Quality improvement formulation of salak chili paste paste based on curly red chili (Capsicum annuum var. hailux) and pondoh salak (Salacca edulis reinw var. semeru) using sensory evaluation, activity evaluation of bioactive compounds and microbiological evaluation

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Abstract. Red Curly Chili (Capsicum annuum) and Salak Pondoh (Salacca edulis Reimw) are one of Indonesia’s agricultural commodities that have high economic value. This research aims to determine the effect of salak chili paste paste formulation on its sensory properties, the effects of salak chili paste paste formulation on the levels of bioactive compounds (capsaicin, flavonoids, phenols, and antioxidant activity, and the effect of salak chili paste paste formulation on total microbes during storage. This research was divided into three stages, which is (1) sensory analysis by trained panelists, (2) analysis of bioactive compounds using a Microplate Reader, (3) analysis of the number of microbes (TPC). The results showed that (1) formulation 37.5 % Curly Red Chili var. Hailux and 12.5 % Salak Pondoh var. Semeru was preffered by the panelists, and had the highest levels of capsaicin bioactive compounds (0.539 mg/g), phenol (0.361 mg GAE/g), and flavonoids (0.462 mg QE/g). (2) formulation 12.5 % Red Chili Curly var. Hailux, and 50 % Salak Pondoh var. Semeru has the highest antioxidant activity (99.87%). (3) formulation of 50 % Salak Pondoh var. Semeru has the antimicrobial activity of 1.4 x 106 CFU/ml.

1. Introduction
Chili and salak commodities are very potential in Indonesia, they need to be processed into products that are liked by the community as an effort to diversify food processed products. The correct formulation of salak chili paste paste needs to be researched to produce the sensory properties that consumers like. Identification of specific sensory properties of salak chili paste paste was also carried out, so as to enrich the characterization of the sensory properties of Indonesian chili sauce. Different formulations of salak chili paste will also produce different chemical compositions and content of bioactive compounds such as capsaicin, flavonoids, phenols and their antioxidant activities. Most
people, especially in Indonesia, do not like the consumption of dry chili (powder) and prefer the consumption of chili in fresh condition (fresh). The freshness level of chili sauce is positively correlated with the type and amount of formulation of its constituent ingredients. The freshness level of chili products is influenced by three factors including (i) color \[1\], (ii) spiciness (capsaicinoids) \[2\] and (iii) phenolic compounds \[3\]. Freshness during the heating process (thermal), storage process, and food safety are strongly influenced by these three factors. Sambal is a product with medium to high water content, so it is very susceptible to damage by both fungi and bacteria. In this study, it was also observed that the microbial population in chili sauce was associated with the presence of bioactive compounds in it.

2. Material and Method

2.1. Materials

The materials used in this study include the main ingredient used for this research is Red Chili Curly var. Hailux (Capsicum annuum L.), obtained from farmers directly in Pagedangan (Turen – Malang). Salak var. semen (Salacca edulis Reinw) obtained from farmers directly in Tirtomarto (Ampelgading – Malang). Chemicals used in the Capsaicin Test, including Ethanol Solvent (Pro Analysis), HCL (1 N), NaOH (0.05 N), Aquadest, Ammonia Solution (2.5%) and Standard Capsaicin. Chemicals used in the Total Phenol Test include Follin – Ciocalteau reagent (10 %), Na2CO3 (7.5 %), and Gallic Acid. Chemicals used in the Flavonoid Test include NaNO2 (5 %), AlCl3 (10%), NaOH (1 M), and Quercetin Standard. The chemical used in the Antioxidant Test is DPPH (1 M). The materials used in the TPC test of salak chili paste are NA agar medium and aquades.

