofed in the manufacture of jack bean sauce.

The purpose of this study is to obtain an optimal formulation for jack bean sauce. Chemical responses analyzed include cyanide acid, protein, and water. Physical responses were analyzed include total dissolved solids and viscosity. Sensory responses were analyzed include flavor, aroma, color, and viscosity.

2. Materials and Methods

2.1. Materials and Tools

The materials used in this study are jack bean (*Canavalia ensiformis L.*), salt, water, *Rhizopus* sp ("Ragi Tempe"), garlic, coriander, papayas, anise, saffron, bay leaf, lemon grass leaf, galangal, MSG , and brown sugar. Chemicals used for the analysis of products include, chemicals for amino acid analysis, the analysis of cyanide acid levels, protein content analysis, analysis of water content, viscosity analysis, and total dissolved solids.

The tools used in this study are basins, jars, incubators, gas stove, pot, scales, pans, strainer, knife, spoon, plastic pan, which has been perfo-

rated plastic, and measuring cup. The tools used for chemical analysis Asama analysis of amino acid levels of cyanide, the analysis of protein content, moisture content analysis, the analysis of viscosity, total dissolved solids and organoleptic testing.

2.2 Research Methods

The research method consists of two phases: a preliminary study and the main study. Preliminary research was conducted to obtain the concentration of "Yeast Tempe" (*Rhizopus* sp) which will be used in the main study. Selected "Yeast Tempe" (*Rhizopus* sp) concentration is a moromi solution with the highest levels of the amino acid leucine that was analyzed using a LCMS (Liquid Chromatography-Mass Spectrometry). Variations in the concentration of "Yeast Tempe" were 0.05%, 0.1%, and 0.5%.

The main research was carried out to obtain the optimal formulation of jack bean sauce according to the chemical response (cyanide acids, proteins, and water), physical responses (total dissolved solids and viscosity), and organoleptic response (taste, aroma, color, and viscosity). This study uses statistical data processing program using Design Expert 7.0.0 Mixture d-optimal Design. The design variables are the jack bean (X1), saline solution (X2), Rhizopus sp ("Yeast Tempe") (X3), garlic (X4), coriander (X5), anise (X6), turmeric (X7), bay leaf (X8), leaves of lemongrass (X9), galangal (X10), MSG (X11), brown sugar (X12), and keluak (x13). The response of this design are cyanide acid levels, protein content, moisture content, total dissolved solids, viscosity, flavor, color, consistency, and flavor. Low showed the lowest value threshold (minimum) and high showed the highest threshold (maximum).

The responses studied were chemical response include the levels of cyanide with Argentometry titration method (AOAC, 1995), protein content using Kjeldhal method (AOAC, 1995), and water content by distillation method (Apriyanto, et al., 1989). Physical responses include total dissolved solids with a refractometer method, the viscosity test with Viscotester. Organoleptic responses made to the jack bean sauce produced are the taste, color, aroma and consistency. Determination of selected optimum formulation uses statistical testing using Design Expert software with D-Optimal method.

3. Research Procedure

3.1 Main research

Description experiment of making the sword lima bean sauce:

1. Washing and sorting

Washing will remove dirt and foreign objects on the jack bean, also to separate good jack bean from the damaged or unsuitable one so that the finished jack bean has good selected physical condition. Washing is done by using clean water at room temperature.

2. Immersion and removal of HCN

Immersion process aims to eliminate cyanide acid levels contained in jack bean using clean water for 3 days and every 6 hours the soaking water is replaced. Comparison of jack bean with water is 1: 4 and soaking conditions is at room temperature. The purpose of soaking process in addition to eliminating HCN or cyanide and also to remove the epidermis which at soaking process, bean seeds absorb water and swell so the peanut shells is broke and easy to release.

3. Peeling skin and downsizing

Peeling skin aims to eliminate jack bean skin that still detached. Then do the downsizing with splitting the jack bean into four parts. The aim of this process is to simplify the process further, especially in the koji fermentation or sauce fermentation.