The equipment used in the three stages of research include Breaker glass Pyrex 100 ml and 250 ml, Pyrex measuring flasks 10 ml, 25 ml and 250 ml, Erlenmeyer Pyrex, Iwaki test tubes, and Pyrex measuring cups 100 ml, Iwaki 250 ml, Pyrex measuring pipettes 1 ml, 5 ml and 10 ml, Herma glass funnel, Petri dish, 100 l and 1000 l micropipettes and tips, filter paper, loop needle, glass and metal spatula, weighing watch, vortex, micro syringe, thermometer, analytical balance. The tool used for extraction is the Microwave Reaction System SOLV (Multiwave Pro) – Anton Paar at the Food Laboratory of the Central Laboratory of Biological Sciences (LSIH) Brawijaya University. Rotary Evaporator IKA RV 10 for concentration of salak chili paste extract. The tool to measure the content of bioactive compounds (Capsaicin, Flavonoids, Total Phenol and Antioxidants) is the Micro Plate Reader (Spectro Star Nano) BMG Lab Tech at the Culture Laboratory of the Central Laboratory of Biological Sciences (LSIH) Brawijaya University. The tools used for TPC testing are autoclave, oven, incubator, Laminar Air Flow (LAF), hot plate stirrer, Bunsen.

2.2. Research phase I- sensory analysis

This study aims to see the effect of salak chili paste formulation on sensory analysis conducted by trained panelists. The research method is carried out using the Quantitative Descriptive Analysis (QDA) method which consists of two separate research stages, namely stage I, panelist selection and stage II, namely training of trained panelists. Sensory attribute exploration was obtained through the description of trained panelists on taste, texture, and aroma \[4\]. The selection stage consists of recruitment, filling out questionnaires, interviews, and sensory selection tests. The second stage was a training test in the form of a basic taste recognition test (sugar and salt solution), a description test of aroma, taste and texture, a hedonic test (level of preference), a threshold test, and a triangle test. The information obtained through is used as a reference in determining the lexicon of salak chili paste paste. Lexicon is the result of the description of the panelists trained in describing the various sensory attributes found in a food product. The description of the salak chili paste paste lexicon formed from trained panelists was then analyzed using the Principal Component Analysis (PCA) method to determine the factors that influence the distribution of responses.
2.3. Analysis of Bioactive Compounds
The analysis of bioactive compounds in this study was carried out using the Micro Plate Reader (Spectro Star Nano) BMG Lab Tech at the Culture Laboratory of the Central Laboratory of Biological Sciences, Brawijaya University. Analysis of bioactive compounds carried out in this study included: capsaicin analysis, flavonoid analysis, phenol analysis, and antioxidant analysis.

2.4. Capsaicin analysis
The principle of capsaicin analysis in this study was carried out in four stages, including:

a. The first stage is the extraction process for the five samples of the salak chili paste formulation (4 grams of each sample dissolved in 40 ml of ethanol solvent) using the Microwave Reaction System SOLV (Multiwave Pro) – Anton Paar at the Food Laboratory, Central Laboratory of Biological Sciences, Brawijaya University for 10 minutes.

b. The second stage after obtaining the salak chili paste extract from each sample is to evaporate the obtained extract using the Rotary Evaporator IKA RV 10 for concentration of salak chili paste extract at a speed of 30 rpm and a temperature of 25°C.

c. The third stage after obtaining a concentrated extract of Salak chili paste, then the next stage is followed by blowing nitrogen (N₂) for approximately 5-10 minutes to remove the ethanol solvent from the salak chili paste extract until a precipitate is formed in the form of a gel / paste.

The last stage (fourth) is the Capsaicin Analysis process using the Microplate Reader (Spectro Star Nano) BMG Lab Tech at the Culture Laboratory of the Central Laboratory of Biological Sciences, Brawijaya University using salak chili paste extract which has been in the form of a gel or paste in the previous stage. The addition of 0.05 N NaOH (10 ml) to the salak chili paste extract increased the pH to alkaline and caused a change in the solubility of the capsaicinoid to become water soluble (will be at the bottom of the solution after centrifugation). Meanwhile, pigments and other phenolic compounds remained in the non-polar phase (at the top of the solution/supernatant).

The standard capsaicin solution was prepared with a concentration of 100 ppm and made with a series of half dilutions in 100% ethanol and carried out twice (duplo), then the absorbance was measured using a Microplate Reader (Spectro Star Nano) BMG Lab Tech at a wavelength of 600 nm. The absorbance value was used as the basis for the preparation of the capsaicin standard curve. The total capsaicinoid content in the sample is calculated by entering the absorbance value of the sample in the linear regression line equation \( y = ax + b \). This equation is obtained from the standard capsaicin curve where the “x” axis is the concentration and the “y” axis is the absorbance of the sample. The results obtained were expressed as mg of capsaicin equivalent per gram of extract (mg CE/g dry weight of extract).

2.5. Flavonoid analysis
The principle of flavonoid analysis in this study used salak chili paste extract with a concentration of 100 l to be added with 30 l 5% NaNO₂, 30 l 10% AlCl₃, 200 l NaOH 1 M and incubated for 15 minutes then the absorbance was measured using a Microplate Reader (Spectro Star Nano) BMG Lab Tech at a wavelength of 415 nm. The quercetin standard was made with a concentration of 100 ppm, and was prepared with a half dilution series and carried out in two replicates (duplo). The total flavonoid content was calculated using the linear equation \( y = ax + b \) where the x-axis is the concentration and the y-axis is the absorbance. The results obtained were expressed as mg equivalent of quercetin per gram extract (mg QE/g BK extract).

2.6. Phenol analysis
The principle of phenol analysis in this study is using salak chili paste extract with a concentration of 100 l which will be added with 50 l of Follin Ciocalteau reagent 10% and 40 l Na₂CO₃ 7.5% and incubated for 15 minutes then the absorbance was measured using a Microplate Reader (Spectro Star Nano) BMG Lab. Tech at a wavelength of 760 nm. Gallic Acid Standard was prepared with a concentration of 100 ppm, and was prepared with a half dilution series and carried out in duplicate
The total phenol content was calculated by entering the absorbance value of the sample in the linear regression equation $y = ax + b$. This equation is obtained from the standard gallic acid curve where the “x” axis is the concentration in ppm and the “y” axis is the absorbance of the sample. The results obtained were expressed as mg gallic acid equivalent per gram extract (mg GAE/g BK extract).

2.7. Antioxidant analysis
The principle of antioxidant analysis in this study using salak chili paste extract used is 100 µl which will be added with 200 µl of ethanol solvent and incubated for 15 minutes then the absorbance is measured using the Microplate Reader (Spectro Star Nano) BMG Lab Tech at a wavelength of 517 nm. DPPH was made with a concentration of 100 ppm, and was made with a series of half dilutions and carried out in duplicate (duplo).

The percentage of inhibition or inhibition can be calculated using the formula below:

$$\text{% Inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100$$

The IC50 value is calculated by measuring the sample at various concentrations of 100 ppm and a half dilution series so that a linear curve is obtained with the equation $y = ax + b$, where the y axis is the % resistance and the x axis is the concentration used.

2.8. Research Phase III - Analysis of Total Plate Count (TPC)
Total Plate Count (TPC) analysis in this study was carried out using the Total Plate Count (TPC) method (Fardiaz, 1992). This method is carried out by inserting a sample (salak chili paste) as much as 4 grams into a test tube by adding 9 ml of Buffered Pepton Water (BPW) diluent, and stirring until homogeneous, and this stage is the first dilution (10-1). Furthermore, 1 ml of the first dilution solution that was homogeneous was put into a test tube containing 9 ml of the dilution solution so that a second dilution (10-2) was formed. The solution was then shaken until homogeneous. This dilution is carried out up to the fourth dilution level (10-4). The result of the last dilution is then taken as much as 1 ml and transferred to Nutrient Agar (NA) media (which has been prepared in advance in a + 20 ml petri dish) and then incubated upside down in an incubator at 37°C for 24 hours.