4. Washing

Jack bean that has been downsized then washed, this process aims to clean up the skin that may still be attached as well as unwanted impurities. The washing process is done by using clean water at room temperature.

5. Boiling

The boiling process is done twice; this process aims to eliminate cyanide acid levels remaining in the jack bean because the cyanide acid is soluble and easy to evaporate. In addition, the boiling process is intended to make the jack bean tender. This process uses the boiling temperature of 100C in 15 minutes of time.

6. Draining

After completion of the boiling process, jack beans are then drained so that water contained in the materials will be reduced. The drained jack beans should be chilled completely because if they are not in a cold state, mushrooming will not work because the fungus will die when sown on it.

7. Inoculation

Inoculation process is the process of adding inoculum using Rhizopus sp ("Ragi Tempe") with a concentration in accordance with the results of the preliminary study.

8. Packaging

The process of packaging used to package jack bean that has undergone a process of inocu-

lation by using a perforated plastic.

9. Incubation

Jack bean that has been mixed with the inoculum and packed by using a perforated plastic, then stored in an incubator for mold fermentation. Mold fermentation conducted for 48 hours or 2 days at a temperature of 30C, the process is often referred to as making tempeh or tempeh fermentation.

10. Separation

Jack bean which has undergone mold fermentation or in other words has become tempeh, separated from one another aims to simplify the process further.

11. Salt Fermentation

Salt fermentation is done by soaking in saline solution for 30 days at room temperature using a solution of salt concentration on a predetermined formula. The result of this fermentation process called moromi.

12. Filtering I

After fermentation is complete then the resulting moromi is filtered to produce a filtrate of raw sauce.

13. Cooking

Raw sauce produced from the filtering process is then cooked to get a consumption ready sauce by adding seasoning and spices according to a predetermined formula.

14. Filtering II

Sauces that has been cooked and then filtered with the aim to separate the filtrate and the residue after cooking, the filtrate is exactly what is called the jack bean sauce. Sauce that is ready to be consumed then being analyzed for levels of it cyanide acid, protein content, water content, viscosity, total dissolved solids, and organoleptic.

3. Results and Discussion

Table 1. Results of Analysis of Amino Acids Leucine From moromi Solution With Multiple Concentration "Yeast Tempe" (Rhizopus sp)

Konsentrasi "Ragi Tempe" (*Rhizopus sp*)	Kadar Asam Amino Leusin (mg/L)
0.05%	202
0.1 %	169
0.5%	200

3.1 Results of Preliminary Research

Preliminary research was conducted to obtain the concentration of "yeast tempe" or scientifically including Rhizopus sp fungus group that will be used in the main study. Selected concentration of "Yeast Tempe" (Rhizopus sp) is a moromi solution with the highest levels of the amino acid leucine. Based on analysis of the amino acid leucine using a LCMS (Liquid Chromatography - Mass Spectrometry) showed that the concentration of the moromi solution of "yeast tempe" (Rhizopus sp) with a concentration of 0.05% was chosen because it has the highest levels of the amino acid leucine that is equal to 202 mg / L .

To obtain the optimal formulation in the main study, the "yeast tempe" concentration needs to be known in advance using the analysis of leucine amino acid from moromi solution, as this result will determine the taste quality of sauce produced from jack bean. Factors that influence the quality of the taste of jack bean sauce is fungus fermentation because in this process the mold will release enzymes that break down substrates into soluble compounds. Those levels of dissolved compounds determine the flavor of soy sauce. The addition of salt in the moromi fermentation serve to attract dissolved nitrogen compounds present in the koji into the salt solution so that the resulting sauce tasty.