Determination of the number of microbes is done by the formula:

$$\text{colony total} = \frac{\text{number of colonies calculated}}{\text{dilution factor}} \times \frac{1}{\text{dilution factor}}$$

3. Results and Discussion
3.1. Research results phase I – sensory analysis
Results of the Selection Stage of Trained Panelists. On the first day, a selection of trained panelists was conducted which was attended by 30 people including 15 men and 15 women. The panelists were asked to fill out a participation agreement sheet as a trained panelist and fill out a research questionnaire on the study of acceptance and level of preference for salak chili paste which consisted of self-identity, medical history, and history of eating habits.

The age distribution of the panelists who took part in the selection stage was 6 people aged 20-29 years, 4 people 30-39 years old, 14 people 40-49 years old, and 6 people > 50 years old. The panelists who took part in the selection stage did not have health problems in the form of food allergies, history of Diabetes Mellitus (DM), history of hypertension, susceptible to colds or were taking certain drugs.

Based on the eating history of the panelists, there were 22 panelists who were fans of spicy food, and 8 panelists who were not fans of spicy food. Based on the panelist distribution data based on the spicy food category above, it can be concluded that there were only 22 trained panelists who met the criteria, while the other 8 panelists did not meet the criteria. Of the 22 panelists who are fans of spicy food, there are 20 panelists who consume chili sauce (> 1 tablespoon) with a frequent frequency (> 5x/week), while 2 panelists rarely consume chili with a frequency of <5x/week (< 1 tablespoon).
Based on the distribution of panelists based on the frequency of consumption of chili sauce, it can be concluded that only 20 panelists met the criteria and proceeded to the next stage, while 2 panelists did not meet the criteria. Panelists who have passed the selection based on the frequency of consumption of chili sauce will then follow the training stage for trained panelists. The 20 selected panelists also have a schedule of activities that are not too busy, so they can follow all stages of the training of trained panelists to completion.

3.2. Test Results Description of Taste, Aroma, and Texture
There were 14 panelists who stated that the most spicy taste in the formulation of Salak chili paste A5. This is because the A5 has the most curly red chili and red chili formulations compared to other formulations, which are 37.5 grams and 12.5 grams, respectively.

The formulation that has the sweetest content is the A1 formula which is the original formulation of salak chili paste. While the formulation that has the most bitter/bitter taste is found in the formulation of A5. The sweet taste that appears in the A1 is due to the higher content of salak (50 grams) so that the sugar content in the chili sauce is higher than other formulations. While the bitter taste that appears in A5 is due to the formulation with the lowest amount of salak (12.5 grams) and the chili composition used is higher than other formulations (50 grams). This is because capsaicin from curly red chili can affect the bitter response on the papillae of the tongue. The results of this study are in accordance with research which showed that high concentrations of curly red chilies can affect the sensitivity of the back of the tongue papilla which affects a high bitter taste response. Research by also mentions that in high concentrations, capsaicin can cause a bitter taste when consumed.

As many as 13 panelists gave a description of the delicious aroma of A1 which is the original formulation of salak chili paste. While the description of the pungent aroma was given by 18 panelists on the A5. The pungent aroma is thought to be caused by capsaicin and volatile phenolic compounds found in chili which are the main components of A5.

The panelists chose the A4 and A5 as the salak chili paste with the roughest texture. While the smoothest texture is found in A1. This is presumably due to the higher chili composition in A4 and A5, so that the resulting texture is coarser than other formulations with lower chili composition.

There were 20 panelists who responded to the level of preference, especially for assessments with neutral criteria, somewhat like, like and really like, dominated by A5.

3.3. Results Research Phase II – Analysis of Bioactive Compounds
The results of the analysis of capsaicin levels. Capsaicin levels ranged from 0.126-0.539 mg/g. The highest capsaicin content of 0.539 mg/g was found in A5, namely the formulation of salak pondoh 12.5 grams, curly red chili 37.5 grams, red cayenne pepper 12.5 grams. While the lowest capsaicin content of 0.126 mg/g was found in A1, namely the formulation of salak pondoh 50 grams and red chili pepper 12.5 grams.

The high capsaicin content in A5 was due to the higher use of curly red chili (37.5 grams) compared to other Salak chili paste formulations. In addition, the low capsaicin content in A1 is due to the absence of curly red chili in the formulation. This is in accordance with [5] which states that the content of capsaicinoids (especially capsaicin and dihydrocapsaicin) in cayenne pepper is higher than other types of chili.