Leucine amino acid content in jack bean is 25,000 mg/kg. After the fermentation process into a moromi solution, leucine amino acid content decreased to 169 mg / L-202 mg / L, which is in line with Astuti, *et al* (2000) research that said the amino acid content would decrease due to Rhizopus sp is wearing amino acids as source of N (nitrogen) for growth. Mold using various sources of nitrogen for growth include the amino acid proline, glycine, aspartic acid, leucine and ammonium salts. In addition to the nitrogen source, molds also need a carbon source such as glucose, fructose, galactose, xylose and mannitol. Mold using jack bean as a substrate for source of carbon and nitrogen sources.

Results of research that was conducted by Nurika, et al (2011) showed that the long incubation affects the total response of amino acids, rather than the molds concentration, it can be seen from the data analysis of variance (ANOVA), where the long incubation significantly affected the total amino acids compared to mold concentration because it has a chance of error <0.01% (chance of error is less than 5%). So it can be said that the total amino acids is influenced by the long incubation compared with concentrations of mold.

3.2 Results of Main Research

3.2.1 Cyanide Acid Levels

Based on the research, indicating that the

twenty formulations were not statistically affect the levels of cyanide, it is because in the 20th formulations have the same treatment done to reduce the content of cyanide. Treatments to minimize cyanide include soaking for 3 days, boiling for 30 minutes, and koji fermentation for 2 days. This is in accordance with Wedhastri's (1990) revelation that the heat treatment, soaking, and koji fermentation will minimize the cyanogenic glucoside compound in jack bean.

The content of cyanide in jack bean is 67.05 mg / kg and after becoming jack bean sauce ranged from 11mg / kg 17mg / kg. According Prastyo and Triadji (2011), lethal dose of cyanide is in the range of 50-90 mg / kg. So that the levels of cyanide from the 20th jack bean sauce formulations are safe, because they are in the consumption limit.

If ingested, HCN is very quickly absorbed by the digestive tract into the bloodstream and it is tied together with oxygen. Dangers of HCN on health, especially in the respiratory system, where oxygen in the blood is bound by the HCN compound and disrupt the respiratory system that causes breathing difficulty (Lisyah, 2012).

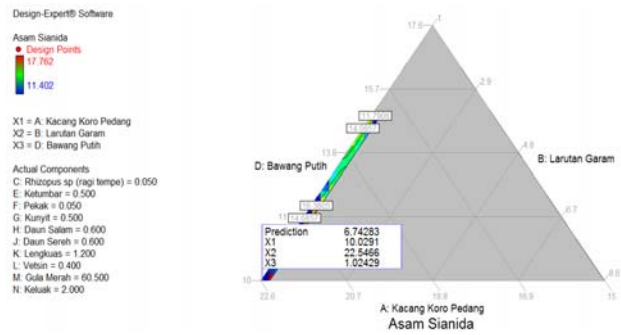


Figure 1. Graph of Optimal Formulation Based on Cyanide Acid Response Levels

3.2.2 Protein levels

Based on the research, indicating that the 20th formulations were not statistically affect the protein content, it is because the protein content in sauce is influenced by the length of moromi fermentation time, while the moromi fermentation time for these 20th formulation is the same with time ferment for 30 days. Levels of protein in sauce is also dependent on the amount of N in the unravel bean. The amount of unravel N will be higher if the reached decay during the first phase of fermentation perfect. The protein contained in this jack bean, by the R.oligosporus and R.oryzae fungal proteinase enzyme hydrolyzed into peptides, followed by hydrolysis of peptides by peptidases into amino acids that are more easily digested by the body. 20th formulations have the same or uniform weathering time that is 2 days or 48 hours.

Protein content of jack bean is 21.89% and after processing into jack bean sauce, it protein content is 3% -4%. Protein levels in this study are in accordance with SNI (01-3543-1999) that the standards for protein in sweet soy sauce are at least 2.5%.

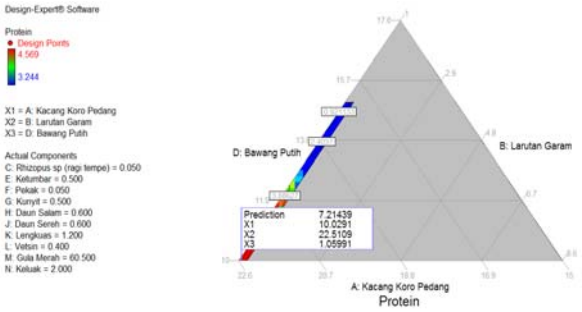


Figure 2. Graph of Optimal Formulations Based On Protein Levels Response

3.2.3 Water Content

Based on the research, showed that the twenty formulations statistically affect the water content, it is caused by more and more sugar is used in the formula will lower the soy sauce water content. That is because the sugar have more opportunity to tie water and more water will be evaporated which can cause the water content of soy sauce to be lower.

The water content of jack bean is 15.46% and after processing into jack bean sauce, it content ranges between 26% -30%. The water content in this study are in accordance with the data from the Palm Plant Research Centre, that the standard of maximum water content in soy sauce is 65%.

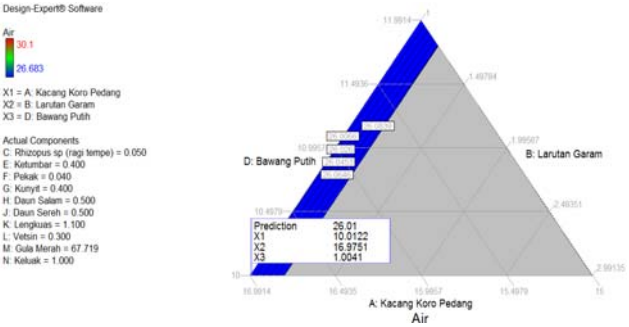


Figure 3. Graph of Optimal Formulation Based On Water Content Response

3.2.4 Total Dissolved Solids

Based on the research, indicating that the twenty formulations statistically affect the value

of total dissolved solids, it is due to the total dissolved solids closely related to the sugar content of the product. it is measured by the percentage of sugar products. High value of total dissolved solids indicating the sugar content in soy sauce is also high. High levels of sugar in the jack bean sauce are due to adding of sugar in the manufacturing process. The higher value of total dissolved solids means more sugar was added.

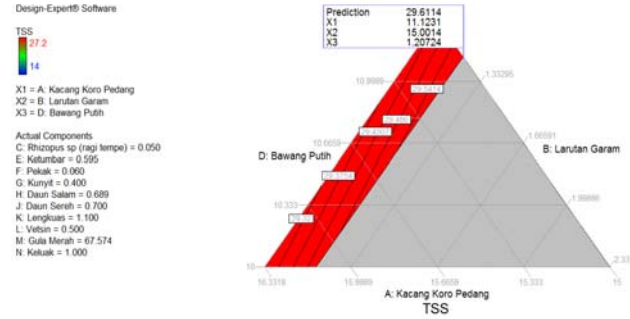


Figure 4. Graph of Optimal Formulations Based on Total Solids Response

3.2.5 Viscosity

Based on the research, indicating that twenty formulations statistically affect the value of the viscosity, it is due to the variation of sugar concentration in the 20th formulations. The higher the viscosity indicating that more amount of sugars are used due to the binding of water by the hygroscopic nature of sugar. The viscosity of jack bean sauce are also affected by cooking process where it done to achieve a certain level of consistency. In terms of the cooking time, the difficulty is to estimate the exact moment to stop the cooking process that associated with the achieved thickness estimate. Viscosity (thickness) is a typical trait in sweet soy sauce and is related to quality. Soy sauce with small viscosity level is said to have a poor quality. According Suprapti (2005), sauce viscosity level is affected by two things: the brown sugar and thickeners. This study did not use thickeners then addition of brown sugar contributing to the jack bean sauce viscosity.