3.4. The results of the analysis of total phenol levels
The highest total phenol of 0.361 mg GAE/g was found in A5, namely the formulation of salak pondoh 12.5 grams, curly red chili 37.5 grams, red cayenne pepper 12.5 grams. Meanwhile, the lowest total phenol of 0.033 mg GAE/g was found in A1, namely the formulation of salak pondoh 50 grams and red chili pepper 12.5 grams.

The total phenol in this study is in accordance with the results of the research by [6] which compared the total phenol and antioxidant activity of C. annuum and C. frutescens, where the results of this study
showed that the ethanolic extract of *C. annuum* had a higher total phenol (272.47±7.38 mg GAE/g) compared to the ethanolic extract of *C. frutescens*.

The high total phenol in Salak A5 is due to the use of curly red chili which is quite large, which is 37.5 grams. The high curly red chili formulation contributes to the high total phenol in the chili sauce, because *Capsicum annum* L is rich in phenolic compounds. This is in accordance with who examined the effect of adding fermented red chili powder to stirred yogurt. The results showed that the yogurt added with fermented red chili powder had a higher total phenol than the control. The results of this study are also supported where the increase in total phenol in chili products is due to the high polyphenol content in chili.

3.5. **The results of the analysis of total flavonoids level**

The highest total flavonoid of 0.462 mg QE/g was found in A5, namely the formulation of salak pondoh 12.5 grams, curly red chili 37.5 grams, red cayenne pepper 12.5 grams. Meanwhile, the lowest total flavonoid of 0.124 mg QE/g was found in A1, namely the formulation of Salak Pondoh 50 grams and red chili pepper 12.5 grams.

The total flavonoids of salak chili paste paste in this study were in accordance with [7] where the total flavonoids of raw *C. annuum* varied between 5.4-544.6 g/g, while the total flavonoids of ripe *C. frutescens* varied between 3.6-425.0 g/g. Research results [8] showed the total flavonoid of raw *C. frutescens* was 45.8 g/g and the total flavonoid of ripe *C. frutescens* varied between 36.5 g/g. The suitability of total flavonoids between curly red chili, cayenne pepper, and chili sauce in this study was due to curly red chili (*C. annum*) and cayenne pepper (*C. frutescens*) as the raw materials for salak chili paste paste. So that the higher the total flavonoids in curly red chili and cayenne pepper, the higher the total flavonoid in salak chili paste. The high total flavonoid in A5 is due to the use of curly red chili which is quite large, which is 37.5 grams. The high curly red chili formulation contributes to the high total flavonoid in chili sauce, because *Capsicum annum* L is rich in flavonoids. This is in accordance with [9] which states that *C. annum* is a good source of flavonoids.

3.6. **The results of the analysis antioxidant activity level**

The highest antioxidant activity of 99.877% was found in A2 salak chili paste, namely the formulation of salak pondoh 50 grams and curly red chili 12.5 grams. While the lowest antioxidant activity of 99.480% was found in A5, namely the formulation of salak pondoh 12.5 grams, curly red chili 37.5 grams, red cayenne pepper 12.5 grams.