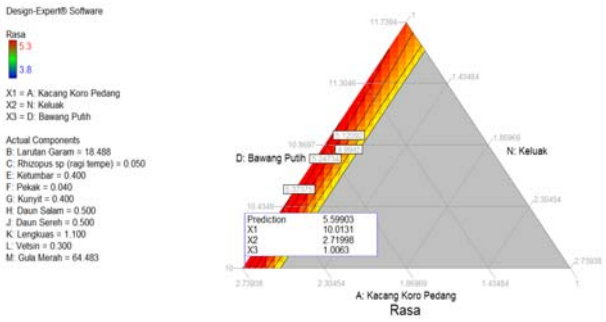


Figure 5. Graph of Optimal Formulations Based on Viscosity Response

3.2.6 Flavor attributes

Based on organoleptic testing showed that all the twenty formulations were not statistically affect the attributes of taste, this is because the range between the minimum and maximum limits used on each of variables or component in the twenty formulations has a short range so that the resulting flavor is not too different from each other. Mixture with spices adds aroma, flavor and the purpose of spices usage in the manufacture of soy sauce is to enhance the delicious flavor and savory, so as to arouse the appetite. Adding spices to the sauce as flavorings and sugar as a sweetener.

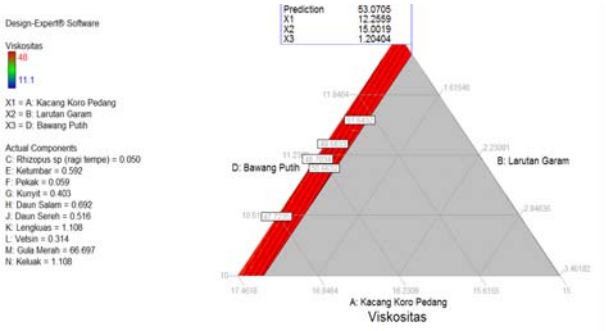


Figure 6. Graph of Optimal Formulation Based On Sense Attributes Response

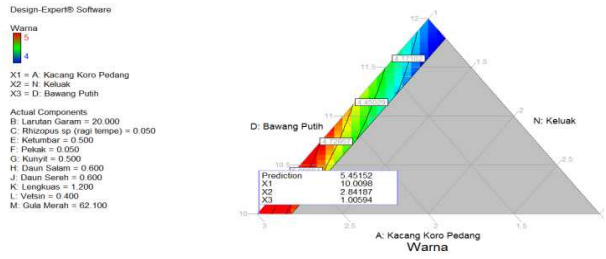


Figure 7. Graph of Optimal Formultions Based On Color Attribute Response

3.2.7 Color

Based on organoleptic testing, indicating that twenty formulations statistically affect the attributes of color, this is because the brownish black color in soy sauce can be obtained from the spices used, one of which is keluwak. Proportion or keluwak formulation added to each formulation is different and it will yield different colors. Maillard reaction leads to the formation of brown color (melanoidin) and also flavor. Caramel flavor in brown sugar allegedly caused by caramelization reactions due to heating during cooking. Caramelization also causes brown color on brown sugar. The addition of high sugar causes the color in the soy sauce become more dark or brown. The increases in the color brightness value of soy

sauce are also caused by the process of caramelization and the addition of spices. In general, a good quality soy sauce is black and also homogeneous.

3.2.8 Thickness

Based on organoleptic test showed that all the twenty formulations were statistically affect the thickness attribute, this is because the viscosity of sauce is affected by two things: the brown sugar and thickeners. In this study does not use thickeners and therefore the factor of brown sugar addition contribute to the viscosity of jack bean sauce. The greater the amount of sugar used, the more viscous the sauce produced, it was resulted from the binding of water by the hygroscopic nature of sugar. Viscosity of sauce is also affected by cooking process when it want to achieve a certain level of consistency. From the side of the length of cooking time, the difficulty is to estimate accurately when to stop the cooking process, its relation to the estimated thickness of the product that has been achieved.

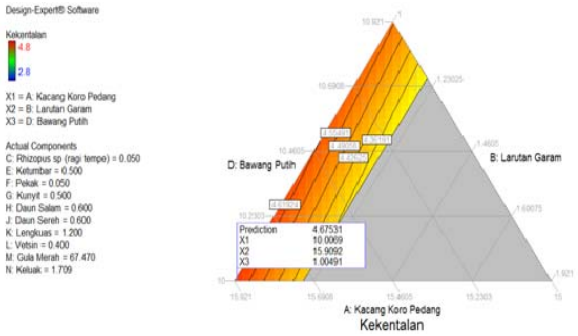


Figure 8. Graph of Optimal Formulation Based On Thickness Attribute Response

3.2.9 Aroma

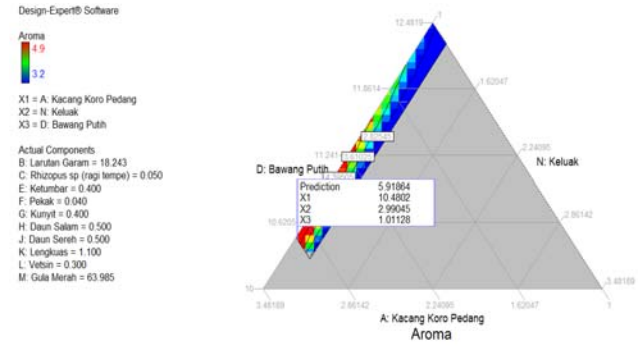


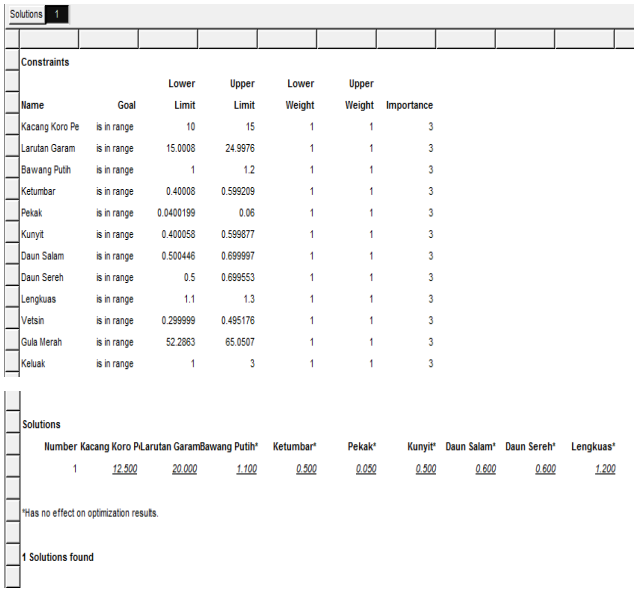
Figure 9. Graph of Optimal Formulation Based On Aroma Attribute Response

Based on organoleptic test showed that the twenty formulations were not statistically affect the aroma attributes, it is because the aroma of spices that are used for the manufacture of sauce also plays a role in the formation of aroma as a whole, all of twenty formulations were prepared using the same seasoning components, just different in its proportions. Protein will be broken down into free amino acids and peptides which will then be converted into the aroma-forming compounds. According Istinah (2001) that brown sugar has a unique flavor because it contains bezil alcohol which is a volatile aromatic compounds.

3.2.10 Selected Optimal Formulation

Selected optimal formulation is an optimal solution predicted by Design Expert software with d-optimal method based on the analysis of physical, chemical and organoleptic responses.

Optimal formulations of jack bean sauce are jack bean 12.50%; 20% salt solution; Rhizopus sp ("Ragi Tempe") of 0.05%; Garlic 1.1%; coriander 0.5%; anise 0.05%; turmeric 0.5%; bay leaves 0.6%; lemongrass 0.6%; galangal 1.2%; MSG 0.4%; brown sugar 60.5%; keluak 2%. This formulation has been predicted by the program with high levels of cyanide 13.79 mg / kg; protein content of 3.46%; moisture content of 28.16%; 20,58°Brix total dissolved solids; viscosity 21.91 d.Pa.S; flavor attribute scores of 4.52 hedonic scale like; score of 4.55 for color attribute hedonic scale like; viscosity attribute scores 3.96 hedonic scale rather like; and a score of 3.63 for aroma attributes with hedonic scale of rather like.