The high antioxidant activity of Salak A2 chili sauce is due to the formulation of curly red chili and salak pondoh which are the main raw materials. This is in accordance with [10] which stated that *C. annum* contains various secondary metabolites that have antioxidant activity. The strong antioxidant activity of Salak chili paste is associated with the content of its bioactive components. Among them are phenols, flavonoids, and capsaicin which are high in *C. annum*. The results of [11] proved that large amounts of phenols, flavonoids, and proanthocyanidins contribute to the antioxidant activity of *C. annum*. This is because flavonoids have an ortho-hydroxylation ring B, free hydroxyl group, C2-C3 double bond in ring C, and a 3-hydroxyl group in their chemical structure so that they can chelate metals, scavenge free radicals, and suppress oxidative stress [12].
The capsaicin level is inversely proportional to the antioxidant activity, where the higher the capsaicin level, the lower the antioxidant activity measured by % inhibition. This is presumably due to heating during the chili processing which causes a reduction in the antioxidant activity of the chili sauce. This is in accordance with [13] who reported that treatment with high temperatures for a long time can cause the decomposition of capsaicin molecules (8-methyl-N-vanillyl-6-nonenamide) through the cleavage of the alkyl groups attached to the amides. The subsequent cleavage and oxidation leads to the formation of vanillin and produces other substituted phenols. This is also supported by the research of Ornelas reported that the boiling process at 96°C caused a decrease in capsaicin, dihydrocapsaicin and nordihydrocapsaicin in different chili cultivars. Furthermore, [14] revealed that the capsaicinoid concentration of chili paste decreased from 21.7 to 28.3% after thermal treatment regardless of the temperature-time variable in the study. This is because cooking can change the chemical structure which affects its ability to trap radicals [15].

Total phenol is inversely proportional to antioxidant activity, where the higher the total phenol, the lower the antioxidant activity measured by % inhibition. Phenol is a secondary metabolite that has antioxidant activity [16]. The increase in total phenol which is inversely proportional to antioxidant activity is thought to be due to phenol being damaged due to cooking. This is in accordance with who suggested that cooking using high temperatures can affect the total phenol. [17] added that phenolic compounds can be degraded during cooking. While the research of [18] concluded that there was no correlation between total phenol and antioxidant activity. However, the research of [19] revealed the opposite, namely phenol and flavonoid compounds actually increased when chili was cooked.
Figure 3 of the Correlation Curve between total flavonoids and antioxidant activity of salak chili paste

Total flavonoid is inversely proportional to antioxidant activity, where the higher the total flavonoid, the lower the antioxidant activity measured by % inhibition. Flavonoids are the constituents of 60% of total phenols. The antioxidant activity of flavonoids is related to their ability to scavenge free radicals [20].

Table 1. Results of the analysis of the antimicrobial activity of salak chili paste paste

| Sample | Standard Plate Counts (CFU/ml) |  
|--------|-------------------------------|  
|        | First Week | Second Week | Third Week | Fourth Week |
| A1     | 1.7 x 10^4 | 8.2 x 10^3 | 1.2 x 10^6 | 1.4 x 10^6 |
| A2     | 1.4 x 10^4 | 6.8 x 10^3 | 9.8 x 10^4 | 1.3 x 10^6 |
| A3     | 1.0 x 10^4 | 5.2 x 10^3 | 7.8 x 10^4 | 1.2 x 10^6 |
| A4     | 5.3 x 10^2 | 3.9 x 10^3 | 5.0 x 10^4 | 1.1 x 10^6 |
| A5     | 2.2 x 10^2 | 2.5 x 10^3 | 3.5 x 10^4 | 1.0 x 10^6 |

Based on the analysis of the total plate count, the highest antimicrobial activity of 1.4 x 10^6 CFU/ml was found in A1, namely the formulation of salak pondoh 50 grams and red cayenne pepper 12.5 grams at week 4. While the lowest antimicrobial activity was 2.2 x 10^2 CFU/ml contained in A5, namely the formulation of salak pondoh 12.5 grams, curly red chili 37.5 grams, red cayenne pepper 12.5 grams.

4. Conclusions

Based on the research that has been carried out, the following conclusions can be drawn:

a. The formulation of A5 is the most preferred by the panelists because it has a spicier taste than other Salak chili paste formulations.

b. The formulation of A5 has the highest levels of capsaicin bioactive compounds (0.539 mg/g), phenol (0.361 mg GAE/g), and flavonoids (0.462 mg QE/g). While the highest antioxidant activity was found in Salak A2 (99.877%).

c. The formulation of A1 has the highest antimicrobial activity of 1.4 x 10^6 CFU/ml compared to other Salak chili paste formulations.

Based on the results of further test analysis using a 5% confidence interval in Minitab, the results showed no significant difference, this was because the results of the treatment data were not significant.
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