Solutions 1

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Kacang Koro Pe	is in range	10	15	1	1	3
Larutan Garam	is in range	15.0008	24.9976	1	1	3
Bawang Putih	is in range	1	1.2	1	1	3
Ketumbar	is in range	0.40008	0.599209	1	1	3
Pekak	is in range	0.0400199	0.06	1	1	3
Kunyit	is in range	0.400058	0.599877	1	1	3
Daun Salam	is in range	0.500446	0.699997	1	1	3
Daun Sereh	is in range	0.5	0.699553	1	1	3
Lengkuas	is in range	1.1	1.3	1	1	3
Vetsin	is in range	0.299999	0.495176	1	1	3
Gula Merah	is in range	52.2863	65.0507	1	1	3
Keluak	is in range	1	3	1	1	3

Solutions

Number	Kacang Koro P	Larutan Garam	Bawang Putih*	Ketumbar*	Pekak*	Kunyit*	Daun Salam*	Daun Sereh*	Lengkuas*
1	12.500	20.000	1.100	0.500	0.050	0.500	0.600	0.600	1.200

*Has no effect on optimization results.

1 Solutions found

Figure 10. Optimal Formulation of Jack bean Sauce

The accuracy of the optimal formulation and the value of each of these responses can be seen in desirability. Desirability is the degree of accuracy

of the solution or the optimal formulation. The closer the value to one, means higher value of optimization accuracy, so it can be inferred based on the desirability value that has reached 1.00, the value of the response has a high accuracy.

The accuracy of the value of the response has been demonstrated by analysis of cyanide levels, protein content, moisture content, total dissolved solids and viscosity of the selected formulation. Based on the analysis, the levels of cyanide 13.04 mg / kg; protein content of 3.15%; moisture content of 29.18%; 23°Brix of total dissolved solids; viscosity of 19.5 d.Pa.s.

4. Conclusions and Recommendation

4.1 Conclusion

The conclusion of this study is as follows:

1. Optimal formulation of selected jack bean sauce are jack bean 12.5%; 20% of salt solution; Rhizopus sp (tempeh) of 0.05%; garlic 1.1%; coriander 0.5%; anise 0.05%; turmeric 0.5%; bay leaves 0.6%; lemongrass leaves 0.6%; galangal 1.2%; MSG 0.4%; brown sugar 60.5%; keluak 2%.
2. The formulation has been predicted by the program with levels of cyanide 13.79 mg / kg; protein content of 3.46%; moisture content of 28.16%; 20,58°Brix of total dissolved solids; viscosity of 21.91 d.Pa.S; scores of flavor attributes 4.52; score of 4.55 on the color attributes; scores on viscosity attributes of 3.96; and score of 3.63 on aroma attributes.
3. Based on the results of the research conducted on all twenty formulations, cyanide acid levels and protein levels do not give effect to the twenty formulations, while the water content, viscosity, and total dissolved solids influence on the twenty formulations.
4. Under the terms of organoleptic, flavor and aroma attributes do not give effect to the twenty formulations, while the viscosity and color attributes do.

4.2 Suggestion

Suggestions that need to be considered in this study include:

1. In addition to the chemical analysis (protein, water, and cyanide), analysis of physical (viscosity and total dissolved solids), and organoleptic, microbiological testing is also recommended because it is mentioned in ISO standards for soy sauce regarding microbial contamination.
2. Jack bean sauce should be further research on its shelf life determination so that we can know the shelf life of this product.
3. Needs further research on this jack bean sauce product on determining the cooking time so we get a good consistency of the produced sauce.

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